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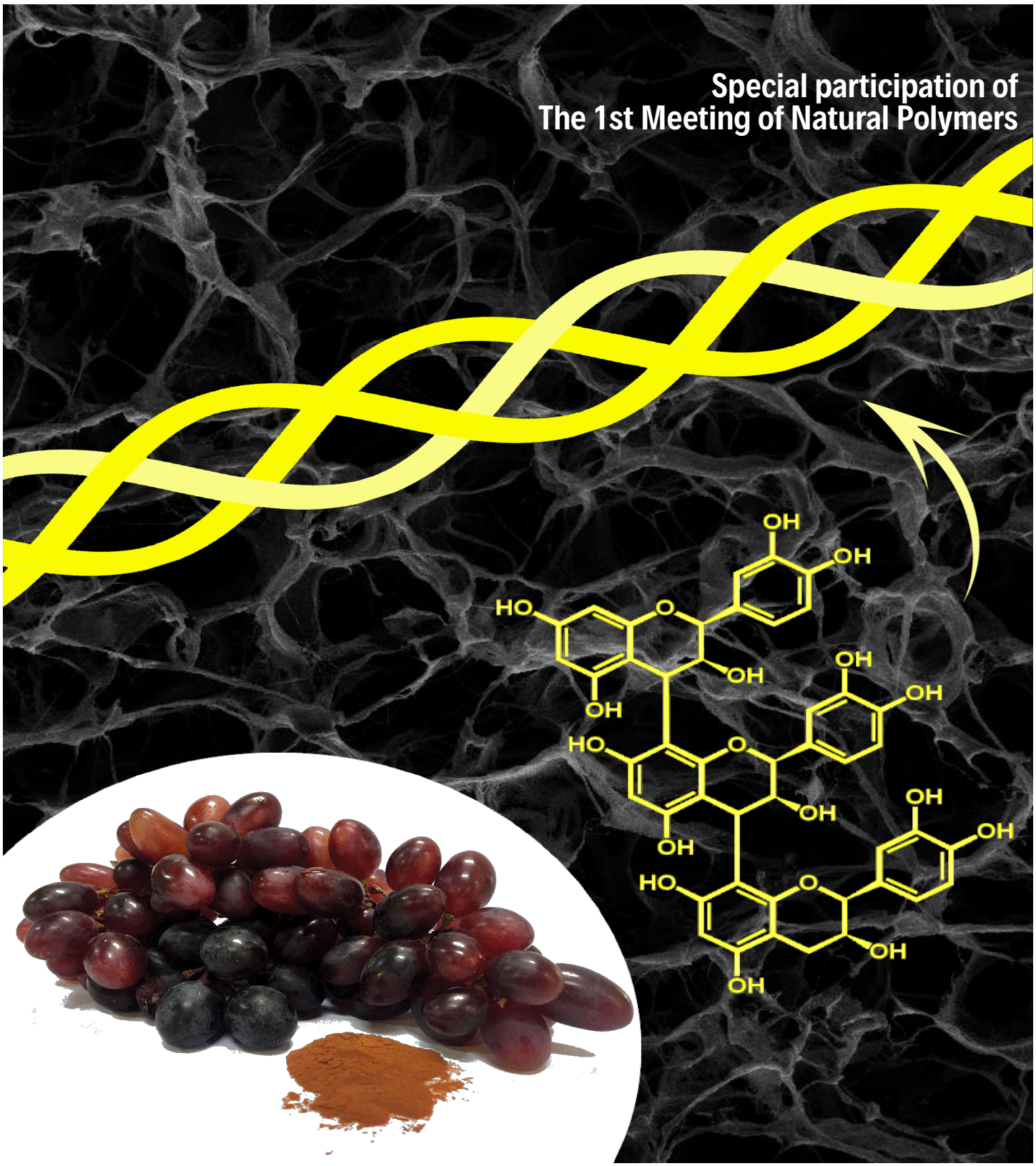


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# Effects of grape seed extract on properties of type I collagen scaffolds

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**Abstract:** To obtain a material with potential for use in tissue engineering, anionic collagen was obtained from porcine serosa (S) and bovine tendon (T) by alkaline hydrolysis for 72h. Part of this collagen was mixed with water to obtain 4 % (weight/weight) collagen suspension and part was solubilized in acetic acid pH 3.5 to obtain 1.5% (w/w) gel. The suspensions were mixed with their respective gels (2:1) (suspension: gel) and grape seed extract, whose main product is proanthocyanidin, was added at concentrations of 0.03% and 0.5%, thus obtaining the scaffolds SC (serosa collagen suspension and gel), TC (tendon collagen suspension and gel), SCP003 (SC with 0.03% extract), TCP003 (TC with 0.03% extract), SCP05 (SC with 0.5% extract added) and TCP05 (TC with 0.5% extract). The materials were analyzed by differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and characterized by phosphate buffered saline absorption assay and in vitro biological stability assay. By DSC it is observed that the addition of 0.5% of extract increases the denaturation temperature (Td) of collagen, indicating that at this concentration the extract acts as polymer crosslinking agent. SEM shows disorganized cross-section pores in all scaffolds, not exceeding 130 μm. Absorption and degradation assays indicated that the addition of 0.5% addition of 0.5% extract increases the absorption of phosphate buffered saline (PBS) by the scaffolds and decreases the degradation percentage by collagenase. These results suggests that the scaffolds can be used for different applications, e.g. as hemostatic agent.

**Keywords:** Biopolymer; Bovine Tendon; Porcine Serosa; Crosslinking.

## Introduction

Topical absorbable hemostatic agents may be used to stop bleeding and retain wound exudate as they initiate platelet aggregation allowing blood coagulation.[1] Different hemostatic agents have been developed and are available to assist surgeons in the treatment of bleeding. Among the various materials used are the microfibrillary collagen, bovine or porcine gelatin sponges, oxidized cellulose, gelatin sealant matrix, thrombin, etc.[1,2,3]

Collagen and its derivatives are chemically attractive in wound healing, with good biological and hemostatic properties. In addition, they have biocompatible, low toxicity and biodegradable characteristics [4]. Its use as a hemostatic agent occurs due to its capacity for platelet aggregation and ability to initiate the cascade of blood coagulation.[5,6]

Collagen is the most abundant structural protein in the body of mammals. It is present in tendons, skin, blood vessels, intestines, bones and teeth and can be extracted from all these sources. The tendons are formed by dense regular connective tissue, which presents fibers arranged in a single direction giving tensile strength and low flexibility, unlike mature bone tissue, which presents collagen fibers arranged in the form of lamellae. Mucosa, muscularis, submucosa, and serosa layers form the intestine, which is composed of loose connective tissue, presenting collagen fibers without order.[7,8,9]

A chemical modification that can be performed with collagen is the selective hydrolysis of the carboxyamide groups of the asparagine and glutamine amino acid residues, leading to an increase of up to 134 negative charges per molecule.[10] This modification allows the production of negatively charged collagen scaffolds at pH 7.4 with high biocompatibility and almost complete absence of chronic inflammatory response.[11,12,13]

Another modification that collagen may suffer is the crosslinking reaction, which increases the resistance to in vitro degradation and alters its mechanical properties.[14] Proanthocyanidins are polyphenolic compounds in the category known as condensed tannins, which can be used as crosslinking agents due to the hydroxyl groups present in their structure, giving stability to hydrogen bonds and thus generating collagen structures with less biodegradability.[15]

Grape seed extract is a byproduct of the production of wines and sparkling wines, presenting several flavonoids such as anthocyanins, flavonols, catechins and proanthocyanidins, the most abundant. Proanthocyanidin has free radical scavenging ability, having superior antioxidant capacity than other oxidants like vitamin C, vitamin E, β-carotene. In addition, it has several pharmacological and biological properties, namely: cardioprotective, anti-tumor, bactericidal and anti-inflammatory.[16]

This study aims to obtain and characterize neutral scaffolds of anionic collagen obtained from two distinct sources (bovine tendon and porcine serosa), crosslinked with grape seed extract and having potential to be used as a hemostatic agent.

## Materials and methods

### Materials

Bovine tendon and porcine serosa were obtained at meat houses, and grape seed extract (*Vitis sp*.) was obtained from a drugstore.

Anionic collagen was obtained from two distinct sources, bovine tendon and porcine serosa, by the treatment in an aqueous alkaline solution (pH ~13) for 72 h using a protocol developed in previous studies in our laboratory.[17] In short, tendon or serosa were placed in an alkaline solution containing Ca(OH)2, KOH,NaOH, Na2SO4, CaSO4, KCl and NaCl for 72 h. Subsequently, the material was equilibrated for 6 h in a solution containing the same salts. Excess salts were removed by rinsing in 3% (w/w) boric acid, deionized water, 0.3% (w/w) EDTA solution, at pH 11 and deionized water up to pH 7.

Treated porcine serosa in water was ground in a blender and later its concentration was defined by lyophilization. Subsequently, it was divided in two parts, the first one was used to prepare a 4 % suspension in water and the second one was solubilized in acetic acid pH 3.5 to obtain a 1% collagen gel.

Treated bovine tendon was lyophilized and grounded in a blender. It was later divided in two parts, the first of which was suspended in water to obtain a 4% suspension and the other one was solubilized in acetic acid pH 3.5 to obtain a 1% collagen gel.

The scaffolds were obtained by mixing the suspension with the collagen gel from the same source, in a 2:1 ratio (suspension: gel) and adding

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or not grape seed extract, which was previously solubilized in 20 mL of 50 % hydroethanolic.

Concentrations of 0.03 % and 0.5 % of grape seed extract were chosen based on previous studies in our laboratory.

The mixtures were placed in Teflon® molds and then lyophilized. They were neutralized using ammonia vapor for a period of 24 h and subsequently left under constant air flow to remove excess ammonia for 72 h. Scaffolds were nominated as shown in Table 1.

| Scaffold | Composition |
|---|---|
| SC | serosa suspension and its gel |
| SCP003 | serosa suspension and its gel + grape seed extract 0.03% (w/w) |
| SCP05 | serosa suspension and its gel + grape seed extract 0.5 % (w/w) |
| TC | tendon suspension and its gel |
| TCP003 | tendon suspension and its gel + grape seed extract 0.03% (w/w) |
| TCP05 | tendon suspension and its gel + grape seed extract 0.5 % (w/w) |

**Table 1 –** Scaffolds denomination

## Methods

### Differential scanning calorimetry (DSC)

DSC curves were obtained with a computer–interfaced differential scanning calorimeter (Model DSC 2010, TA Instruments, New Castle, DE, U.S.A.). Samples of 20 mg were sealed in hermetic aluminum pans and equilibrated at the initial temperature. The scanning was carried out with a heating rate of 10°C $min^{-1}$ from 5 to 120 °C and performed under a nitrogen atmosphere. Denaturation temperature (Td) was determined as the inflection point value of the corresponding endothermic effect.

### *Scanning Electorn Microscopy (SEM)*

Samples were coated with a thin layer of gold of 6 nm. The specimens were examined with a Leo 440, LEO Electrorn Microscopy Ltd. (Cambridge, England) with an accelerating voltage of 20 keV. For the cross section analysis, the scaffolds were frozen in liquid nitrogen, fractured and lyophilized. To measure the pore size, ImageJ software was used and for each scaffold, 31 determinations were made using the approximation of the Martin's diameter and an image magnification of 500x.[18]

### *Absorption kinetics in phosphate buffered saline (PBS)*

Scaffolds were placed in vials containing phosphate buffered saline (PBS). At specific time intervals, scaffolds were removed from the PBS and the excess of solution was removed using a 2 cm x 2 cm filter paper. The scaffolds were weighed and returned to the vials for further time taken. The process was made in quintuplicate. The amount of PBS absorbed was calculated by averaging the results found using equation (1), where $w_{humid}$ is the mass of the swollen scaffold and $w_{dry}$ is the mass of the scaffold before swelling.

$$\%\ absorption = \frac{(w_{humid} - w_{dry})}{w_{dry}} \text{ x } 100 \quad (1)$$

### *In vitro biological stability (collagenase)*

For *in vitro* biological stability determination, collagenase solution in 10 mmol $L^{-1}$ tris–HCl buffer at pH 7.4 containing 25 mmol $L^{-1}$ $CaCl_2$ was prepared. Collagenase solution (10 U $mg^{-1}$of collagen) in tris–HCl buffer, was added to scaffolds known weights which were subsequently placed in a bacteriological oven at 37 °C for 2 h. After this, the samples were washed deionized water, frozen and lyophilized to constant weight. The percentage of degraded collagen (% degradation) was determined by the difference in collagen mass before ($w_{initial}$) and after enzymatic degradation ($w_{final}$) by equation (2), and at least three determinations were made for each scaffold.

$$\%\ degradation = \frac{(w_{initial} - w_{final})}{w_{initial}} \text{ x } 100 \quad (2)$$

### *Statistical Analysis*

The analysis results were analyzed using ANOVA. A value of $p<0.05$ was considered statistically significant.

## Results and discussion

The presence of grape seed extract is noted for its beige color in TCP003 and SCP003 scaffolds and brown in TCP05 and SCP05 scaffolds. The scaffolds without extract (TC and SC) are white and all of them presented touch–resistant appearance and reversibility to small deformations.

DSC curves showed a transition that appears as a discontinuity at baseline, giving the collagen denaturation temperature (Td). Denaturation occurs due to heating and can be defined as the transition of the collagen triple helix to a form in which intramolecular bonds are broken down, changing from a highly organized structure to a disorganized state called gelatin.[19] The values of collagen denaturation temperatures are shown in Table 2.

Native tissues (tendon and serosa) had a denaturation temperature of 63.1°C, values close to those found by Willett[20] and Hizaji[21], who analyzed the Td of bovine tail tendon collagen and obtained values of 62.4°C and 63.3°C, respectively. Hirata[22] evaluated the Td of bovine serosa and obtained a value of 64.6°C, close to those found in this study.

Therefore, a reduction in Td of 9.8 and 3.2°C is observed for native tissues compared to their respective TC and SC scaffolds, respectively. This indicates that alkaline treatment destabilizes the triple collagen helix, and that this destabilizing effect is greatest in tendon matrices.

Alkaline hydrolysis also modifies the macromolecular aggregation of native collagen[23], which is different for tendon and serosa tissue.[7] Differences in Td of SC and TC may indicate that the structure of the serosa is more resistant to destabilization, keeping part of its intrinsically cross–linked loose connective tissue structure.

The denaturation temperature of TC and TCP003 are very close, as well as for SC e SCP003 suggesting that crosslinking by grape seed extract was not effective at 0.03 % concentration.

Scaffolds containing 0.5 % of extract had an increase in denaturation temperature when compared to the scaffolds without extract, being of 14.7°C for the bovine tendon scaffolds and 6.4°C for the serosa scaffolds, indicating that in both cases 0.5 % of extract is effective as a crosslinking agent.

In addition, the 0.5% extract cross–links collagen to the extent that the Td of SCP05 and TCP05 is larger than that of their respective originating tissues. This is because there are still crosslink available sites in the tendon and serosa and the proanthocyanidin present in the extract binds to available sites of the aminoacid proline group present in collagen, causing crosslinking. [24]

Scanning electronic microscopy allows to define the morphology of the scaffolds microstructures, such as pore size, distribution, porosity and interconnectivity, which are major factors in the interaction of the scaffolds with physiological environment, promoting or not the absorption of physiological liquids.

| Scaffold | Td (°C) |
|---|---|
| Bovine tendon | 63.1 |
| Porcine serosa | 63.1 |
| TC | 53.3 |
| SC | 59.9 |
| TCP003 | 53.7 |
| SCP003 | 60.8 |
| TCP05 | 68.0 |
| SCP05 | 66.3 |

**Table 2** – Denaturation temperature (Td).

Photomicrographs (Figures 1–6) show the presence of surface pores and in the cross–section for all samples. Table 3 shows the average pore size, with the respective standard deviations (sd) and the coefficient of variation (Cv).

No statistical difference was observed between TC and TCP05 pore sizes, indicating that the extract addition is not a major factor in pore size in bovine tendon scaffolds. However, for serosa scaffolds, the addition of extract at concentrations of 0.03 % and 0.5 % causes the emergence of pores pores on the scaffolds surface.

The histograms show that TC scaffold (Figure 2), with 74.0% Cv, has a bimodal system with a pore size frequency between 10–20 μm and 30–40 μm. In the case of the SCP003 scaffold (Figure 3), it also has a bimodal system with higher pore size frequencies between 20–30 μm and 30–40 μm. The other scaffolds have systems with one mode, and for the scaffolds TCP003 (Figure 4), SCP05 (Figure 5) and TCP05 (Figure 6) the largest frequency of pore size occurs between 40–50 μm, 20–30 μm, and 30–40 μm, respectively. In the cross section, it is observed that only the TCP003 scaffold has a bimodal system, with a higher incidence of pores between 40–50 μm and 50–60 μm. Only two scaffolds showed statistical difference between the pore size of the surface and the cross section. SC scaffold showed no pores on the surface and SCP05, which presented great pore variation in its cross section, presenting Cv of 54.2%.

Nyberg[25] (2017) and Zhang[26] (2018) show that pores smaller than 100 μm facilitate the diffusion of physiological fluids within the scaffold and promote a capillary force that improves cell attachment in the periphery of the scaffold and can induce cell penetration.

The ability of a scaffold to retain water and electrolytes is an important property for evaluating its effectiveness as a hemostatic material, so the absorption test is essential. Absorption of saline phosphate buffer by collagen scaffolds arises due to various electrostatic interactions. When the first water molecules enter the pores of the scaffold, they bind (hydrate) the hydrophilic groups present in collagen and grape seed extract. The

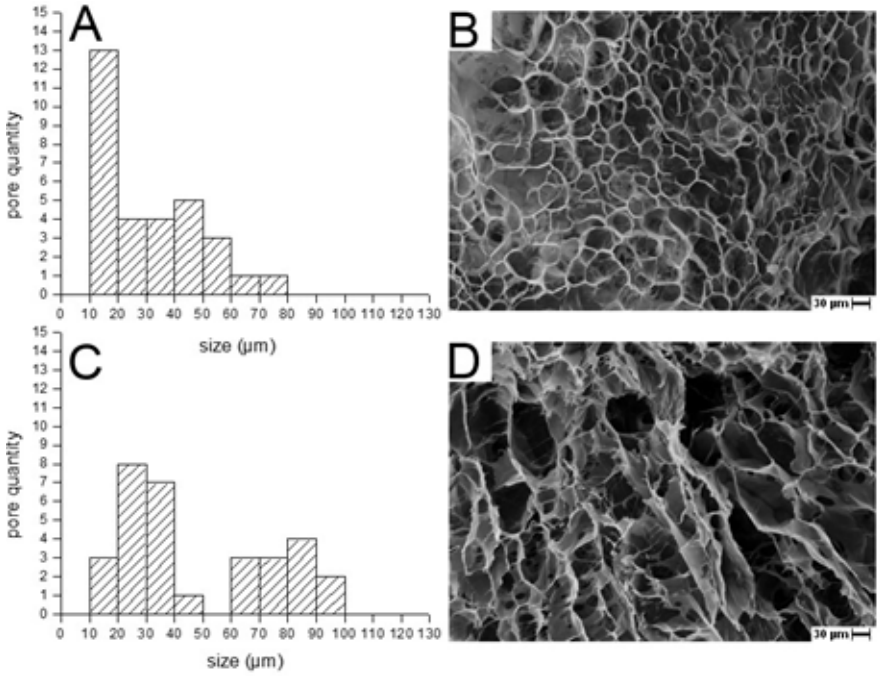


**Figure 1 –** SC scaffold: (A) surface pore size distribution histogram; (B) surface photomicrograph; (C) cross–sectional pore size histogram (D) cross section photomicrograph.

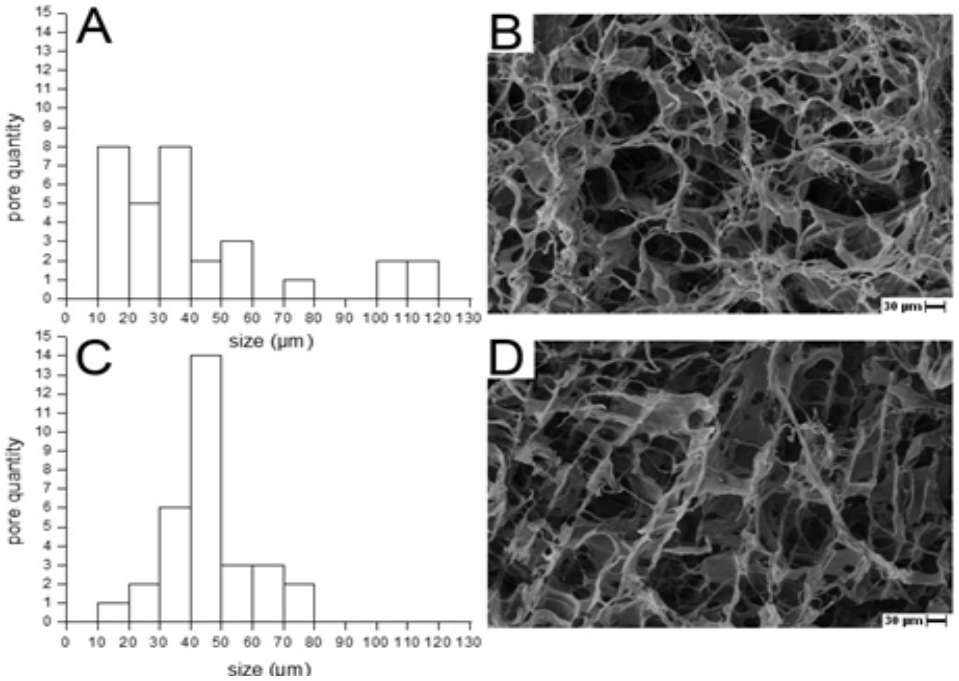


**Figure 2 –** TC scaffold: (A) surface pore size distribution histogram; (B) surface photomicrograph; (C) cross–sectional pore size histogram (D) cross section photomicrograph.

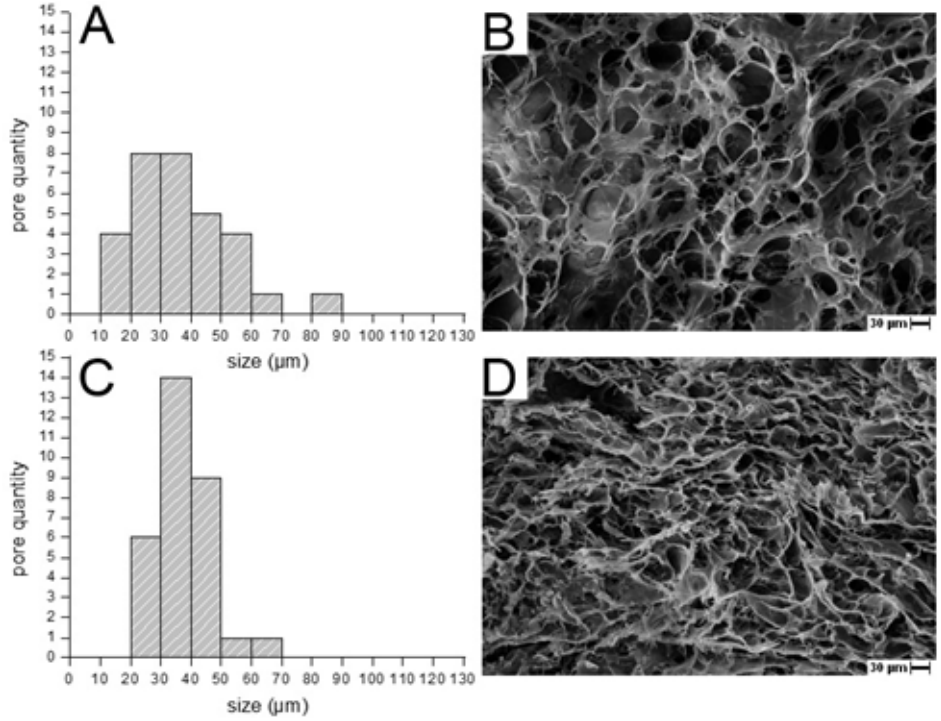


**Figure 3 –** SCP003 scaffold: (A) surface pore size histogram; (B) surface photomicrograph; (C) cross–sectional pore size histogram (D) cross section photomicrograph .

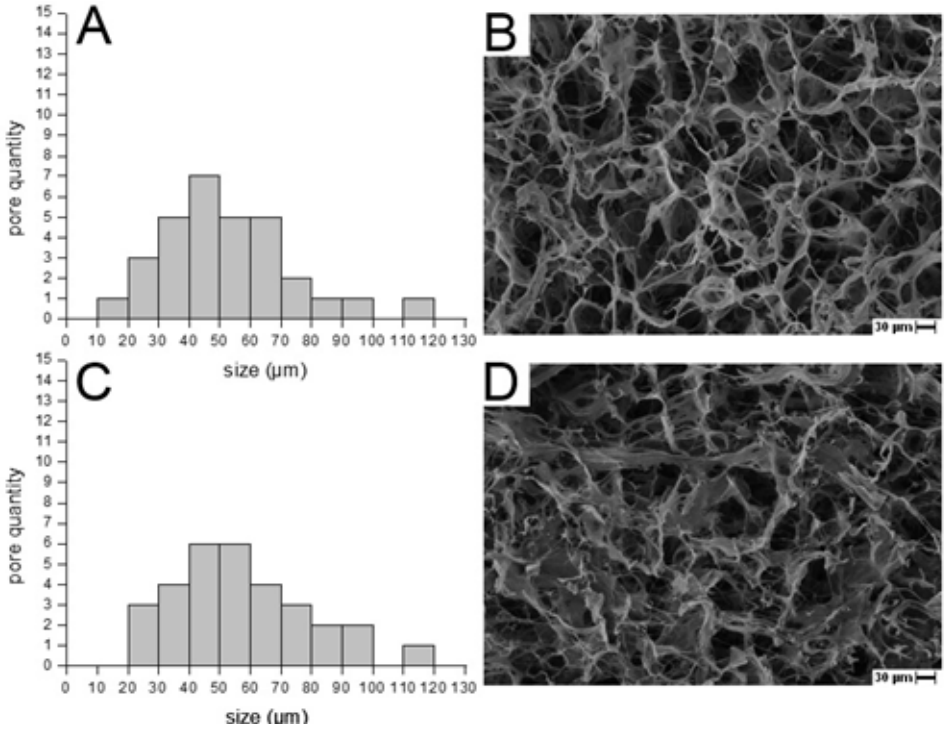


**Figure 4** – TCP003 scaffold: (A) surface pore size distribution histogram; (B) surface photomicrograph; (C) cross–sectional pore size histogram (D) cross section photomicrograph.

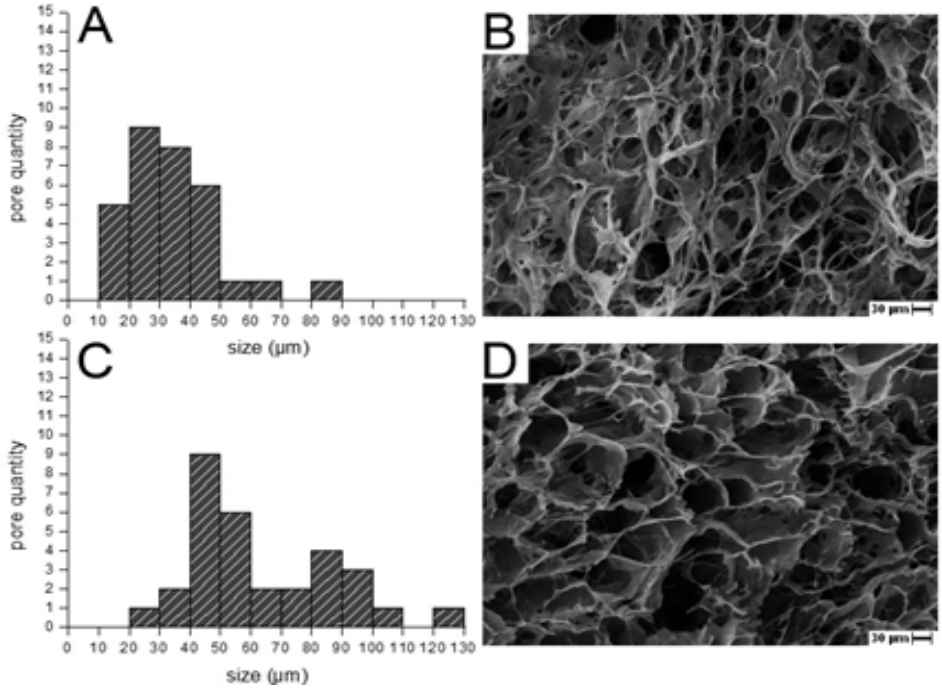


**Figure 5** – SCP05 scaffold: (A) surface pore size histogram; (B) surface photomicrograph; (C) cross–sectional pore size histogram (D) cross section photomicrograph.

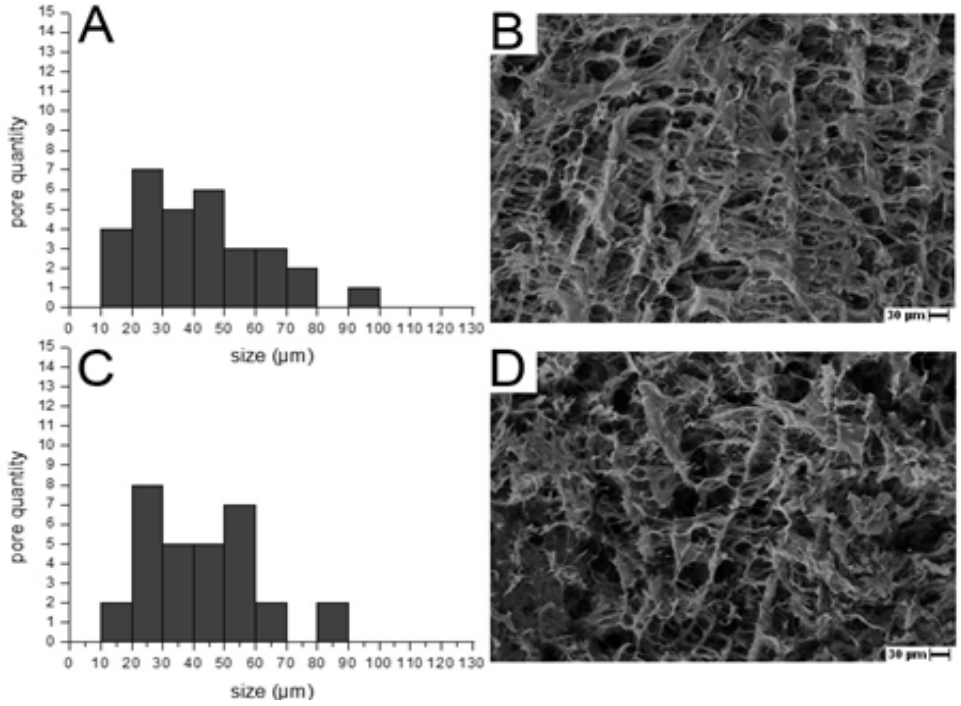


**Figure 6** – TCP05 scaffold: (A) surface pore size distribution histogram; (B) surface photomicrograph; (C) cross–sectional pore size histogram (D) cross section photomicrograph.

| Scaffolds | Mean ± sd (μm) | | | |
|---|---|---|---|---|
| | Surface | Cv (%) | Cross section | Cv (%) |
| TC | 41.1 ± 30.4 [a, b] | 74.0 | 43.2 ± 13.7 [b, d] | 30.3 |
| SC | 31.1 ± 17.8 [b] | 57.1 | 48.4 ± 26.2 [b, c] | 54.2 |
| TCP003 | 52.9 ± 21.0 [a] | 39.7 | 56.7 ± 22.2 [a, b] | 39.2 |
| SCP003 | 36.4 ± 16.6 [b] | 45.5 | 38.3 ± 9.3 [c, d] | 24.2 |
| TCP05 | 41.9 ±19.5 [a, b] | 46.4 | 41.2 ± 17.5 [c, d] | 42.5 |
| SCP05 | 32.4 ± 14.5 [b] | 44.6 | 63.1 ± 23.8 [a] | 37.7 |

In the same column, same letter means statistically equal numbers ($p<0.05$) sd: standard deviations. Cv: coefficient of variation (%).

**Table 3** – Surface and cross section pore size of the scaffolds.

adhesive interactions between the various polar aminoacids residues present in collagen and PBS are stronger than the cohesive forces of the buffer itself. This causes the buffer to be absorbed throughout the scaffold, causing it to swell. PBS absorption at 30, 60 and 1200 min is shown at Table 4.

Figure 7 shows the percentage of absorption at different times. TCP05 and TCP003 had their maximum absorption at 2750 % in the first minute, different of TC, which had maximum absorption at 1920% after 1200 minutes.

Serosa scaffolds absorbed their maximum around 30 minutes, which SC and SCP003 had statistically equal absorption around 1400% and the SCP05 absorbed around 2750 %.

Scaffolds TCP05 and SCP05 have statistically equal absorption around 2700%, almost 2 times higher than the scaffolds without extract. This is due to the large number of hydroxyls groups present in proanthocyanidin, which make the material most hydrophilic.[27]

Swelling of a material is hampered by inter and intramolecular bonding, intrinsic stiffness and degree of crosslinking.[28] Then, a difference in the absorption of the TC and SC was expected, since the serosa scaffold showed higher thermal stability, which may be due to a larger number of bonds in its structure (intrinsic cross–linking).

This difference is only observed between TCP003 and SCP003, since the presence of 0.03 % extract is not able to crosslink collagen to the point of increasing its Td, but it contributes to the hydrophilicity of the material, making the scaffold TCP003 more hydrophilic than SCP003, which is difficult to absorb due to its intrinsic cross–linking.

### *In vitro* biological stability (collagenase)

Collagenase biological stability assays were designed as a relative indication of the biodegradability of post implant scaffolds. In vitro degradation of collagen by collagenase occurs in three stages: i) they are diffusion, in which collagenase in the solution diffuses through the substrate. In this case, it is collagen itself; ii) absorption, in which the enzyme is adsorbed onto the collagen surface and, iii) degradation, in which the molecule is broken into minors fragments.[29]

Since scaffolds, TCP003 and SCP003, did not show an effective cross–

linking as observed by their denaturation temperature, only TC, SC, TCP05 and SCP05 were used in the degradation assays, shown in Table 5.

The scaffold TC presented the higher percentage of degradation, being 74.0 ± 5.0 and, therefore, having lower biological stability in vitro. The scaffold SC presented a degradation percentage of 9.3 ± 2.1, indicating greater stability of serosa collagen against collagenase, probably due to the higher number of intrinsic serosa tissue crosslinks, as already mentioned.

With the addition of 0.5% extract, the percentage of tendon scaffolds degradation decreased from 74.0 ± 5.0 to 15.3 ± 0.4 due to crosslinking caused by proanthocyanidin and other flavonoids present in grape seed extract.

Degradation percentage for SC and SCP05 were expected to be statistically different, however this was not observed. Maybe another test using a higher degradation time could result in a difference of degradation between serosa scaffolds.

Ma[30] evaluated the biodegradability of collagen of 0.25% glutaraldehyde crosslinked bovine tendon observing that there was a reduction of biodegradability of approximately 87 %, close to the observed in this study with tendon scaffold that when cross-linked with 0.5 % extract showed reduction of approximately 80 %.

Rodrigues[31] also evaluated trypsin enzymatic stability of glutaraldehyde cross-linked collagen. Glutaraldehyde cross-linking reduces the degradation percentage of collagen scaffolds too. This indicates a possible substitution of glutaraldehyde for the extract, as glutaraldehyde presents high cytotoxicity.[32]

| Scaffold | Absorption (%) ± sd | | |
|---|---|---|---|
| | 30 min | 60 min | 1200 min |
| TC | 1575 ± 217 [b] | 1753 ± 239 [b] | 1921± 188 [b] |
| SC | 1374 ± 131 [b] | 1397 ± 124 [b] | 1489 ± 152 [b] |
| TCP003 | 2739 ± 192 [a] | 2791 ± 179 [a] | 2872 ± 186 [a] |
| SCP003 | 1469 ± 106 [b] | 1511 ± 087 [b] | 1579 ± 051 [b] |
| TCP05 | 2342 ± 305 [a] | 2531 ± 205 [a] | 2572 ± 214 [a] |
| SCP05 | 2813 ± 580 [a] | 2855 ± 524 [a] | 2806 ± 519 [a] |

In the same column, same letter means statistically equal numbers ($p<0.05$) sd: standard deviations.

**Table 4** – PBS absorption kinetics (%).

| Scaffold | Degradation (%) ± sd |
|---|---|
| TC | 74.0 ± 5.0 [a] |
| SC | 9.3 ± 2.1 [b, c] |
| TCP05 | 15.3 ± 0.4 [b] |
| SCP05 | 3.7 ± 2.5 [c] |

In the table, same letter means statistically equal numbers ($p<0.05$) sd: standard deviations.

**Table 5 –** Scaffolds degradation ± sd.

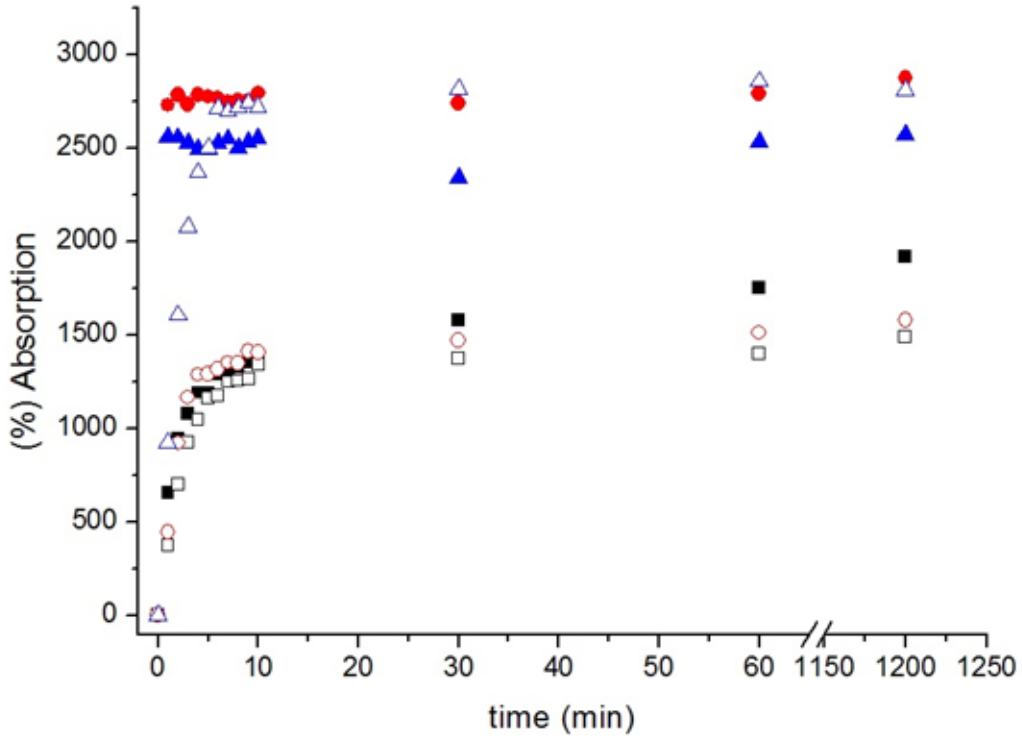


**Figure 7 –** (%) absorption of the scaffolds (■) TC;(●) TCP003; (▲) TCP05; (□) SC; (○) SCP003 and (△) SCP05.

## Conclusion

Photomicrographs showed the presence of surface pores and in the cross–section for all samples, not larger than 130 µm. Cross–linking by 0.5% extract increases collagen denaturation temperature, reduces degradation percentage 12 and increases the swelling, which may result in scaffolds for different applications such as hemostatic agent or soft tissue regeneration.

## Conflict Of Interests

There are no conflicts to declare.

## Acnlowledgements

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# Effect of crosslinker and nanostructure on adsorption and release of paraquat herbicide from different natural hydrogel nanocomposites

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**Abstract:** Hydrogels based on natural polymers have been applied in different sectors due to their biocompatibility, biodegradability, relatively low cost, and atoxicity properties. The aim of this study was to prepare alginate/starch hydrogels crosslinked with different ions ($Mn^{2+}$, $Zn^{2+}$ or $Ca^{2+}$), containing zeolite or nanoclay and to evaluate the effect of crosslinker and nanostructure on adsorption and release behavior of paraquat herbicide. The hydrogels and their nanocomposites were prepared by immersing of alginate/starch, alginate/starch/nanoclay or alginate/starch/zeolite solutions in recipients containing the crosslinker solution ($MnCl_2$, $ZnCl_2$ or $CaCl_2$) at 25°C. All hydrogels presented good adsorption capacity, mainly the nanostructured hydrogels with nanoclay and zeolite loaded. The polar characteristics allow high interaction with paraquat molecules. The release behavior was also very interesting, being that the amount zeolite in the hydrogel nanocomposites-forming solution can control the paraquat release, indicating that these materials can be used as carrier vehicles in the controlled release system.

**Keywords:** Herbicide; Paraquat; Controlled Release; Nanoclay; Zeolite.

## Introduction

The environmental concern due to the scarcity of fossil resources and the high pollution index caused by non-biodegradable materials, linked to the concept of sustainability, has directed researchers to focus their research on the development of renewable and ecologically materials. In this sense, the polymeric materials have gained great space in the different technological sectors, because it is chemically inert and have in their structure highly reactive groups that allow the modification of their properties, increasing their application.[1-3]

The use of polymers has revolutionized the market sectors by increasing agricultural productivity, food technology, medical treatments, aircraft performance and reducing fuel consumption through vehicles made of lighter materials.[4] Even with this revolution, these polymers are mostly derived from fossil sources (petroleum), so efforts are being made to replace by natural polymers.

Natural polymers are macromolecules obtained from the extraction of plants, animals and natural waste such as peelings, fruit marc, and stems. It is considered renewable materials in which proteins such as collagen, gelatin, hyaluronic acid, silk and zein, and polysaccharides such as starch, sodium alginate, cellulose, chitosan; terpenes in the case of natural rubber and lipids.[4,5]

The technological properties of natural polymers are numerous, allowing its application in many areas. Among these properties are biocompatibility, biodegradability, atoxicity, low cost, renewability, and availability. From these properties, there are already advances in the area of technology and food packaging,6 in biomedical through the drug controlled delivery7 and bone regeneration,8 in agriculture in soil water retention and agrochemicals controlled delivery9, and also in construction as natural soil stabilizer.[4]

One of the materials prepared from natural polymers that improved the applicability in the biomedicine and agriculture fields are the hydrogels. These classes of materials are characterized by absorbing a high amount of water into the interior by having three-dimensional networks, and which are formed by the crosslinking process.[10-12] They can be prepared from a combination of various synthetic or natural materials and polymers, such as sodium alginate and starch.

Sodium alginate (SA) is a polysaccharide with a high capacity to form hydrogels when immersed in a solution containing di or trivalent ions. It is chemically structured by glycosidic units known as blocks. M and G blocks consist of molecules of β-D-mannuronic acid and α-L-guluronic acid, respectively, which are linked by α (1, 4) glycosidic bonds.[10,13]

Starch is also characterized as an interesting polysaccharide, mainly due to its availability and hydrophilic characteristics. Structurally consists of repetitive units of glucose in different conformations, being amylose and amylopectin. Amylose is a short-chain linear polymer in which the units of α-D-glucopyranose are connected from α 1→4 glycosidic bond and is identified as the unbranched, hydrophobic part of starch. Like amylose, amylopectin is a long-chain polymer with repetitive units of α-D-glucopyranose linked to each other by α 1→4 glycosidic bond, but the effective α 1→6 bonds between the amylopectin molecules confer high ramifications, which differ one substance from another.[14,15]

Despite all these interesting properties, hydrogels, films or any other material based on natural polymers have precarious mechanical properties, which can limit their application in some areas.[16,17] Thus, researchers are investigating the effect of the incorporation of fillers in the polymeric matrices. Among the different reinforcement agents used, clay and zeolite aluminosilicates stand out because they are also natural and mainly non-toxic. Besides the mechanical reinforcement, these materials increase the absorption capacity of the hydrogels and can control the release of actives.[18,19]

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Therefore, the objective of this work was to develop hydrogels from natural alginate and starch polymers incorporated with inorganic nanostructures and to investigate the effect of the crosslinker and the zeolite and/or nanoclay on the paraquat herbicide adsorption and in vitro release behaviors.

## Material And Methods

### Materials

Corn starch – A4001 (Amidex® 4001 MW = 324,000 g/mol) and sodium alginate (SA) (MV = 85,000 g/mol; M / G ratio = 2.1) were obtained from Ingredion and Cromoline (Química Fina), respectively. Pure anhydrous calcium chloride ($CaCl_2$), manganese chloride ($MnCl_2$) and anhydrous zinc chloride ($ZnCl_2$) were acquired from Vetec (Química Fina, MM: 110.99 g/mol), Synth (MM: 197.90 g/mol) and Neon (MM: 136.30 g/mol), respectively. The nanostructures used were the zeolite – Clinoptilolite ZK406 H (St. Cloud Zeolite) and the nanoclay – Cloisite–$Na^+$ (Southern Clay Products®). All reagents were used as received without any purification.

### Preparations of solution

The concentration of the polysaccharides was optimized in our previous study[10] and the final concentration for sodium alginate, and starch was 1 and 2% w/v, respectively. Firstly, the starch was initially dissolved in distilled water under magnetic stirring at 70 °C. Then, SA powder was solubilized into the starch solution using mechanical stirring for 4 h. After, the final solution was stored in the refrigerator to inhibit microbial growth.

The solutions of the crosslinker agents ($MnCl_2$, $ZnCl_2$ or $CaCl_2$) were obtained in the concentrations of 1% w/v. For this, each crosslinker agent was dissolved separately in distilled water under magnetic stirring at 25 °C.

### Preparation of hydrogels

The sodium alginate/starch, alginate/starch/nanoclay or alginate/starch/zeolite solution was separately placed in an 8 $cm^2$ mold and immersed in a recipient containing the crosslinker solution during 24 h. Then, the prepared hydrogel or their nanocomposite was removed from mold and added in a recipient containing distilled water for dialysis for 24 h. Finally, the hydrogels were 40 °C oven–dried for 24 h and characterized.

### Characterizations

### Paraquat (PQ) adsorption in aqueous solution

The herbicide adsorption behavior was analyzed by inserting a certain mass of previously dried hydrogel (w) in an herbicide solution of known concentrations ($C_0$).

The herbicide concentrations in the solution were determined by using UV–Vis spectrophotometer at λ = 257 nm and based on the calibration curve. The herbicide amount absorbed ($q_t$) was determined using Equation 1:

$$q_t = \frac{[(C_0 - C_t)]\text{x V}}{\text{w}} \qquad \text{Eq. 1}$$

*where* $C_t$ is the herbicide concentration at time *"t"* and *V* is the paraquat solution volume.

### *In vitro* assays herbicide release

The PQ release behavior was studied at pH 7.4 buffer solution (200 mL of $KH_2PO_4$ 8 g/L solution + 800 mL of $K_2HPO_4$ 9.5 g/L solution). The hydrogel loaded with paraquat was immersed into 20 mL of buffer solution. The kinetic of the PQ amount released was investigated by monitoring the variation of absorbance peak at λ = 257 nm, using the same methodology described above. The quantity of paraquat release percentage was determined from Equation 2:

$$\text{Paraquat released (\%)} = (\text{Mt} / \text{M}\infty)\text{ x } 100 \qquad \text{Eq. 2}$$

where Mt is the herbicide amount released by the hydrogel at time "t" and M∞ is the total herbicide amount loaded by the hydrogel.

### Statistical analysis

Statistical analysis was performed using a SISVAR software (version 5.7) by using the Tukey´s tests at 95 % confidence level.

## Results and Discussion

### Adsorption of paraquat herbicide

### Starch–alginate composite hydrogels

From Figure 1, it was possible to see that the all composite hydrogels, crosslinked with different ions, have good adsorption capacity. This great affinity is mainly related to the presence of polar groups in the polymeric backbone.

As the hydrogel is constituted of an anionic polysaccharide (SA), and paraquat presents cationic characteristics, the adsorption can occur through electrostatic interactions. This interaction occurs mainly between the $-COO^-$ groups and the $H_3C - N^+$ linked to the 2 pyridinic rings of the paraquat molecule (Figure 2).

Additionally, the presence of –OH groups in the chemical structure of both alginate and starch polysaccharides also favored the pesticide adsorption, as well as the hydrophobic interactions.[20,21] The $sp^2$ hybridization of the pyridinic ring becomes the structure with weakly polar characteristics whereas this hybridized form is more electronegative than $sp^3$ hybridization, also present in the pesticide structure. This factor also improved paraquat absorption.

It is noted that the hydrogels presented the same absorption equilibrium time (approximately 10 hours of study), independently of the crosslinker agent. Additionally, the hydrogels crosslinked with $Mn^{2+}$ absorbed more than those crosslinked with $Zn^{2+}$ or $Ca^{2+}$. This tendency is probably related to both factors: ion size and quantity of free $-COO^-$ groups. It is known that the electrostatic interactions between the crosslinker ions and $-COO^-$ groups are non–directional. As the $Mn^{2+}$ ions are the smallest concerning to the others, their interaction with $-COO^-$ groups is less effective, consequently, there is a greater amount of $-COO^-$ free to interact with paraquat (Figure 2).

On the other hand, the increase in ion size promotes a more effective electrostatic interaction because the species are closer, and the $-COO^-$ groups are no longer free to interact with the herbicide, since they are attracted by the ions to form the three–dimensional networks.[22] Because it is size, there are more effective repulsive interactions between the $Ca^{2+}$ ions and the cationic regions of paraquat, decreasing their adsorption.

Another interesting factor to be highlighted is the low effectiveness in the interaction of the $Mn^{2+}$ and $-COO^-$, making the chain more flexible (high expansion degree by relaxation process). This factor guarantee a sorption of greater amount of paraquat in its structure.

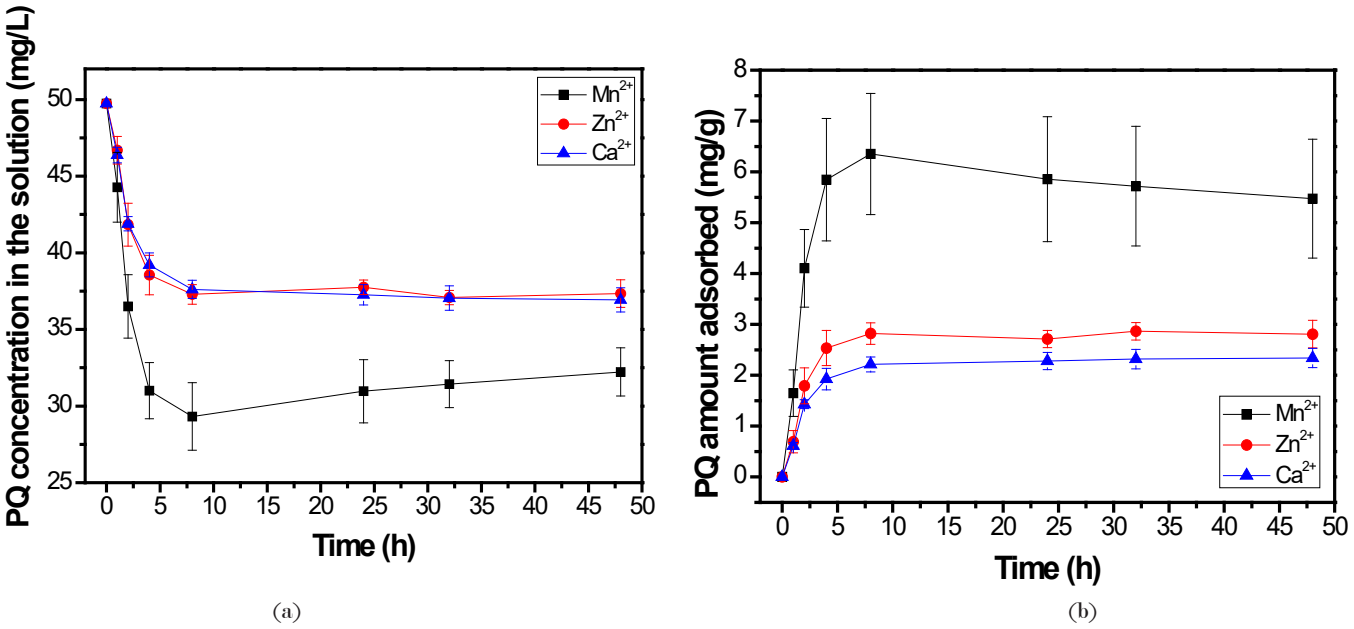



**Figure 1 –** Adsorption profiles of composite hydrogels. (a) paraquat concentration in the study solution, and (b) paraquat amount adsorbed in mg/g hydrogel.

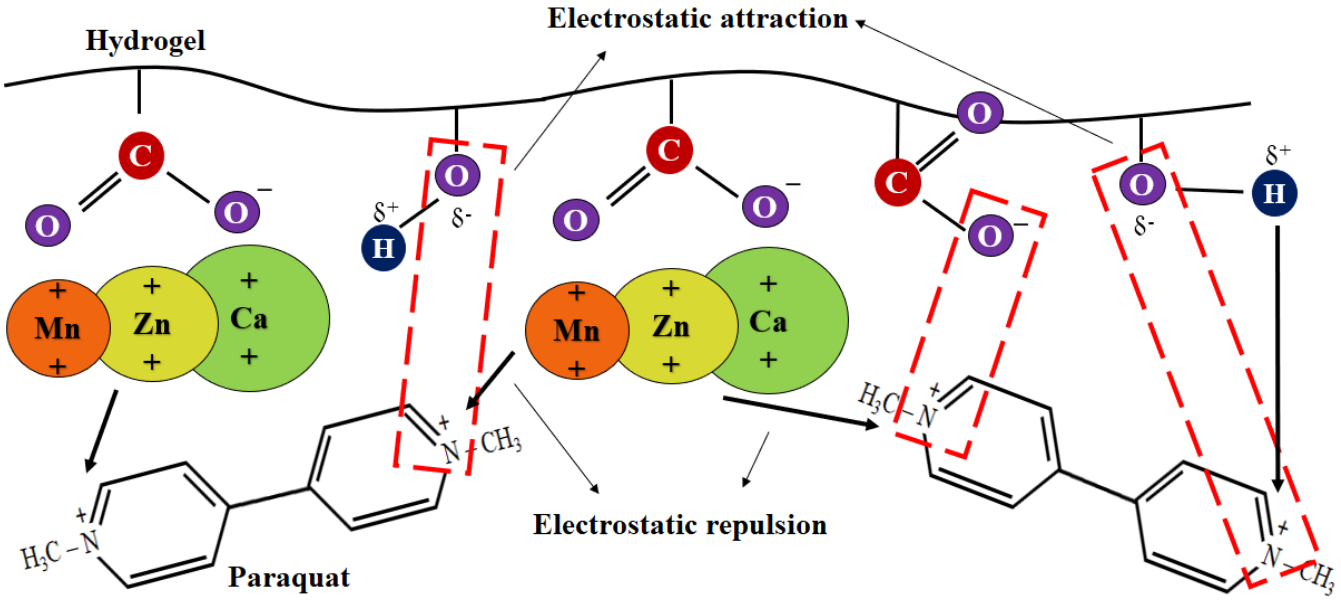



**Figure 2 –** Representative model of the interactions between the hydrogel matrix and the paraquat molecules.

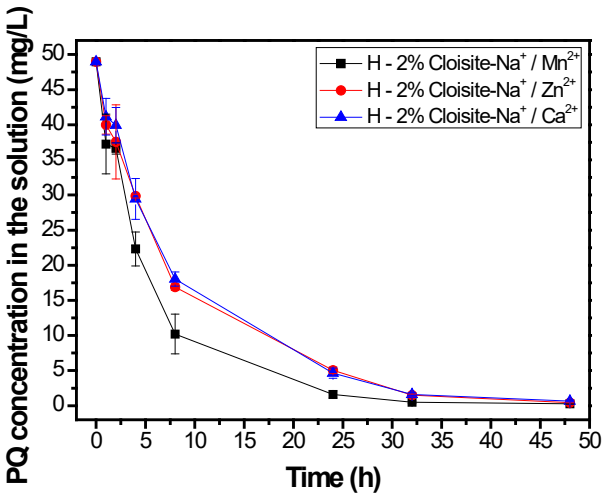
**Adsorption behavior of nanocomposite hydrogels**

From Figure 3, it was possible to confirm that the incorporation of the nanostructures significantly improves the pesticide adsorption capacity of the matrices.

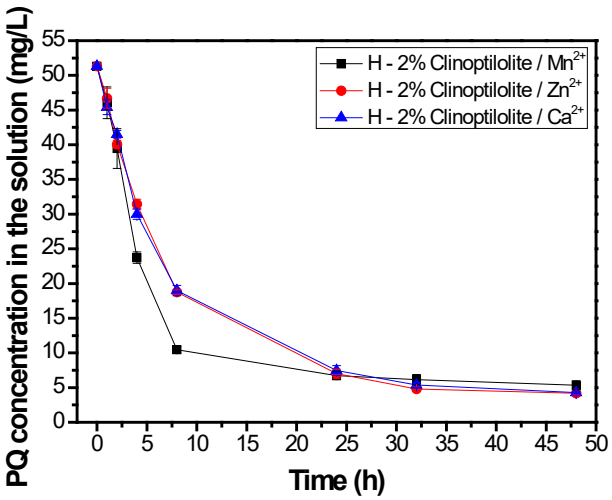
In the nanocomposites, the polar characteristic of their structures is increased because the aluminosilicates (nanoclay or zeolite) have atoms with different electronegativity in its chemical structures. This difference in electronegativity creates permanent dipoles in the molecule, which are responsible for the Coulombic interaction with the paraquat molecules ($H_3C - N^+$).[23,24] A simple model representing theses specific interactions between nanostructure–pesticide is represented in Figure 4.

For zeolite–hydrogel nanocomposites, an important fact to be highlighted is that paraquat molecules may not have diffused between the channels of the zeolite structure because their molecules have dimensions of approximately 15 Å and the zeolite pores channels have sizes around 4 Å.[21,25]

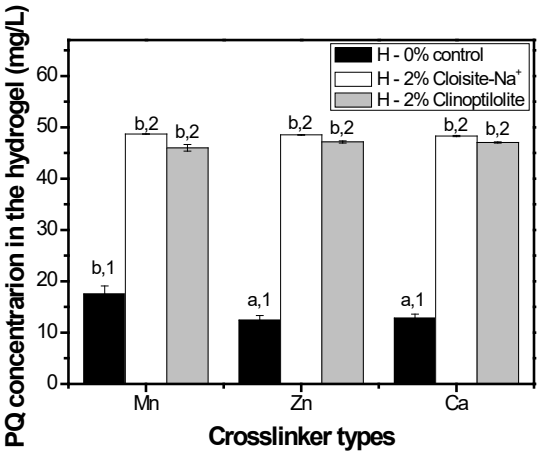
It is not observed a very significant effect on the adsorption capacity of nanocomposite hydrogels caused by crosslinker type. This no variation is possibly being masked by the addition of nanostructures, showing a competitive interaction sites, i.e., polymeric chains–paraquat and zeolite/nanoclay–paraquat.




(a)

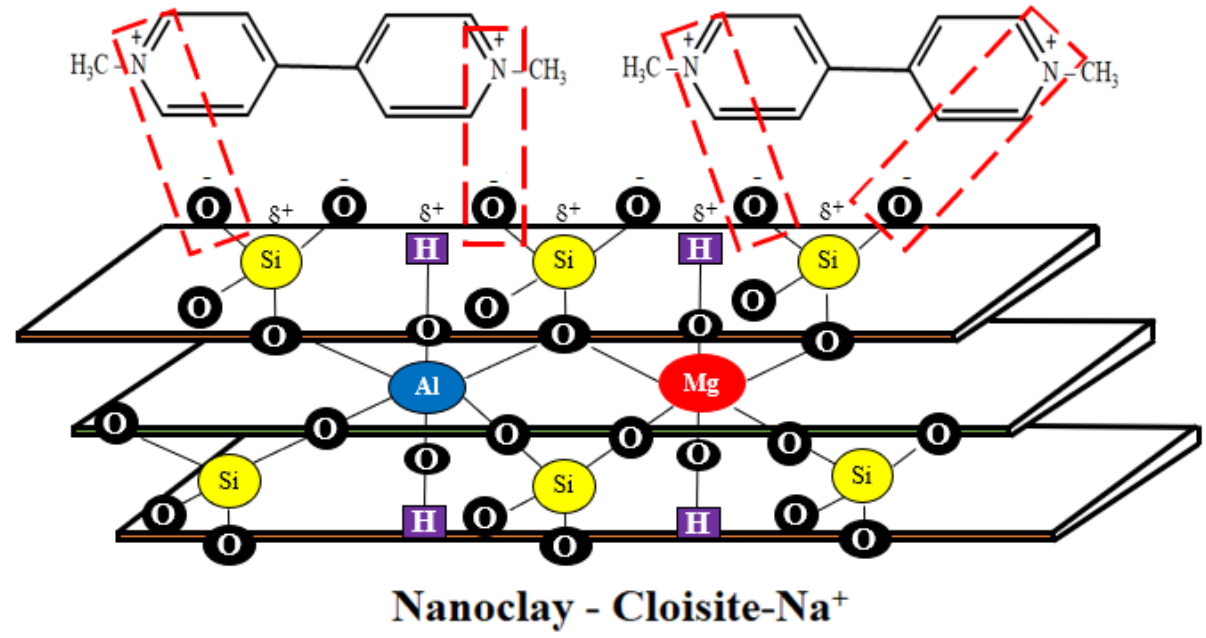



(b)

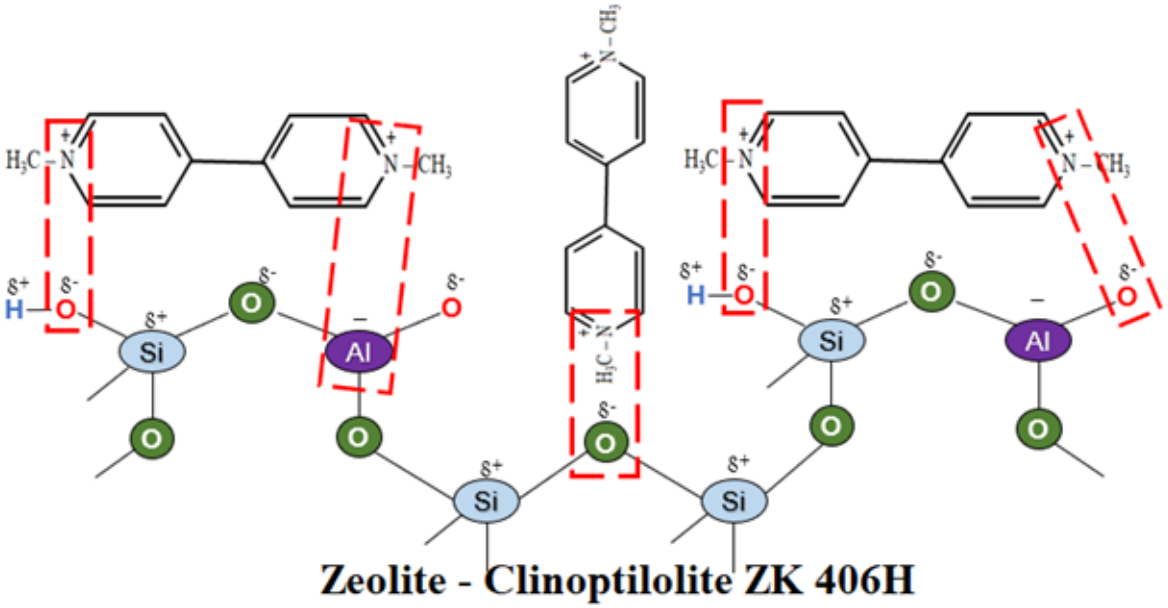



(c)

**Figure 3 –** Adsorption kinetic behaviors: Paraquat concentration in the solution for hydrogels incorporated with (a) nanoclay and (b) zeolite, and (c) paraquat concentration adsorbed at equilibrium for composite and nanocomposite hydrogels. Different letters and numbers indicates significant difference at $P < 0.05$ in the different crosslinkers and different concentration, respectively. All experiments were done in triplicate (n = 3).




(a)




(b)

**Figure 4 –** Proposed model representing the electrostatic interactions between paraquat and nanostructures (a) nanoclay and (b) zeolite.

**Desorption of paraquat herbicide**

**Composite hydrogels release behavior**

As observed in the Figure 5, the composite hydrogels reached equilibrium around 8 hours in which there was total release of the paraquat adsorbed. We believe that the amount above 100% is due to the paraquat amount that was adsorbed on the surface of the hydrogel not quantified in the removal process from the solution.

The active controlled release process from polymeric structure can occur by different ways depending on the bond type formed between these species. Thus, if they are joined by primary bonds, i.e., covalent or ionic bond, this active release must occur, mainly, by disintegration/erosion of the polymeric structure. Already when joined by physical interactions, the release occurs mainly by diffusion process, being closely influenced by the swelling capacity of the hydrogel as well as the pore sizes.[26–28]

It was highlighted in our previous study[10] that in the swelling process the chains of composite hydrogels crosslinked with $Mn^{2+}$ expands to a higher degree, due to the less effective interactions between these ions and the $-COO^-$ groups. Besides, the pore size of these supracitated hydrogels is probably larger than the hydrogels crosslinked with other ions, which facilitated the herbicide diffusion. The good solubility of paraquat in water also facilitated their diffusion to desorption medium (buffer solution at pH 7.4).

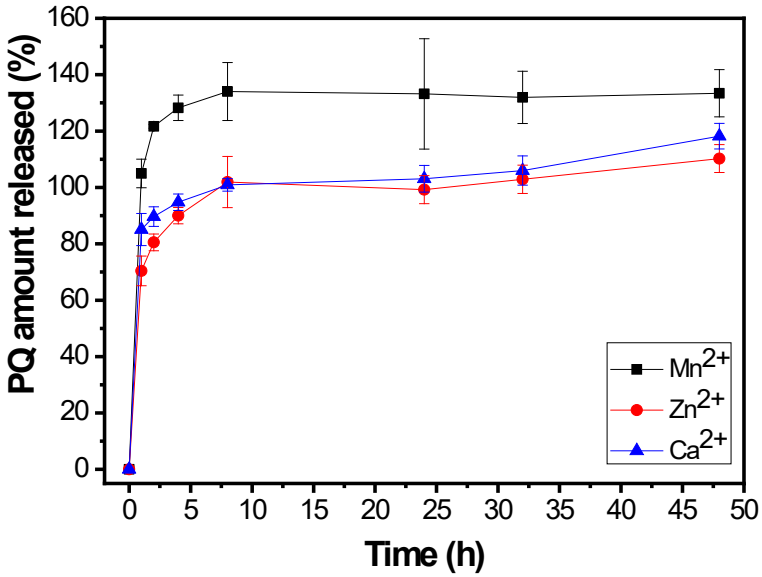



**Figure 5 –** Paraquat amount released in percentage from starch/sodium alginate hydrogels crosslinked with different ions.

**Nanocomposite hydrogels release behavior**

Regarding to nanocomposite hydrogels containing nanoclay and loaded with paraquat, no significant pesticide release was observed (Figure 6). It is believed that the low amount released may be related to the strong intermolecular interactions between the negative surface of the nanoclay platelets and paraquat. Furthermore, as previously reported, the paraquat molecules diffusion from hydrogels is mainly governed by the swelling process, which for these hydrogels is attenuated. This effect is related to the fact of the nanoclay acts as a physical crosslinker in the polymeric matrix, reducing their expansion capacity and making it more difficult for herbicide release.[10]

For the nanocomposites prepared from hidrogel and zeolite, it was observed a good release capacity (Figure 7). The better pesticide de–sorption behavior presented by hydrogels containing zeolite over nano–clay–hydrogel nanocomposites is probably related to the fact of the paraquat no penetrated into zeolite channels, since its molecules are larger than the zeolitic pores.

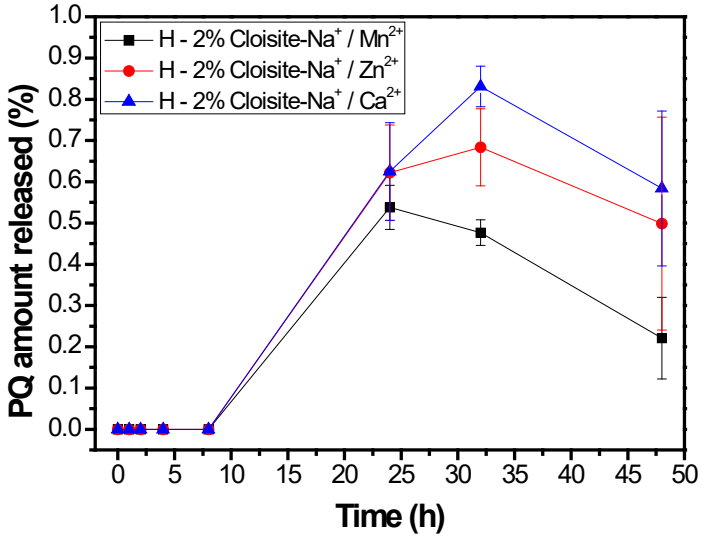



**Figure 6 –** Paraquat amount released in percentage from starch/sodium alginate hydrogels containing 2% nanoclay and crosslinked with different ions.

The increase in zeolite concentration decreased the amount of paraquat released (Figure 7d) due to the increase in the quantity of polar regions that are probably interacting with the paraquat molecules. Additionally, based on the swelling results of the previous study[10], both zeolite and nanoclay is acting as a physical crosslinker, what decreased the polymeric relaxation during the swelling process, reducing the amount of paraquat released from nanocomposites. Finally, the type of the crosslinker influenced the velocity of the pesticide desorption. For instance, the time for release of 20 % of paraquat (Figure 7d) were 1.0 h; 3.3 h, and 5.2 h for $Mn^{2+}$, $Ca^{2+}$ and $Zn^{2+}$, respectively.

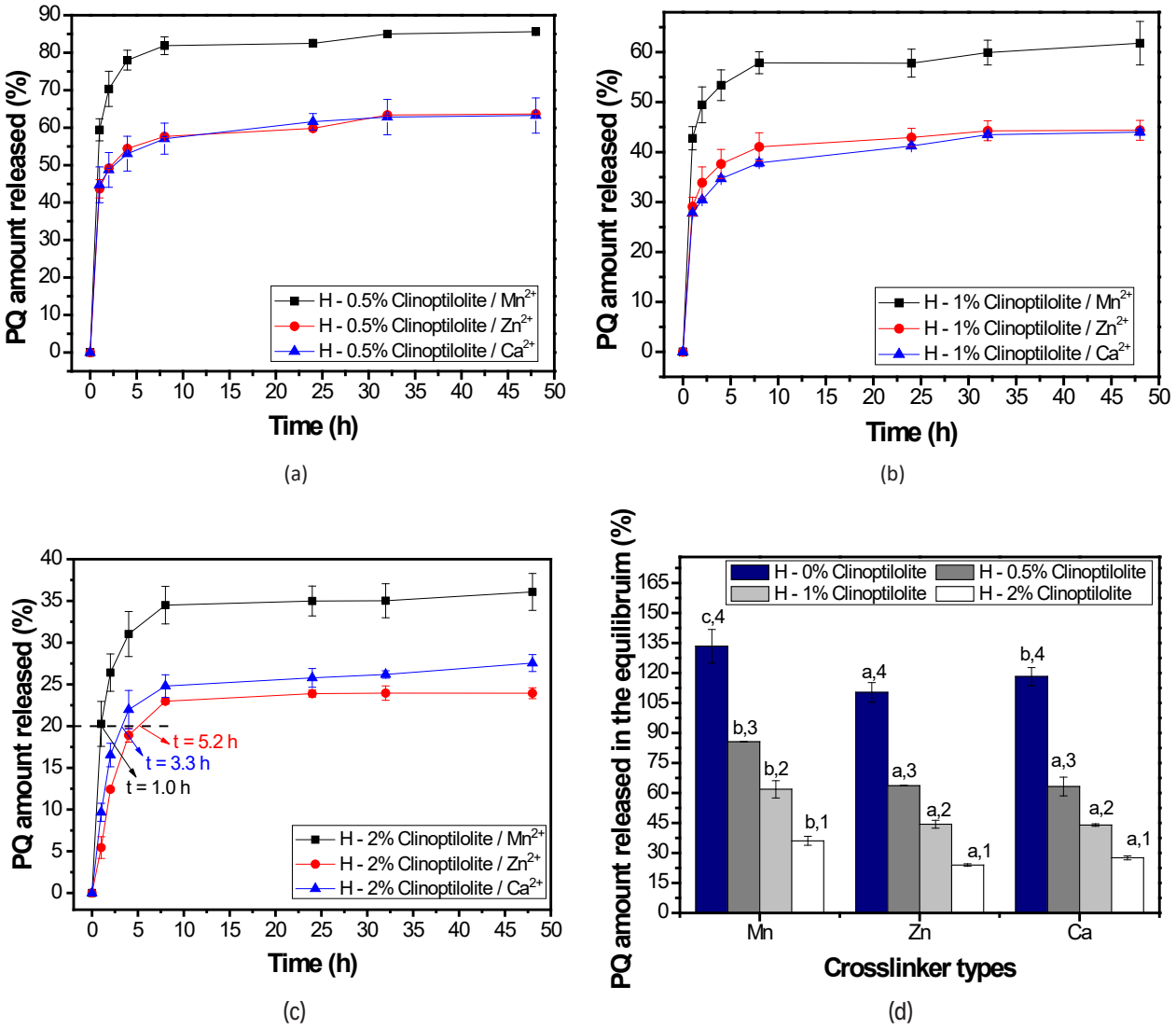



**Figure 7 –** Paraquat release behavior from starch/alginate hydrogels containing different zeolite concentrations. (a) 0.5% (b) 1% (c) 2% zeolite, and (d) paraquat amount released in the equilibrium stages. Different letters and numbers indicates significant difference at $P < 0.05$ in the different crosslinkers and different zeolite concentration for the same crosslinker, respectively. All experiments were done in triplicate (n = 3).

## Conclusion

The composite and nanocomposite hydrogels based on alginate and starch natural polymers were successfully obtained by ionotropic gelation using three different crosslinkers. The different sizes of the crosslinkers altered the interaction between pesticide–matrix, reflecting in different profiles of adsorption and desorption pesticide. The addition of nanostructures also increased the hydrogel adsorption capacity. In relation of nanostructures, the presence of zeolite considerably reduced the paraquat amount released. On the other hand, interactions between paraquat and nanoclay were so effective preventing the release of herbicide. The results of this study show that hydrogels can be prepared with different crosslinkers and nanostructures and thus having desired sorption and desorption properties. In this way, hydrogels and their nanocomposites can be used in controlled release systems, not only in the paraquat release, but also in the drug release, since the natural polymers used are also biocompatible and biodegradable.

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MINI-REVIEW

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# Surface-Modified Bacterial Cellulose with Mercaptosilane as a Multifunctional Platform

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**Abstract:** Cellulose synthesized by bacteria has unique properties such as high water retention capacity, biocompatibility, biodegradability and flexibility. Nevertheless, modification of this biomaterial is required in order to obtain multifunctional materials, which may be applied in several high-value added products, as catalytic and cell culture platforms. The surface of bacterial cellulose (BC) can be modified by several approaches, namely: (i) physical treatment by plasma, (ii) adsorption of molecules onto BC surface, and (iii) chemical modification. In this sense, the aim of this study was to modify the BC surface by silanization reaction at room temperature using a mixture of ethanol and water, using two different protocols. Thus, BC membranes synthesized by Komagataeibacter xylinus were modified by adding the thiol (SH) functional group with (3-mercaptopropyl) trimethoxysilane under mild conditions. The produced materials were analyzed by elemental analysis, ATR-FTIR, TGA and SEM, and the successful modification was proven by elemental analysis and SEM.

**Keywords:** Bacterial cellulose; Surface Modification; Silanization; Mercaptosilane.

## Introduction

Cellulose is the most abundant biopolymer on planet and it can be produced by green plants, fungi and bacteria. Cellulose consists of β-D-glycopyranose units linearly arranged by β-(1-4) glycosidic bonds. Plant-derived cellulose is usually associated with several components as hemicellulose, lignin and pectin, while bacterial cellulose (BC) is obtained chemically pure. Besides, BC presents nanometer-sized fibers once it is excreted by a microorganism, therefore BC is also known as bacterial nanocellulose[1]. BC has several unique properties such as biocompatibility, mechanical strength, biodegradability, high water retention capacity and flexibility[2], hence BC is a promising material whenever these properties are required.

Because of its distinct properties, BC has gained prominence in the scientific field, especially in the biomedical area as wound dressings, matrix for controlled drug release and temporary skin substitute[2]. However, the introduction of either charged or hydrophobic moieties onto the bacterial nanocellulose is necessary in many applications [3-4]. Ideally, the modifications should be effective without altering the biomaterial nanostructure, once it accounts for the material distinct properties.

Chen et al.[3] have functionalized the BC surface with amidoxime; the researchers have produced a nanohybrid based on the amidoxime surface functionalized BC and gold nanoparticles, which had shown excellent catalytic activity. Taokaew et al.[4] have chemically modified the BC surface by grafting methyl and amine terminated organosilanes to evaluate attachment, spreading and growth of normal human dermal fibroblasts; it was observed that the cells improved their spreading and attachment on the amine-modified BC. Li et al.[5] have introduced the thiol group into cellulose chains, creating a platform capable of undergo further modifications via thiol-ene reaction.

Traditionally, surface modification protocols to impart hydrophobic properties[6] onto nanocellulose do not respect the green-chemistry principles. Besides being time-consuming, most of them use to employ previous solvent-exchange steps with hazardous aprotic organic solvents such as dimethyl sulfoxide and *N*,*N*-dimethylformamide in addition to heat curing process after the chemical treatment[7].

This study aims to evaluate the efficiency of BC surface modification through silanization reaction under room temperature and using a mixture of ethanol and water as solvent through two simple protocols which do not require specific apparatus. Particularly, two different silanization methods were employed in order to modify the BC surface by adding the thiol (SH) functional group, an active site for further modification, with (3-mercaptopropyl)trimethoxysilane (MPTMS): Method A, according to the protocol described by Frone et al. [8], and Method B using $NH_4OH$, as described by Lu et al.[9].

## Experimental Procedures

### BC Production and Surface Modification

The strain used for the BC production was *Komagataeibacter xylinus*. The bacteria were cultured under static conditions in Hestrin-Schramm (HS) media, composed of D-glucose, yeast extract, peptone, disodium hydrogen phosphate, citric acid, and distilled water.

Dried BC membranes of surface area and thickness of 1.5 $cm^2$ and 0.05 mm, respectively, were treated by silanization reaction with MPTMS by two different methods. A mixture of ethanol and water 10:1 (v/v) was used as solvent.

For Method A (sample BC-SH-A), it was used 0.538 $mmolmL^{-1}$ of MPTMS. The system was kept at stirring for four hours at room temperature. Then the excess of solution was discarded and the membranes were washed three times with acetone and dried at room temperature for three days[8].

For Method B (sample BC-SH-B), the membranes were put in contact with the solvent for 5 minutes. Then $NH_4OH$ was added to medium followed by the addition of siloxane – the final medium concentration was 0.163 $mmolmL^{-1}$ of MPTMS, three times lower than that used in the first method. The system was kept at stirring for 12 hours at room temperature. Then, as the process done for the previous method, the excess of solution was discarded and the membranes were washed three with acetone and dried at room temperature for three days[9].

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### Characterization

The membranes were characterized by elemental analysis, Attenuated Total Reflectance Fourier Transform Infrared (ATR–FTIR), Scanning Electron Microscopy (SEM) and Thermogravimetric Analysis (TGA).

### Elemental Analysis

Hydrogen, sulfur, nitrogen and carbon contents were determined by dry combustion in a *Perkin Elmer* elemental analyzer, model 2400 series II.

### ATR–FTIR

The transmittance spectra were obtained in the infrared region by attenuated total reflectance (ATR–IR) in a *Cary 630* Agilent spectrometer in the range of 4,000–650 $cm^{-1}$.

### SEM

The morphological characterization was performed using a *Joel* JSM 7500F Field Emission Scanning Electron Microscope. The samples were covered with a thin layer of conductive carbon. The images were taken at 10,000 times magnification.

### TGA

Thermogravimetric analyzes were performed on a *TA Instruments* SDT Q600 thermal analyzer under the following conditions: heating rate of 10 °C/min with a synthetic air flow of 100 mL/min, from 30 °C to 600 °C. Alumina pan was used as reference.

### Results and Discussion

In terms of chemical reaction of silanization, water has the function of induce siloxane hydrolysis to silanol (Figure 1, Step 1), while ethanol favors the materials drying process. The interaction between hydroxyls favors the condensation reaction (Figure 1, Step 2). Thus, in this type of modification the condensation reaction occurs between silanol groups (Si–OH) as well as between the silanol and the BC hydroxyls[10]. The condensation step can also be induced by employing strong or weak bases such as NaOH[7] and $NH_4OH$[9].

The efficiency of BC surface modification by the applied methods was verified by elemental analysis of pristine BC, used as reference, compared to the treated samples (Table 1).

Modifying the material with MPTMS necessarily implies increasing sulfur (S) content once this siloxane carries the thiol functional group. BC–SH–A has a sulfur percentage very close to the native (reference) while BC–SH–B has a sulfur percentage almost 4 times higher compared to the native. This indicates that only method B was indeed efficient in modifying the BC surface through the covalent Si–O–Si bond formed between the silane moiety and the polymeric matrix.

FTIR–ATR spectrum (Figure 2) of native BC and BC treated by Methods A and B have the same profile. In all of them, there is a weak band at 900 $cm^{-1}$, attributed to vibrational modes of the C–O–Cβ (1→4) glycosidic bonds between glucose units, a band at 1,054 $cm^{-1}$ attributed to the C–OH stretch, and a band at 1,163 $cm^{-1}$, attributed to asymmetric deformation of the glucose pyranose ring C–O–C. The 3,340 $cm^{-1}$ band is attributed to the O–H stretch of water adsorbed to the cellulose surface[11]. This analysis was not sensitive attest the surface functionalization even for the BC treated by Method B, once it was not possible to observe the band corresponding to the deformation of the Si–O–Cellulose group in the region between 1,100–1200 $cm^{-1}$ [8].

In the SEM images obtained from the sample surfaces (Figure 3), it is observed that the native BC has a three–dimensional nanometric structure with randomly arranged nanofibers. It can be seen that the surface of the sample submitted to Method A did not change (there was no modification), unlike the surface of the sample submitted to Method B, where a distinct structure material deposited on the nanofibers is observed. These results corroborate with the data obtained by elemental analysis.

According to the thermogravimetric curves (Figure 4), it can be seen

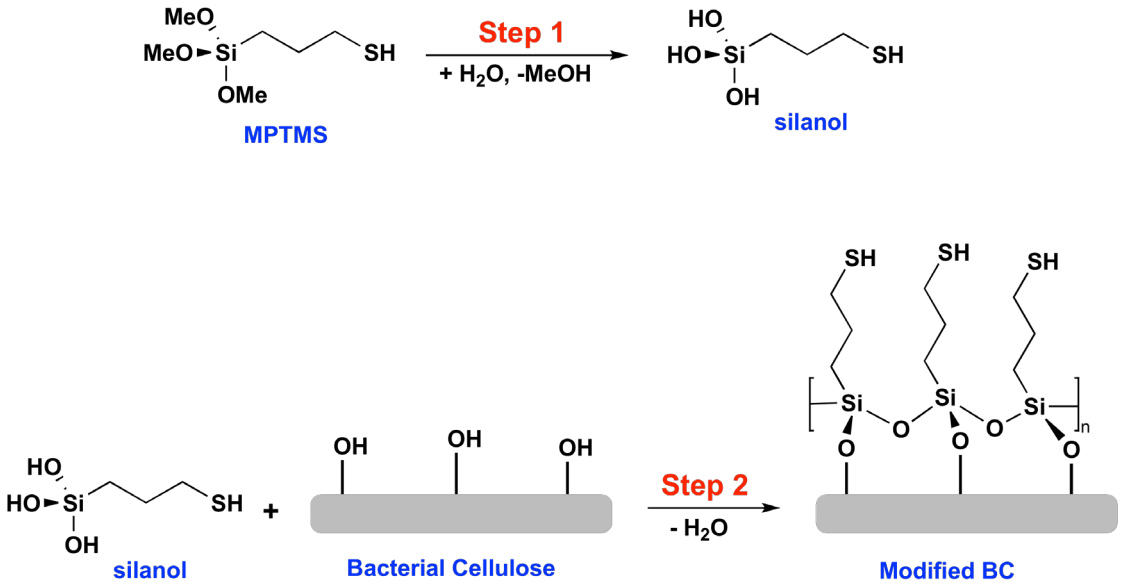


**Figure 1** – Silanization reaction with MPTMS.

| Sample | Hydrogen (%) | Nitrogen (%) | Sulfur (%) | Carbon (%) |
|---|---|---|---|---|
| Native BC | 5.75 | 0.87 | 1.04 | 42.45 |
| BC–SH–A | 5.95 | 0.94 | 1.37 | 42.23 |
| BC–SH–B | 6.26 | 0.91 | 3.3 | 40.16 |

**Table 1 –** Elemental composition of samples.

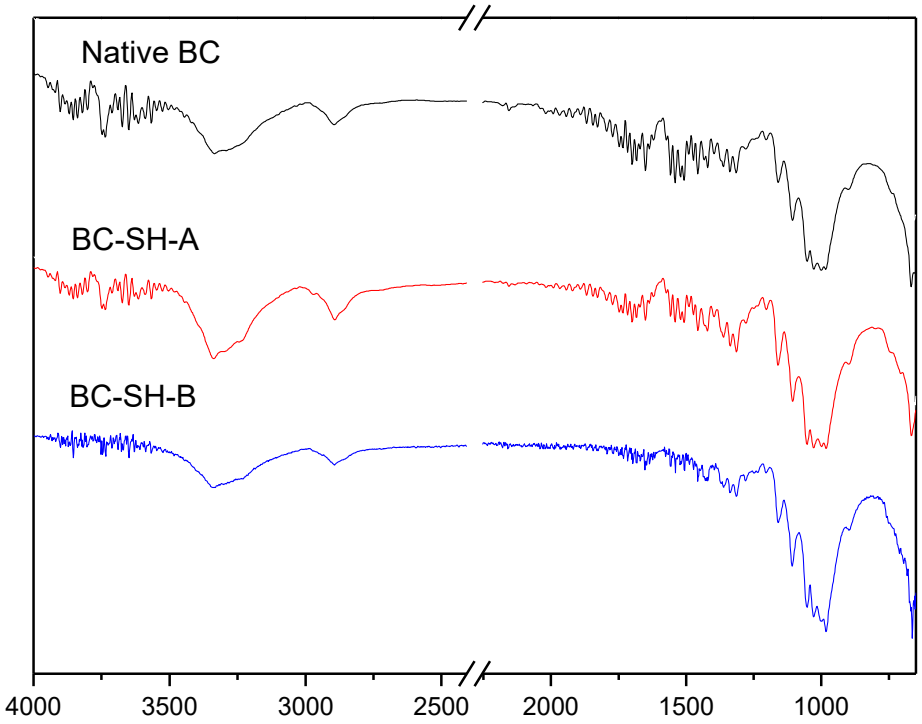


**Figure 2 –** ATR–FTIR spectrum of Native BC, BC–SH–A, and BC–SH–B.

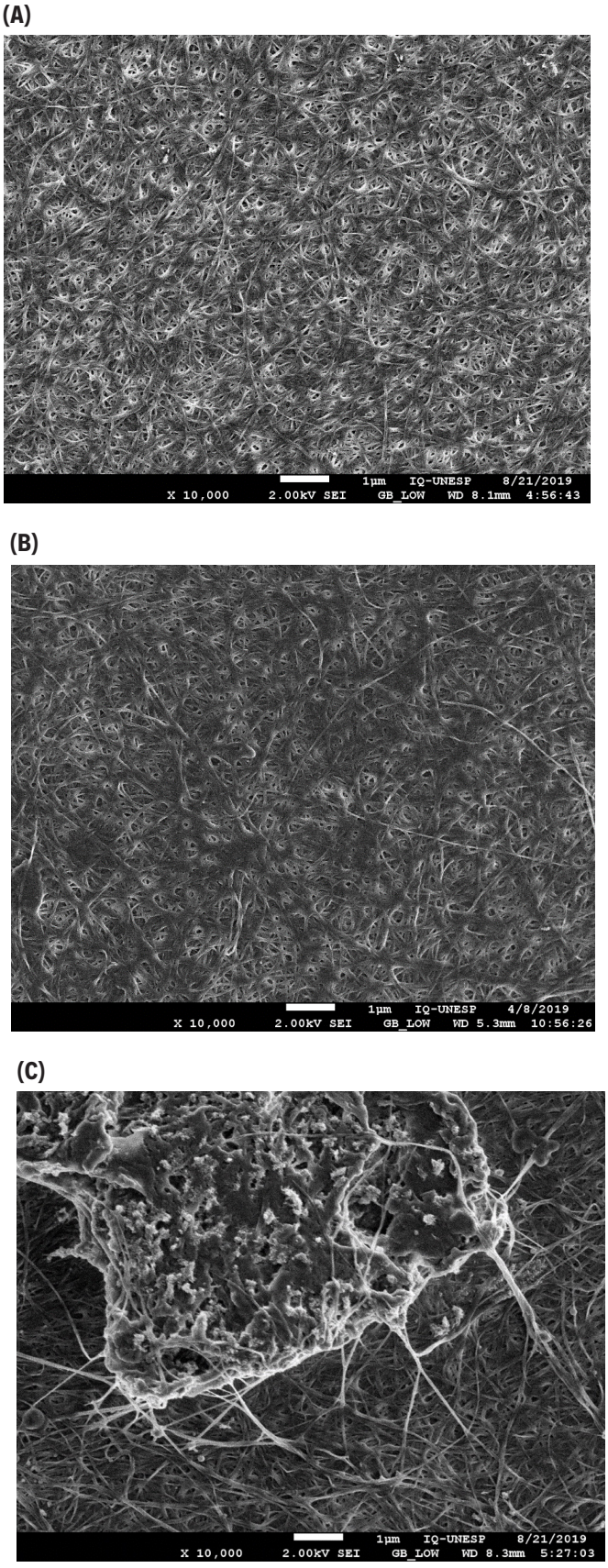


**Figure 3 –** Surface image by SEM of (A) Native BC (B) BC–SH–A and (C) BC–SH–B.

that the membranes treated by both Methods show mass losses with more pronounced inflection points in relation to native BC. In addition, the percentage of surface water loss is lower for treated membranes: about 3% for treated samples and 5% for BC. As well as the FTIR analysis, the TGA was not sensitive to verify the BC functionalization once it was not observed mass increase by 600°C due to silicon oxide formation.

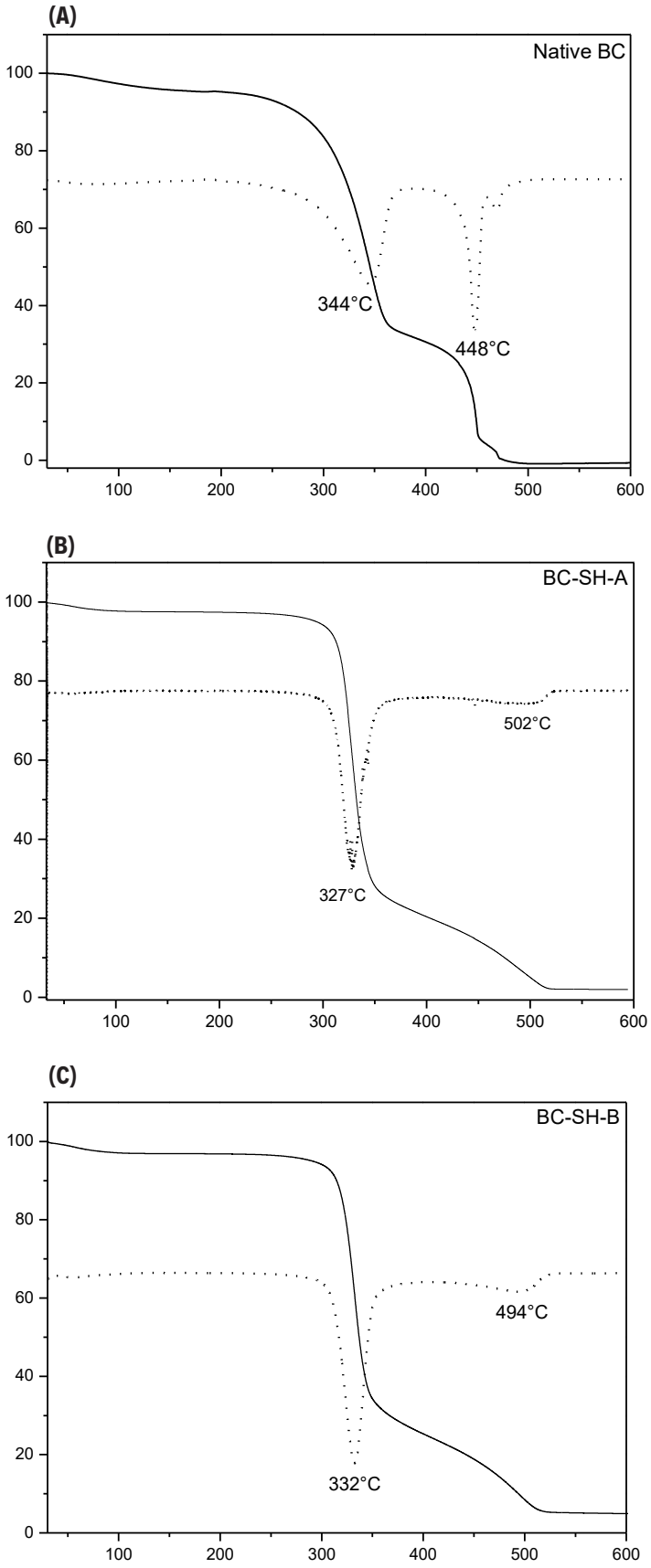


**Figure 4 –** TGA and DTG curves of (A) Native BC (B) BC–SH–A and (C) BC–SH–B.

## Conclusions

Among the silanization methods employed in this study for BC surface modification, only the one whose condensation step was induced by employing $NH_4OH$ was indeed effective. Elemental analysis and SEM analysis confirmed the efficiency of this particular surface functionalization using MPTMS.

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# Effects of calcium phosphates incorporation on structural, thermal and drug–delivery properties of collagen: chitosan scaffolds

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**Abstract:** In this study, we evaluated how different procedures of calcium phosphate synthesis and its incorporation in collagen:chitosan scaffolds could affect their structural and thermal properties, aiming the obtention of homogeneous scaffolds which can act as drug delivery vehicles in bone tissue engineering. Therefore, three different scaffold preparation procedures were developed, changing the order of addition of the components: in CC–CNPM1 and CC–CNPM2, calcium phosphate synthesis was performed in situ in the chitosan gel (1%, w/w) followed by mixture with collagen (1%, w/w), with changes in the reagents used for calcium phosphate formation; in CC–CNPM3 procedure, calcium phosphate was synthesized *ex situ* and then incorporated into the collagen gel, in which chitosan in powder was mixed. In all procedures, 5% (in dry mass) of ciprofloxacin was incorporated. FTIR analysis confirmed the presence of calcium phosphate in all scaffolds. DSC curves showed that collagen denaturation temperature (Td) increased with calcium incorporation. SEM photomicrographs of scaffolds cross–section revealed porous scaffolds with calcium phosphate grains internally distributed in the polymeric matrix. XRD diffractograms indicated that the calcium phosphates obtained are hydroxyapatite. The pore size distribution was more homogeneous for CC–CNPM3, which also stood out for its smaller porosity and lower absorption in PBS. These results indicate that the *in situ* or *ex situ* phosphate incorporation in the scaffolds had a great influence on its structural properties, which also had consequences for ciprofloxacin release. CC–CNPM3 released a smaller amount of antibiotic (30%), but its release profile was better described by all the tested models.

**Keywords:** Calcium phosphate; Collagen; Chitosan; Bone Regeneration; Ciprofloxacin Release.

## Introduction

Tissue engineering is an interdisciplinary field of sciences which uses principles and methods from engineering, biology, chemistry and physics in order to replace, keep or increase biological functions of tissues or damaged organs.[1] Tissue engineering studies aim to eliminate some of the main disadvantages of conventional clinical treatments, such as high cost, excessive recovery time and possibility of infections.

One of the major areas of tissue engineering is the bone tissue engineering. Bones consist of inorganic compounds such as calcium hydroxy phosphate ($Ca_5(PO_4)_3OH$), also known as hydroxyapatite (HA), calcium carbonate, as well as small quantities of bicarbonate, citrate, magnesium, potassium and traces of other metals. All this inorganic composition represents 65% of bone. 25% is composed of organic compounds as collagen type I, proteoglycans, carbohydrates, besides other proteins. The 10% left correspond to water.[2]

Bone regeneration involves the selection and migration of osteoprogenitors cells, followed by their proliferation, differentiation, matrix formation and bone remodulation.[3] Due to the many *in vitro* steps involved in the process, numerous variables can affect it, such as pH, fluid flow, mechanic and biochemical stimuli, culture medium, temperature, immunologic and inflammatory process, enzymes, as well as number, origin, mobility and activity from cells.[3,4] For this reason, the latest studies in tissue engineering focus on the development of biomaterial–based scaffolds capable of absorbing and releasing bioactive compounds and drugs, besides accelerating the healing response and promoting proper tissue formation.[4]

Scaffolds have been widely used in bone regeneration as a support throughout the recovery process, mimicking the porous architecture of the bone.[5] Besides the support, scaffolds must promote tissue growing, nutrients transport, oxygen diffusion and integration with the host bone, presenting a good biocompatibility with the osteoblasts. During the structural development of the scaffolds, mechanical and biological properties as porosity, pores size, water absorption, mechanical strength and pores shape need to be evaluated and controlled, as they will dictate the ability of the developed material to be applied as an implant or bone substitute.[6,7]

Among the main materials used in the development of scaffolds are natural polymers such as chitosan and collagen, as well as ceramics and minerals needed to mimic the structure and composition of a natural bone.[8,9] Calcium phosphates are one of the most widely used, as they have properties such as nontoxicity, noninflammatory response, and the ability to bind directly to the host bone.[10] Moreover, calcium phosphates as hydroxyapatite can be used in implants and as bone substitutes due its excellent biocompatibility, bioactivity, affinity to biopolymers and osteoconductivity.[11,12] These phosphates can be prepared in many forms, as a dense ceramic, in powder, in particles of micrometric or even nanometric size, according to their applications.[13] HA as nanometric particles has a similarity with the morphology of mineral grains found in bone. Nanometric particles have a greater surface area in relation to volume, which causes an increase in proteins absorption and cellular adhesion in the scaffolds and improves biological and mechanical properties.[14]

Regarding to the natural polymers, the applicability of collagen in tissue engineering brings advantages as its excellent biocompatibility, biodegradability and cellular adhesion properties.[9] In addition, as al–

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ready mentioned, type I collagen is the most abundant protein in the extracellular matrix of bone, which justifies its widespread use in bone tissue engineering. Many studies have already involved the development of scaffolds, composites and other types of collagen–based materials, associating them with calcium phosphates for bone regeneration: Keeney et al. (2010),[15] developed collagen and calcium phosphate scaffolds as vectors of plasmid DNA transfer; Inzana et al. (2014),[16] developed a 3D printing study of scaffolds based on collagen and calcium phosphate, characterizing them in relation to their cytocompatibility and cell viability.

However, this field still has limitations related to collagen–scaffolds faster degradation and weaker mechanical properties than natural bone.[17] To minimize these disadvantages, some effort has been concentrated in the development of collagen chemically reticulated and/or combined with natural and synthetic polymers. In this sense, the addition of a polymer such as chitosan to the scaffold is an alternative due its biocompatibility, biodegradability, plasticity, adhesiveness and osteoconductive properties.[13] For an example, Zugravu et al. (2012)[5] developed scaffolds based on chitosan/collagen/calcium phosphate microparticles, evaluating the advantages brought by the association of collagen with chitosan over ***in vitro*** compatibility and material biodegradability.

Besides obtaining the scaffolds, one of the biggest problems in surgical interventions is related to the possibility of infections, what takes to the systemic use of antibiotics. Antibiotics administration involves toxicity and a high incidence of antibiotic resistance.[18,19] To avoid these problems a rational use of these drugs is required, avoiding excess or yet the local use instead of systemic treatments. So, a release system that controls the antibiotic rate and keeps its therapeutic concentration during a long period is an advantage over the conventional methods.

To the best of our knowledge, despite numerous reports in the literature on the development of scaffolds and other types of collagen, chitosan and calcium phosphate–based materials, there is no study evaluating different procedures for the incorporation of calcium phosphate salts in the polymer matrix. The order–of–addition of component materials should have effects on the structural properties such as porosity and pore size, thermal properties such as collagen denaturation temperature and the ***in vitro*** antibiotic release, all of them evaluated in this study. We also model the release curves of ciprofloxacin, evaluating the release kinetics.

## Materials And Methods

### Materials

All solvents and reagents were of analytical grade and used without further purification. The bovine tendon used for collagen extraction was obtained at Casa de Carnes Santa Paula, São Carlos – SP. ***Doryteuthis*** spp. squid pens were obtained at Miami Comércio e Exportação de Pescados Ltda in Cananéa–SP and used as source of β–chitin for chitosan preparation.

### Methods

#### *Collagen obtention*

To obtain collagen, tendon bovine was treated in an alkaline solution containing salts (chlorides and sulfates of $Na^+$, $K^+$ and $Ca^{2+}$) for 72 h at 25°C, according to the procedure described by Horn et al. (2009).[20] The excess salts were removed by washing in solutions of boric acid ($H_3BO_3$) and deionized water, followed by washings in EDTA solution and deionized water. Collagen was extracted in pH 3.5 acetic acid (HAc) solution. Collagen gel concentration of 0.98 ± 0.23% was determined by lyophilization.

#### *Chitosan obtention*

Chitosan was prepared from squid pens (***Doryteuthis*** spp.) following the procedure of deproteinization and deacetylation adapted from Horn et al. (2009).[20] The reaction yield was 27.8%, which agrees with a previous study.[21] For chitosan characterization, its acetylation degree (6.7%)[22] was determined in a previous study by proton nuclear magnetic resonance spectroscopy ($^1$H NMR), according to the method developed and validated by Lavertu et al. (2003).[23] Chitosan molecular weight (327 kDa)[22] was also previously determined by capillary viscosimetry procedure, according to Rinaudo (2006).[24] 1% (w/w) chitosan gel was obtained in a 1% (w/w) HAc solution, under stirring for 24 h.

#### *Preparation of collagen:chitosan:calcium phosphate scaffolds*

In this study, three different procedures for calcium phosphate synthesis and incorporation in collagen and chitosan scaffolds were developed, by changing the reagents used, the order of addition of scaffold components and the ***in situ*** or ***ex situ*** synthesis of phosphate. In all of them, the collagen:chitosan ratio was kept in 1:1 and the amount of calcium phosphate incorporated was the same (35% in relation to the total dry mass of the polymers), being controlled by the molarity and the volume of reagents added in the system. The three procedures developed are described as following:

#### *Procedure 1 (CC–CNPM1)*

$CaCl_2$ and $(NH_4)_2HPO_4$ at the concentrations of 0.2 mol $L^{-1}$ and 0.12 mol $L^{-1}$, respectively, were added in the 1% (w/w) chitosan gel, followed by stirring for 24 h. The pH was raised to 9.0 with 1.0 mol $L^{-1}$ $NH_4OH$ and the stirring kept for more two days. Excess salts of material were removed with water and the chitosan containing the calcium phosphate synthesized was solubilized in HAc solution pH 3.5.

#### *Procedure 2 (CC–CNPM2)*

A 0.3 mol $L^{-1}$ solution of $H_3PO_4$ was added in the 1% (w/w) chitosan gel (in HAc) under constant stirring. Then, a 0.5 mol $L^{-1}$ $Ca(OH)_2$ ethanolic solution was slowly dropped in the mixture, keeping the pH 9.0 for two days. Excess salts of material were removed with water and the chitosan containing the synthesized calcium phosphate was solubilized in HAc solution pH 3.5.

In the two procedures described above, the material obtained ***in situ*** synthesis of calcium phosphate in chitosan was added to the collagen gel (1% w/w, in HAc pH 3.5) and kept under stirring for two days, in order to obtain homogeneous mixtures. The air was removed from the samples, which were placed in Teflon® molds, frozen and freeze–dried. The scaffolds obtained were washed in phosphate buffer saline (PBS) pH 7.4 and in deionized water, frozen and lyophilized.

#### *Procedure 3 (CC–CNPM3)*

Nano–calcium phosphate (NCP) was synthetized according to the procedure described by Gopi et al. (2015).[10] In brief, 0.05 mol $L^{-1}$ $CaCl_2.2H_2O$ was added to a 0.15% pectin solution, under stirring for 1 h. 0.03 mol $L^{-1}$ $(NH_4)_2HPO_4$ was dropped into the mixture, under vigorous stirring for 3 h. The pH was adjusted to 9.0 with 1.0 mol $L^{-1}$ $NH_4OH$, and after 24 h the white precipitate was dried at 80°C, washed with deionized water and ethanol. Finally, calcination eliminated the pectin matrix, ensuring that the powder obtained was only calcium phosphate.

The synthesized calcium phosphate was added to the 1% (w/w) collagen gel (in HAc pH 3.5), keeping the same ratio of the calcium phosphates incorporated on scaffolds of Procedures 1 and 2 (35%). The mixture was kept under stirring for 60 min. Chitosan powder was then added to the mixture, kept under stirring for another 24 h. Thus, this procedure changed not only calcium phosphate ***ex situ*** synthesis, but also the order of salt addition, being initially added to collagen for later incorporation of chitosan powder (and not chitosan gel, as in the other procedures).

The resultant mixture was placed in Teflon® molds, frozen and freeze–dried. The scaffolds were washed in PBS pH 7.4 and in deionized water, frozen and lyophilized.

#### *Ciprofloxacin incorporation*

Once the three procedures for obtaining the scaffolds were developed, the antibiotic ciprofloxacin was incorporated into the mixtures. To

this end, about 15 g of the mixtures were separated before being frozen and lyophilized, and to this amount of material 5% (w/w, relative to the dry mass of the polymers) of ciprofloxacin powder, corresponding to 7.5 mg, was added. Thus, the final antibiotic concentration in relation to the collagen:chitosan:calcium phosphate mixture was 0.5 mg $g^{-1}$. These new mixtures were homogenized by stirring, placed in Teflon® molds, frozen and lyophilized. Scaffolds were neutralized in ammonium vapor for 24 h and aerated under constant air flow for 72 h, being denominated CC–CNPM1–C, CC–CNPM2–C and CC–CNPM3–C.

***Fourier transform infrared spectroscopy (FTIR)***

CC–CNPM1, CC–CNPM2 and CC–CNPM3 samples were diluted in HAc pH 3.5 (in the ratio of 1:3), placed in Teflon® molds and dried under air flow, in order to form films by the casting method. The spectra were obtained in a FTIR Shimadzu IR Affinity – 1 at a 400 a 4000 $cm^{-1}$ interval with 4 $cm^{-1}$ of resolution.

***Thermal stability***

Denaturation temperature of collagen present in the scaffolds was obtained by differential scanning calorimeter (DSC) in $N_2$ atmosphere using a DSC–2010 (TA Instruments). A sample of 20 mg was used, and the heating rate was 10°C $min^{-1}$, with a temperature range from 5 to 120°C. For the quantification of residue in the scaffolds, thermogravimetric curves (TG) were obtained with a TGA–Q50 (TA Instruments). A sample of 10 mg was used, with a heating rate of 10°C $min^{-1}$ and the temperature range was from 25°C to 800°C, under synthetic air atmosphere.

***Scanning electron microscopy (SEM)***

The scaffolds morphology was observed using a ZEISS LEO 440 (Cambridge, England) equipment, with an OXFORD detector (model 7060) and an electron beam of 20 kV. Before the analysis, the scaffolds were affixed in stubs with conductive carbon tape and covered with a 6 nm gold layer. The software UTHSCSA Image Tool was used to measure the scaffolds pores. For each one, 20 measures were performed.

***X–rays diffraction (XRD)***

X–rays diffraction provides a better understanding of the size and nature of synthesized calcium phosphate grains, checking for the presence of crystalline structures in the scaffolds developed. The analysis was performed using monochromatic radiation of CuK, 1,5406 Å, 50 kV, scanning speed of 2° $min^{-1}$ and 2θ between 5 and 80°. Using the diffractogram and the Scherrer's equation (1),[25] the crystal size was calculated:

$$L_{002} = \frac{K\,\lambda}{\beta \cos\theta} \quad (1)$$

In this equation, K is a constant related to the grain size, being its value close to the unitary; λ is the x–ray radiation wavelength (nm); β is the widening of the diffraction peak 002, measured at half the maximum peak intensity, in radians, and θ is the Bragg diffraction angle, in degrees.

***Porosity***

Scaffold porosity was determined in quintuplicate, according to the procedure described by Nwe et al. (2009).[6] Scaffolds were soaked overnight in water; tissue papers were dried overnight, and their weights were measured. The wet scaffolds had their diameter and thickness measured, so their volume could be calculated. They were placed on the top of the tissue papers and centrifuged at 4,500 rpm for 5 min. The mass of the wet tissue papers was measured, and then the volume of the water absorbed by the scaffold was determined. The porosity was calculated by the relation between this volume ($V_2$) and the volume of each scaffold ($V_1$), according to Equation (2).

$$Porosity\ (\%) = \left(\frac{V2}{V1}\right) x\ 100 \quad (2)$$

***Absorption in PBS***

The study of scaffolds absorption in PBS pH 7.4 was performed in quintuplicate. The dried scaffolds were weighted, placed in PBS buffer and reweighted at specific times. At the end, the percentage of buffer absorbed by each scaffold was calculated using Equation (3).

$$\%\ PBS\ absorbed = \left(\frac{mw - md}{md}\right) x\ 100 \quad (3)$$

Where $m_w$ is the mass of the wetted scaffold, in specific times, and $m_d$ is the initial mass of dried scaffold.

***Ciprofloxacin release***

The ciprofloxacin release study was made in quintuplicate, by the immersion of scaffolds in 100 mL of PBS pH 7.4, under 100 rpm of stirring and at 37°C. In specific time intervals, aliquots of 1.0 mL were collected, being replaced by 1.0 mL of PBS. Absorbance was read at 271 nm, using a UV spectrophotometer (model HITACHI U–3000).

***Statistical analysis***

The Shapiro–Wilk test was used to verify data distribution. Pore sizes and porosity results were examined using analysis of variance (ANOVA), followed by Tukey's test. Significance level was set at 5 % in all cases.

**Results And Discussion**

All scaffolds obtained were porous, homogeneous and white in color, without visible calcium phosphate precipitates. The presence of ciprofloxacin antibiotic did not bring visible changes in the appearance of scaffolds.

**Fourier transform infrared spectroscopy (FTIR)**

FTIR analysis aims to verify the presence of calcium phosphate characteristics bands in the samples, the first of a series of analysis that allow the confirmation of its incorporation in the scaffolds by the tested procedures. In addition, it can also endorse the presence of typical bands of the polymers used as the scaffolds matrix (collagen and chitosan). FTIR spectra of the samples (CC–CNPM1, CC–CNPM2 and CC–CNPM3) and of the calcium phosphate used in Procedure 3 are shown in Fig. 1.

The spectrum of calcium phosphate confirmed the presence of several characteristics bands: the band at 560 $cm^{-1}$ refers to the deformation of P–O bond in the phosphate group ($PO_4^{2-}$), while the band at 1041 $cm^{-1}$ is related to P–O stretching.[26] The same phosphate bands (560 and 1041 $cm^{-1}$) were present in the scaffolds spectra, which also presented large bands at 3300 $cm^{-1}$ referring to the O–H deformation, and characteristics bands of the polymers (collagen and chitosan), as observed at 1630 $cm^{-1}$, 1540 $cm^{-1}$ and 1230 $cm^{-1}$ (amides I, II and III, respectively).[27] At 1410 and 1450 $cm^{-1}$ can also be observed bands referring to the carbonate group ($CO_3^{2-}$), usually present in calcium deficient hydroxyapatites as a form of charge compensation.[28] All spectra were similar to each other, with no emphasis on any bands that emerged or differentiated between them.

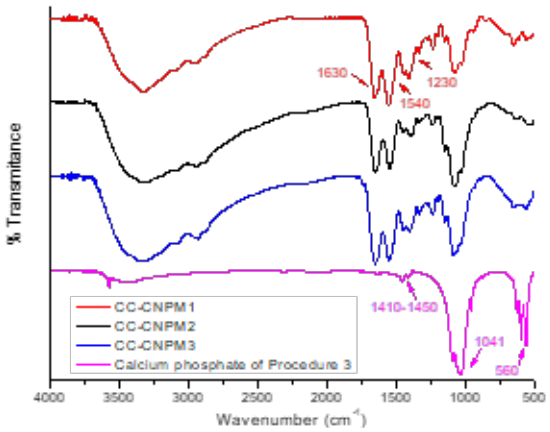


**Figure 1** – FTIR spectra of CC–CNPM1, CC–CNPM2, CC–CNPM3 and of the calcium phosphate used in Procedure 3.

### Thermal stability

The denaturation temperature (Td) obtained by DSC are presented in Table 1. The analysis was also performed for collagen used in the preparation of scaffolds, and its Td was determined to be 44.8°C. According to Table 1, it is possible to observe an increase in the values in relation to this collagen, which indicates that the calcium phosphate incorporation increased collagen thermal stability, requiring more than 63°C to denature its triple helix.[29]

Thermogravimetric curves for CC–CNPM1, CC–CNPM2 and CC–CNPM3 revealed similar profiles, as shown in Fig. 2. Three stages of weight loss can be observed: from 25 to 200°C, the loss of structural water takes place; the second stage, from 200 to 400°C, is related to the thermal degradation of the collagen structure; finally, the third step refers to the components decomposition and carbonization (400–700°C).[29] Slight differences can be observed in each one of the steps. In general, it is observed that the CC–CNPM1 presented the lowest mass loss values in these three steps.

Furthermore, in all cases it can be observed residues at 700°C related to inorganic components of the scaffolds, especially the calcium phosphate which does not decompose in this temperature range.[30] As shown in Fig. 2, calcium phosphate shows a slight loss of water at the beginning of its curve and remains stable without decomposition over almost the entire working temperature range. Comparing the residual values found for the scaffolds, it can be said that CC–CNPM1 stood out with the highest calcium phosphate content, almost the double of the scaffolds obtained by the other procedures. Thus, it is believed that Procedure 1 generated the largest amount of calcium phosphate in its synthesis *in situ*. Thermogravimetry may have been a first indication of the influence of the different adopted procedures on the properties of the obtained scaffolds.

| Scaffold | Td (°C) | Weight loss (%) | | | Residue (%) |
|---|---|---|---|---|---|
| | | 25–200°C | 200–400°C | 400–700°C | 700°C |
| CC–CNPM1 | 65.5 | 9.9 | 30.3 | 14.9 | 44.8 |
| CC–CNPM2 | 63.8 | 17.1 | 38.1 | 23.6 | 20.9 |
| CC–CNPM3 | 67.9 | 10.5 | 37.0 | 29.0 | 22.5 |

**Table 1** – Thermal parameters and residue content at 700°C of CC–CNPM1, CC–CNPPM2 and CC–CNPM3 scaffolds.

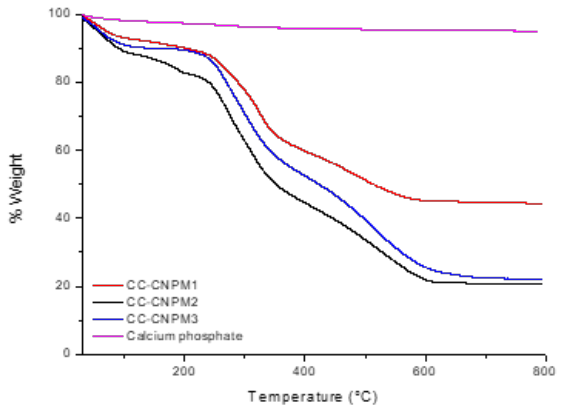


**Figure 2 –** Thermogravimetric curves for CC–CNPM1, CC–CNPM2, CC–NPM3 and synthesized calcium phosphate.

**Scanning electron microscopy (SEM)**

The photomicrographs obtained by SEM are shown in Fig. 3. The scaffolds were porous, with calcium phosphate grains internally distributed throughout the polymer matrix and no larger than 300 nm. However, differences can be observed regarding the quantity and size of scaffold pores, as well as regarding the morphology of the grains and their quantity.

From Fig. 3A, 3C and 3E, all with the same magnification (25,000x), there is a distinct difference with respect to the morphology of the scaffolds: although CC–CNPM3 presents itself as a more compact scaffold and its pores are more homogeneous and better distributed, the same does not occur in CC–CNPM2, in which no well–defined pores are observed.

Regarding to calcium phosphate (Fig. 3B, 3D and 3F), the formed grains are clearly different from each other. In CC–CNPM1, their needle–like shape resembles CC–CNPM3 grains, but in the latter case they are smaller and better distributed along the scaffold cross section. Li et al. (2010)[31] reported obtaining needle–shaped apatite formed on the chitosan surface after a mineralization process by immersion in saline solutions. However, the grains obtained by them were about 7 times larger than the calcium phosphates formed in CC–CNPM1. On the other hand, the calcium phosphate grains in CC–CNPM2 are spherical in shape, aggregated and poorly distributed in the scaffold. The same spherical shape of calcium phosphates can be observed in the work of Zhao et al. (2008),[32] who mineralized scaffolds based on collagen and chitosan, obtaining calcium phosphates of different shapes and sizes, according to the calcium ion concentration.

ImageJ software was used to measure the size of scaffold pores using SEM photomicrographs with 500x of magnification. Table 2 shows the average surface and cross–sectional pore size of the scaffolds. In general, all samples presented at least 40% of their surface pores with sizes that varied within the same range, which were larger for CC–CNPM2 (between 30 and 40 µm) than for the other two cases (Fig. 4).

CC–CNPM3 presented smaller surface and cross–sectional pores, with approximately the same size range (5 to 40 µm), unlike CC–CNPM1 in which the cross–sectional pores were almost twice the average pore size of the surface pores. On the other hand, CC–CNPM2 showed a more heterogeneous pore size distribution, both at surface (range 10 to 65 µm) and cross sectional (range 10 to 55 µm). Thus, from the analysis of SEM images and of the surface and cross–sectional pores of the samples, it can be concluded that the ***ex situ*** calcium phosphate incorporation adopted in CC–CNPM3 was the procedure that resulted in more homogeneous scaffolds with better defined pores.

According to Karageorgiou & Kaplan (2005),[33] pores with sizes around 100 µm are favorable for cell growth and nutrient transport. Although the scaffolds developed by the three procedures presented pore sizes smaller than 100 µm, studies involving ***in vitro*** evaluation of osteoblasts growth in collagen scaffolds with smaller pores have already been successfully conducted.[34]

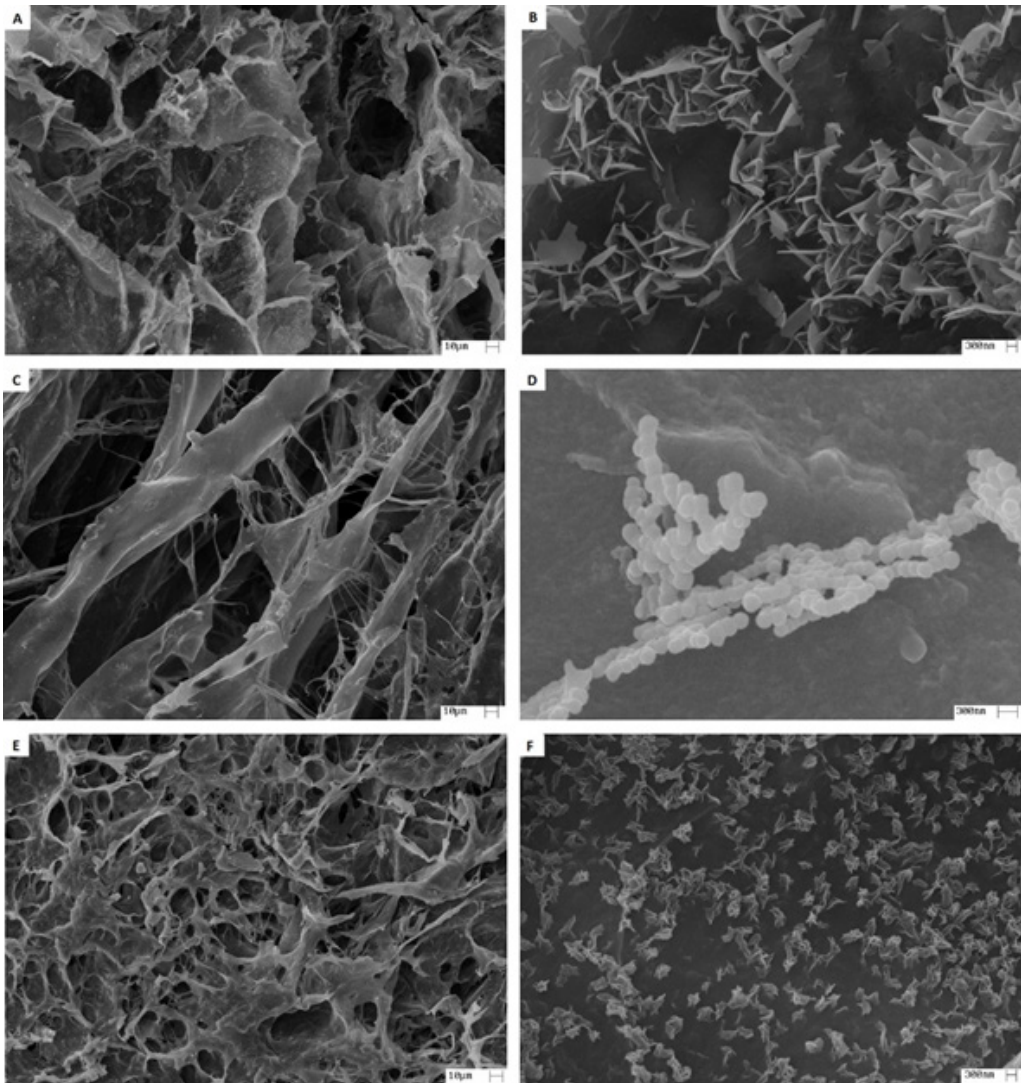


**Figure 3 –** Photomicrographs by SEM of cross section of: (A) and (B) CC–CNPM1, (C) and (D) CC–CNPM2, (E) and (F) CC–CNPM3. Magnitude of 1,000x (A), (C) and (E); 25,000x (B) and (F); 30,000x (D).

| Scaffold | Pores sizes ± SD (μm) | |
|---|---|---|
| | Surface | Cross–section |
| CC–CNPM1 | $17.7 \pm 6.8^{b}$ | $42.8 \pm 9.2^{a}$ |
| CC–CNPM2 | $38.2 \pm 14.3^{a}$ | $31.2 \pm 13.7^{b}$ |
| CC–CNPM3 | $21.1 \pm 8.5^{b}$ | $18.4 \pm 8.7^{c}$ |

In the same column, values with the same superscript letter (a–b) were not significantly different ($P > 0.05$).

**Table 2** – Pores sizes by SEM photomicrographs for CC–CNPM1, CC–CNPM2 and CC–CNPM3 scaffolds.

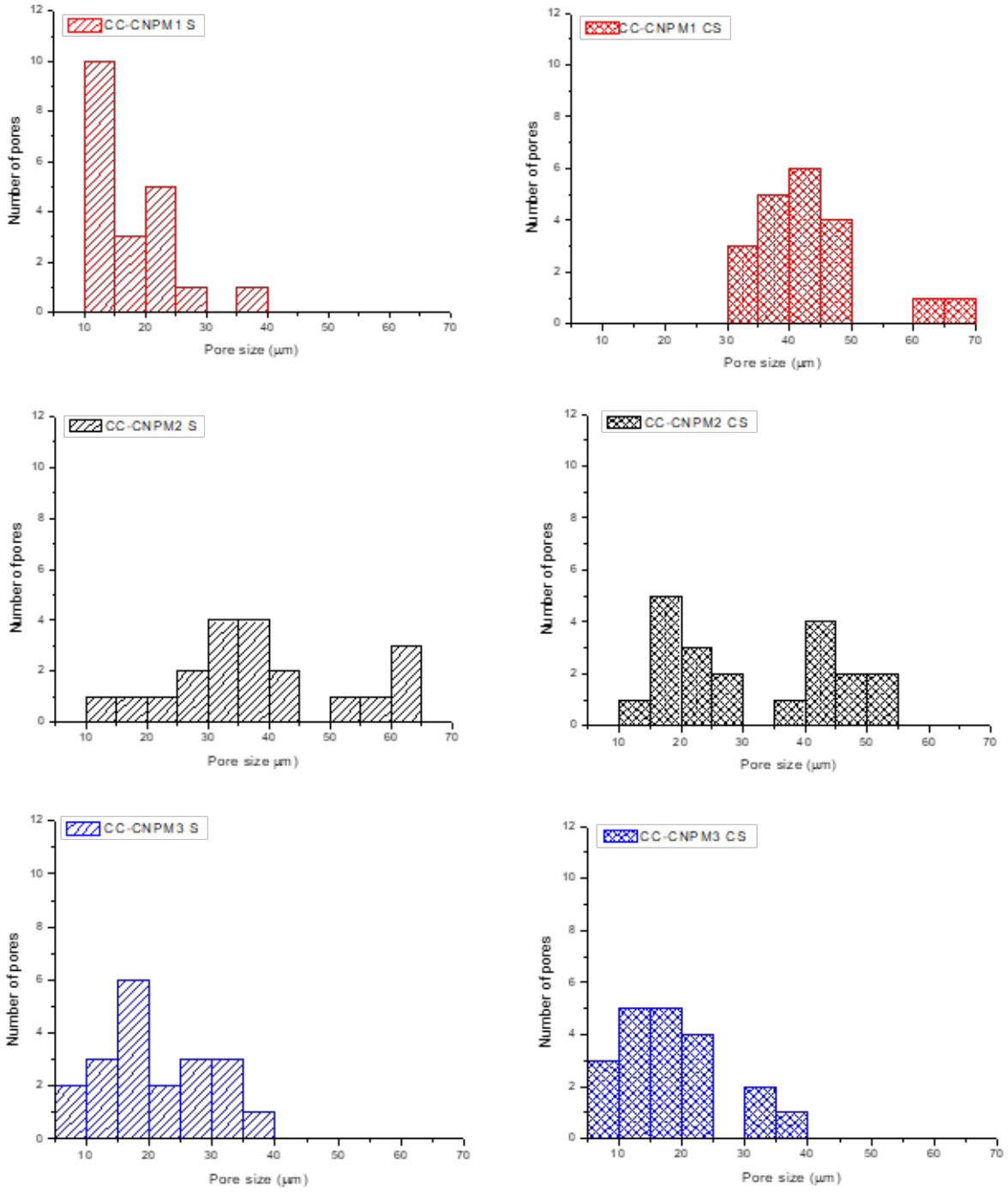


**Figure 4 –** Histograms of pore size distribution for surface (S) and cross–sectional (CS) SEM images.

### X-rays diffraction (XRD)

X-rays diffraction analysis was performed to identify the crystalline phases present in the scaffolds developed, as well as in the calcium phosphate synthesized *ex situ* for Procedure 3 (Fig. 5).

As can be seen in Fig. 5, the peaks at 2θ=32° characteristic of the (211) plane of hydroxyapatite[35] appear in all diffractograms presented, with greater intensity and better resolution in the case of isolated calcium phosphate diffractogram. The well-marked peaks confirm that the calcium phosphates obtained in the procedures developed is this study are believed to be hydroxyapatite.

In the scaffold spectra, the presence of collagen and chitosan interferes with the crystallinity of the mineral content. Zugravu et al. (2012),[5] studied the effects of different collagen concentrations on the calcium phosphate crystallinity incorporated in composite microparticles. They reported that at a 25% collagen concentration, calcium phosphate peaks completely disappeared from the spectra. Furthermore, in the scaffold spectra two additional peaks can be observed, at 2θ=10° and 2θ=20°, referring to the chitosan hydrous and anhydrous peaks, respectively.[35]

The grain size of HA synthesized in Procedure 3 was measured by the enlargement of diffraction peak 002, indicated in the spectrum. Scherrer equation's (1) was used to calculate the grain size, and the value obtained was 21.4 nm, which confirms HA synthesis in nanometric dimensions.

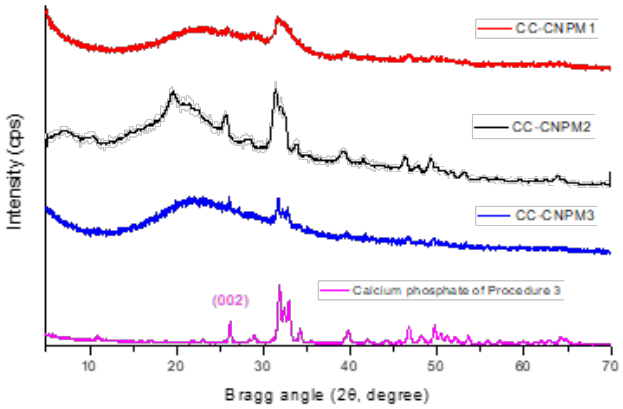


**Figure 5** – Representative XRD spectra of collagen/chitosan/calcium phosphate scaffolds compared to the calcium phosphate synthesized in Procedure 3.

### Scaffolds porosity and absorption in PBS

Scaffolds porosity, in addition to pores size and morphology, is an essential factor to be studied when considering the application of these materials in bone regeneration, as it influences processes such as water absorption, PBS absorption, migration and cell growth. Table 3 shows the porosity values obtained for the developed scaffolds, as well as their relative absorption percentage in PBS pH 7.4.

It can be observed from the porosity values presented that, although there are no significant statistical differences between the procedures, the scaffolds obtained by Procedures 1 and 2 (*in situ* procedures) showed a tendency to be more porous than the scaffolds prepared by Procedure 3, in which phosphate incorporation was *ex situ*.

PBS absorption study is critical to evaluate whether prepared scaffolds can be used as controlled drug delivery vehicles. Fig. 6 shows the mean absorption curves in PBS as a function of time for CC-CNPM1, CC-CNPM2 and CC-CNPM3 scaffolds.

The percentage of PBS absorbed, as well as the absorption profile, differ between the scaffolds. While CC-CNPM1 and CC-CNPM2 both stabilized in 1,500% or more and in approximately 1 min, CC-CNPM3 stabilized their absorption in 1,100% after 10 min. The absorption results may be related to the scaffold porosity: as seen, CC-CNPM3 was the least porous scaffold among the three developed, and it was expected that its PBS absorption was also the lowest. Nevertheless, all prepared scaffolds absorbed large amounts of buffer over a short period of time, which is a promising result for their use as drug carriers and delivery systems.

PBS absorption and porosity results, when combined with scaffolds pore size distribution data, allow an important conclusion to be drawn as the direction of this study: the order of addition of scaffold components, as well as the way calcium phosphate is incorporated, have a greater influence on the porous structure of the final material than the simple reagents change evaluated in the *in situ* procedures developed. Thus, CC-CNPM3 scaffolds were the least porous and absorbed less PBS but presented a more homogeneous pore distribution and smaller surface and cross-sectional pores. CC-CNPM1 and CC-CNPM2, in turn, despite having different pore size distributions, presented similar porosities and absorption profiles.

| Scaffold | Porosity ± SD (%) | % PBS absorbed |
|---|---|---|
| CC-CNPM1 | 30.6 ± 5.3[a] | 1,600 |
| CC-CNPM2 | 30.1 ± 2.7[a] | 1,495 |
| CC-CNPM3 | 23.9 ± 4.8[a] | 1,062 |

In the same column, values with the same superscript letter (a) were not significantly different (P > 0.05).

**Table 3 –** Porosity and PBS absorption for CC-CNPM1, CC-CNPM2 and CC-CNPM3.

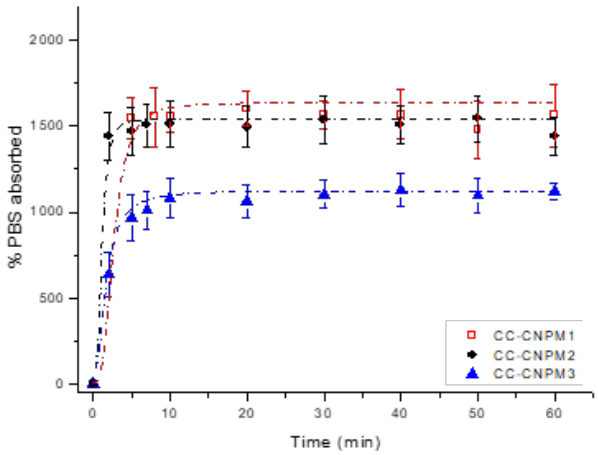


**Figure 6 –** PBS absorption profiles for CC–CNPM1, CC–CNPM2 and CC–CNPM3.

**Ciprofloxacin release**

Fig. 7 shows the mean release profile of ciprofloxacin by the scaffolds. As expected, CC–CNPM3–C presented the most distinct profile, releasing approximately 30% (0.4 mg) of their incorporated antibiotic after about 2 h of assay. CC–CNPM1–C and CC–CNPM2–C released 40% (0.45 mg) and 63% (0.7 mg) of their ciprofloxacin, respectively, stabilizing these percentages after about 1 h of assay.

Although the release time was well balanced in all cases and stabilization occurred at acceptable time intervals, the maximum release percentages were relatively low. The reason for this may be related to the structure of ciprofloxacin: the antibiotic is a fluorquinolone, which can complex with calcium ions of phosphate present in scaffolds, making it difficult to release (Fig. 8).[36,37] This fact also explains why CC–CNPM1–C released less antibiotic, despite having a PBS absorption profile similar to CC–CNPM2–C: its inorganic content was the highest, which implies a possible greater complexation between its HA incorporated therein and ciprofloxacin.

Despite the released concentrations are low in relation to the total antibiotic incorporated, depending on the application to which the scaffold is submitted (application site, type of injury) the amount of ciprofloxacin released may still be enough or even higher than the required therapeutic concentration. According to the FDA, the recommended dose of ciprofloxacin to be consumed in a day is 250 to 1000 mg, but a concentration of 2 μg $mL^{-1}$ is already enough to inhibit a huge range of microorganisms, as *E. coli* and *S. aureus*.[38] Thus, all scaffolds developed in this study released larger amounts of ciprofloxacin in a much shorter time period than 24 h.

In order to better understand which drug release mechanisms were involved for each of the prepared scaffolds, different release models were tested, including: first order, second order, Hixson–Crowell, Baker–Lonsdale, Korsmeyer–Peppas, Higuchi and Weibull.[39,40] Only the three last models showed satisfactory results for the samples developed in this study, and their equations and parameters are described in Table 4.

Table 5 lists the main parameters obtained by modeling the ciprofloxacin release curves for CC–CNPM1–C, CC–CNPM2–C and CC–CNPM3–C samples, according to the three models described above. As can be observed, CC–CNPM3–C was best fitted in all tested models, presenting the highest R values. The release curves of CC–CNPM1–C and CC–CNPM2–C were best adjusted by the Korsmeyer–Peppas model. This model is used in systems in which more than one process is involved in drug release: for samples with $0.5 < n < 1.0$, the drug transport mechanism is called anomalous or non–Fickian, and its release mechanisms can be by diffusion or by relaxation of polymeric chains.[40] Otherwise, if $n < 0.5$ the transport drug mechanism in denominated Quasi–Fickian diffusion, and its kinetics of diffusion is completely different from the non–Fickian mechanism. In this case, it is believed that the polymer chains have more mobility, releasing a greater amount of drug by diffusion than the non–Fickian model would release.

According to Table 5, CC–CNPM1–C and CC–CNPM2–C showed Quasi–Fickian diffusion mechanisms, while CC–CNPM3–C presented a non–Fickian mechanism, releasing ciprofloxacin by diffusion and/or relaxation in the polymeric matrix. Different mechanisms of diffusion were really expected for this sample, since the profile of its release curve was very different from the others (Fig. 7), as well as its maximum amount of ciprofloxacin released. Thus, it can be said that although CC–CNPM3–C was the sample that released the least amount of antibiotic, its release was the most controlled of the three cases, and it would be chosen as the best release model for ciprofloxacin.

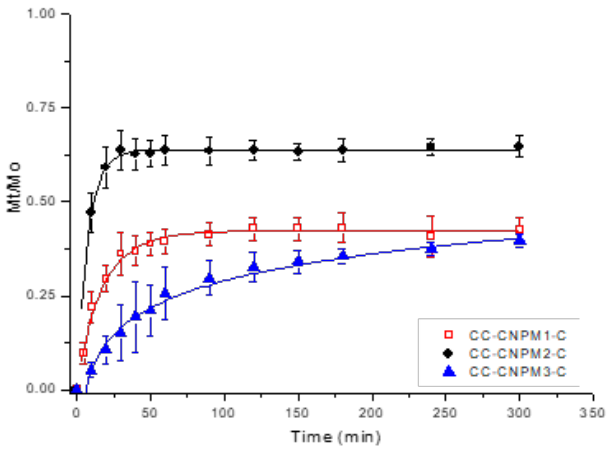


**Figure 7 –** Ciprofloxacin release profile of the scaffolds. Release conditions: 37°C, 100 rpm, absorbance reading at 271 nm.

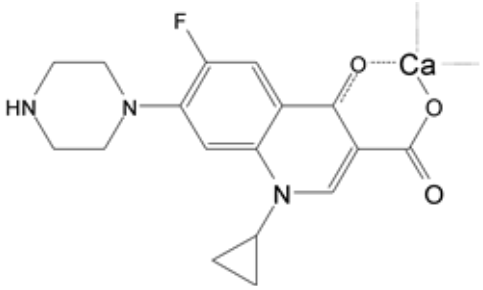


**Figure 8 –** Complexation between the ciprofloxacin and calcium ions from phosphates.

| Model | Equation | Parameter(s) |
|---|---|---|
| Higuchi[38] | $Mt/Mo = k.t^{0.5}$ | **k** is Higuchi kinetic constant; **Mt** is the amount of drug dissolved in time **t**; **Mo** is the initial amount of drug |
| Korsmeyer–Peppas[38] | $Mt/Mo = k.t^{n}$<br>$\ln(Mt/Mo) = \ln(k) + n.\ln(t)$ | **k** is Korsmeyer–Peppas kinetic constant, **n** is the release exponent, indicative of the drug release mechanism |
| Weibull[38] | $\frac{Mt}{Mo} = 1 - e^{-[(t-T)^b]/a}$<br>$\log[-\ln(1-M\_t/M\_0)] = b.\log(t) - \log(a)$ | **T** is the location parameter, represents the lag time before the onset of the dissolution or release process and in most of the cases will be zero; **a** is the scale parameter defines the time scale of the process; **b** shape of dissolution curve as either exponential (b=1), *S*–shaped (b>1) or parabolic (b<1) |

**Table 4 –** Model equations used for fitting ciprofloxacin release data.

| Models | Scaffolds | | |
|---|---|---|---|
| | CC–CNPM1–C | CC–CNPM2–C | CC–CNPM3–C |
| Higuchi | | | |
| k | 0.02136 | 0.01476 | 0.03188 |
| R | 0.91934 | 0.87986 | **0.98727** |
| Korsmeyer–Peppas | | | |
| k | 0.16483 | 2.89548 | 0.00986 |
| n | 0.21357 | 0.09724 | 0.74079 |
| R | **0.95788** | **0.93737** | **0.98087** |
| Weibull | | | |
| a | 4.1468 | 2.04207 | 60.9116 |
| b | 0.16327 | 0.08449 | 0.61416 |
| R | 0.88836 | 0.87812 | **0.96201** |

**Table 5 –** Parameters obtained by modeling ciprofloxacin release curves for CC–CNPM1–C, CC–CNPM2–C and CC–CNPM3–C.

## Conclusion

This study revealed an unprecedented study of the development of three distinct procedures for the synthesis and incorporation of calcium phosphates in collagen and chitosan–based polymeric systems, aiming to obtain homogeneous scaffolds that could act as controlled release vehicles of ciprofloxacin. The materials were structurally characterized, presenting internally distributed calcium phosphate grains and pores of different shapes and size distributions. Studies of scaffolds porosity, pore size and absorption in PBS were conducted, and all results pointed in the same direction: CC–CNPM1 and CC–CNPM2, in which calcium phosphate synthesis occurred ***in situ***, presented porosities and absorption profiles in PBS quite different from CC–CNPM3, in which phosphate incorporation was by simple mixture, confirming that the order of addition of scaffold components actually interferes with their final structural properties. Finally, the release study of ciprofloxacin revealed by modeling the data that CC–CNPM3–C stood out as the sample that released this antibiotic by both diffusion and relaxation of the polymeric matrix, although it released a smaller amount of the drug. It is expected that this study opens new possibilities of application of the developed materials, once some of the main important aspects for their application in the area of bone regeneration have been characterized.

## Conflict of Interests

There are no conflicts to declare.

## Acnlowledgements

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## SPECIAL SESSION IN HONOR OF ANTONIO CARLOS MASSABNI

*Vera Regina Leopoldo Constantino*
*Pedro Paulo Corbi*

The present issue of International Journal of Advances in Medical Biotechnology (IJAMB) introduces a set of five papers elaborated by Prof. Antonio Carlos Massabni and students that have attended a graduate course nominated Seminars of Integration from the Postgraduate Program in Biotechnology of the University of Araraquara (UNIARA), Araraquara, Brazil. The authors have focused on the impact of Industry 4.0 technologies on Biotechnology as well as the chance for future professionals and the work relations in this context. In other papers, the importance of biotechnology in the development of vaccines, antibiotics, hormones and scaffolds for regenerative medicine are discussed. This issue is dedicated in honor of Prof. Massabni for his commitment with teaching, research and administrative duties.

Born in the city of Pirangi in the interior of the state of São Paulo–Brazil, in November 1944, Prof. Dr. Antonio Carlos Massabni obtained the Licentiate degree and later the Bachelor degree in Chemistry at the Faculty of Philosophy, Letters and Sciences of Araraquara, Brazil, (FFCLA) in the years 1966 and 1967, respectively. In 1973, as a professor at FFCLA, he received the degree of Doctor in Chemistry under the supervision of Prof. Dr. Osvaldo Antonio Serra, working on the synthesis of phosphine oxide complexes with Co(II). In 1981, he became Associate Professor at the Institute of Chemistry at UNESP–Araraquara (IQ–UNESP) and, in 1986, received the Full Professor title in the same institution, a position held until 1998. Since then, Prof. Massabni acts as senior collaborator professor at IQ–UNESP.

Prof. Massabni contributed actively to the construction and development of IQ–UNESP, acting dedicatedly as professor, researcher and in administrative positions. He was head of the Department of Chemistry at the FFCLA and of the Department of Inorganic Chemistry at IQ–UNESP, and he also coordinated the Postgraduate Program in Chemistry at the IQ– UNESP in early 1980s. Prof. Massabni was director of IQ–UNESP from 1998 to 1992, and president of the Research Committee of IQ– UNESP. Furthermore, he served as Consultant to the Rectory of Graduate Studies and Research of UNESP and Director of Fundunesp (Foundation for the Development of the State University of São Paulo). The important contribution of Prof. Massabni at IQ–UNESP was recognized in a ceremony in which he received the title of Professor Emeritus of the institution, which is one of the highest honors for those who dedicated their career to the strengthening of teaching and university research. Nowadays, Prof. Massabni is member of the Regional Chemistry Council (CRQ–IV), São Paulo, Brazil.

Acting as advisor, Prof. Massabni supervised sixteen Master and nine Doctoral studies. Currently, as a professor of the Postgraduate Program in Biotechnology in Regenerative Medicine and Medicinal Chemistry at UNIARA, Prof. Massabni remains committed in training new researchers. As a result of his scientific career, Prof. Massabni has published about one hundred articles in specialized journals and about two hundred papers in scientific meetings. He co–authored one book in nomenclature of Inorganic Chemistry, three patent applications and has participated in the evaluation of more than fifty master's and doctoral formal examinations.

These registers show part of the career and work of Prof. Massabni as a professor and researcher. However, his contribution goes beyond the information presented here. There are a great number of students who have attended his courses and who, like us, had the opportunity to be supervised by him. Today, as professors at recognized research institutes in the national and international panorama, we can take some of what we learned from Prof. Massabni in the time when we shared his fellowship. Currently, we bring values to our students such as ethics in science and respect for the public investment received for the development of our research. In this way, we have forwarded the knowledge received, searching to contribute so that new knowledge be generated and shared by everyone looking for a better quality of life and for the welfare of our society.

**Thank you, Master Massabni!**

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# Biotechnology applied for sustainable development: social responsibility in the Industry 4.0

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**Abstract:** This research was conducted from a review of bibliographic content on Biotechnology, sustainable development, social responsibility and Industry 4.0. The goal endows the understanding of the role of Biotechnology as a science in sustainable development in this historical phase experienced by humanity, the Fourth Industrial Revolution, verifying what would be the social responsibility of Industry 4.0 in this context. Dialectical and historical methods were used to systematize the obtained data. The importance of maintaining the environmental balance through sustainable practices in the daily life of Industry 4.0 has been demonstrated to comply with the constitutional principle of the social function of property. However, in order to achieve sustainable development, the economic and social aspects, besides the environmental, must be considered. The relevance of Biotechnology in this process has been proven as a driving force for sustainable development. It is hoped with this research to mobilize the academic community and the society in the fight against environmental degradation, bringing knowledge about the role of Biotechnology in this process, in the context of Industry 4.0, and demonstrating the need for companies, professionals and governments to adapt to this new and unknown reality in order to face the problems that are already emerging, always taking into consideration the protection of human rights, especially the healthy and balanced environment, safety, life and dignity of the human person.

**Keywords:** Biotechnology; Sustainable development; Industry 4.0.

## Introduction

Industry 4.0 reflects technological implementations and contemporary business management practices making the production process increasingly efficient, agile, autonomous and customizable through technological innovation. It will be necessary to rethink the old and already obsolete business models, developing research and technologies for industrial production.

However, the same mistake made centuries ago cannot repeat, in which the development of industry has bring environmental degradation and scarcity of natural resources by the unbridled and unthinking use in the production process.

The planet still suffers from the inconsequential acts of the past and, today, the concern is to degrade as little as possible, maintaining the fauna and flora and stabilizing the hole in the ozone layer, responsible for climate change resulting from the environmental imbalance resulting from pollution and destruction of the environment by the mankind.

Under this perspective, this work presents important definitions, including the performance of Biotechnology for environmental protection, applying the results obtained by research and development of innovative technologies capable of promoting sustainable development, ensuring the environment to future generations, as a fundamental right of citizens.

As for the materials, the research will include studies of literary works, scientific articles, legislation and other means of registration and dissemination of data that proved to be necessary for the pursuit of the proposed objectives.

## Objectives

It is intended to demonstrate the importance of maintaining the environmental balance through sustainable practices inserted in the daily life of Industry 4.0, a social responsibility imposed on companies to contain degradation in the production process and marketing of goods and services, reaffirming, or better, proving the relevance of Biotechnology in the fight for environmental preservation.

## Methodology

This research was conducted using the following methods: dialectical, to understand social reality, approaching it through argumentative and conflictive dialogue, and historical, analyzing the data from a historical perspective. This is a literature review research, in which several literary works, scientific articles and the legislation applicable to the case were studied to identify the social responsibility of Industry 4.0 and the application of Biotechnology to achieve sustainable development.

## Discussion

### Preliminary notions about biotechnology

The United Nations defines Biotechnology as "any technological application that uses biological systems, living organisms, or derived beings, to manufacture or modify products or processes for specific use".[1] Maria Antonia Malajovich defines Biotechnology as "an activity based on multidisciplinary knowledge, which uses biological agents to make useful products or solve problems". The author states that this definition encompasses, in a very comprehensive way, various activities such as engineering, chemistry, agronomy, veterinary, microbiology, biology, medicine, law, economics, among others[2]. For Emilio Muñoz, Biotechnology is understood as a set of techniques that allow the application of the properties of living beings to produce goods and services. These techniques are very old, being used for the fermentation of food through microorganisms, originating other products and/or helping in its conservation.[3]

Biotechnology involves numerous scientific areas such as biolo-

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gy, chemistry, biochemistry, microbiology, informatics and robotics. Its methods range from simple procedures for modifying living organisms, such as domestication of animals and cultivation of plants, to complex processes, such as biolixiviation, contemporary methods involving genetic manipulation, cell and tissue culture, and nanotechnology.[2]

The Biotechnology sector covers countless business segments, but the activities required for the development process of biotechnological products require high investment for research, testing, performance evaluation, among other crucial stages until the final development of the product, which becomes an obstacle and restricts its use by micro and small companies. In order to obtain competitive advantages, it is essential for these companies to have professionals in the Biotechnology area, formalize partnerships with research and technology centers, in addition to financial resources with the contribution of investors – which is not easy for the small entrepreneur.[2]

In 2007, the Biominas Foundation conducted a survey with Brazilian Biotechnology companies nationwide. The survey identified 181 companies in the bioscience sector, 71 forming the Biotechnology sector. In 2009, in a new research, Biominas verified a growth of 40%, in which there were 253 bioscience and 91 biotechnology companies, distributed in seven branches: human and animal health, agriculture, environment, bioenergy, mixed and inputs. The research revealed that many of these companies, with Biotechnology projects, have other main activities, therefore they would not be considered biotech companies. Bioscience companies, on the other hand, are related to the areas of human and animal health, agriculture and the environment, reaching the consensus that every bioscience company is a biotechnology company, however, not every bioscience company is a biotechnology company.[4]

Precisely, Biotechnology multidisciplinary character makes it a science composed of several areas, which were classified in colors according to their fields of application: yellow, blue, white, gray, gold, orange, brown, green, red, black and purple.[5]

Table 2 shows some possibilities for biotechnology-based products and services in various sectors of the economy.

It is important to mention some advantages and also disadvantages of Biotechnology. In order to facilitate understanding, the information was set out in Table 3.

| BIOTECHNOLOGY |
|---|
| **Yellow:** intended for food production and nutritional control. |
| **Blue:** applied in the exploitation of marine biological resources; marine biotechnology. |
| **White (also called industrial):** dedicated to the production of energy; used in the manufacture of products by means of methods less harmful to the environment. |
| **Grey:** studies and optimizes recycling processes and other related processes. |
| **Golden:** used in bioinformatics and computational techniques. |
| **Orange (or educational biotechnology):** is dedicated to the information, dissemination and dissemination of biotechnology and its applications. |
| **Brown:** dedicated to obtaining components and making use of desert resources. |
| **Green:** it is related to plants and their products; it is applied in agriculture in order to develop more sustainable conditions. |
| **Red:** applied to both human and animal health; used in medicine to improve treatments and medicines, including genetic manipulation. |
| **Black:** reflects the set of actions aimed at biodefense to prevent the use of pathogens for bioterrorism; it is also used for warfare purposes (e.g., production of biological weapons). |
| **Purple:** identifies the legal aspects of biotechnology and its application. |

**Table 1 –** Representative colors of the areas of Biotechnology. Source: Dasilva, 2004[5]

| SECTORS | PRODUCTS OU SERVICES |
|---|---|
| **Agriculture** | Fertilizer, silage, biopesticides, biofertilizers, seedlings of disease–free plants, seedlings of trees for reforestation. Plants with new incorporated characteristics (transgenic): higher nutritional value, resistance to pests and adverse cultivation conditions (dryness and salinity). |
| **Food** | Bakery (bread and biscuits), dairy products (cheeses, yoghurts and other dairy beverages), beverages (beers, wines and distilled beverages) and various additives (shoyu, sodium monoglutamate and sweeteners); single cell protein (PUC) for rations, foods of transgenic origin with new properties. |
| **Energy** | Ethanol, biogas and other fuels (from biomass[6]). |
| **Industry** | Butanol, acetone, glycerol, acids and vitamins (sugar–alcohol production). Numerous enzymes for other industries (textile and detergents). |
| **Environment** | Oil recovery, bioremediation[7] (e.g. of water and waste), bioconversion[8] of waste from agriculture, production of biofuels and biodegradable plastic. |
| **Livestock** | Embryos, animals with new characteristics (transgenic), vaccines and medicines for veterinary use. |
| **Health (Medicine/Farmacy/Biomedicine)** | Antibiotics and drugs for various diseases, hormones, vaccines, reagents and diagnostic tests, new treatments, production of laboratory antibodies for immunodeficient patients, gene therapy, stem cell research, manipulation of animals for organ use in transplants, printing of 3D prostheses and bioprinting of organs in 3D. |

**Table 2 –** Examples of products and services of biotechnological origin by sectors of the economy. **Source**: Adapted from Malajovich.[2]

| BIOTECHNOLOGY | |
|---|---|
| **ADVANTAGES** | **DISADVANTAGES** |
| Improved harvesting (increased production and increased resistance to pests); reduced world hunger. | Dependence on technologies produced by developed countries; increase in the concentration of income and wealth. |
| Durability of transgenic foods – less waste. | Increase in the occurrence of diseases caused by transgenic products. |
| Production of more nutritious or biofortified foods (content of proteins, fibers, oils, carbohydrates, vitamins and mineral salts) – OGM/transgenic. | Biodiversity loss; impact on nontarget organisms of the technology, consequently impacts on biological diversity. |
| Decrease in the use of pesticides. | Intensive use of inorganic fertilizers and pesticides. |
| Use of bioremediation to control and decontaminate environments. | Creation of infertile GM seeds. |
| Reduction of energy in the production process. | Interference with the balance of nature – effects on the food chain or food chain (network). |
| Use of products with lower environmental impact – less pollution and degradation; production of biodegradable products. | Uncertainties regarding the biosafety of the products generated – potential risks and their probability of occurrence, in environmental aspects and effects on human and animal health, especially those of a long–term nature. |
| Reduction of contagious diseases. | Genetic pollution – it is not possible to control the effects of the spread of genetically modified organisms (GMOs) on the environment. |
| Development of more precise therapeutic devices and techniques for the prevention, diagnosis and treatment of diseases, including incurable diseases with ineffective treatments such as cancer. | Ethical issues and permissibility to clone living beings. |

**Table 3 –** Advantages and disadvantages of Biotechnology. **Source**: Adapted from Malajovich.[2]

Biotechnology still arouses countless discussions about innovations. Technologies are the result of scientific knowledge, but considered by some people as an unnatural and dangerous activity. When discussing whether biotechnology is progressive or reactionary, good or bad, it is forgotten that what characterizes a technology is its use.[2]

**Overview on Industry 4.0**

The name Industry 4.0 began to be used at the Hannover Fair (2011 edition), from a German government's initiative to promote partnerships with technology companies, universities and research centers with a view to changing the paradigm of the production process of industries[9]. This concept encompasses technological innovations in both automation and control, management and information technology in the production and logistics chain of companies.

The 4.0 industry will demand a gradual adoption of several emerging technologies for industrial automation and information technology, with information digitalization and direct communication between systems, machines, products and people. This integration is now known as the Internet of Things (IoT). This process will generate manufacturing environments with high flexibility and self–adjusting to the growing demand for increasingly customized products.[9]

Industry 4.0 is the fruit of the Fourth Industrial Revolution, which is based on a digital revolution defined by more extensive mobile Internet access, by decreasing the size and price of sensors in return for increased capacity and power, and by artificial intelligence and automatic learning that invaded all sciences, promoting frightening advances and unimaginable times such as genetic sequencing, nanotechnology, renewable energies, 3D printers, among others[10].

According to Klaus Schwab, the Fourth Industrial Revolution will monumentally impact the global economy, because it is expected that every major macrovariable, such as gross domestic product (GDP), consumption and employment, will be affected.[10]

Companies that do not adjust to this new reality will certainly be doomed to failure and will not survive the savage capitalism promoted by globalization.

But how to achieve development and stay in the market fulfilling the social responsibility in the preservation of the environment?

The adoption of good practices in the productive process when the subject is environment is not enough, the need of business models with strategies of adaptation to the scarcity of natural resources is urgent. They need to think in short, medium and long terms, because all business activity provokes environmental impacts, some with greater intensity and others in lesser degree.

Natural resources are finite assets, environmental degradation and overexploitation of these resources cause damage to society as a whole, often irreparable and irreversible. Therefore, the model of production and consumption must be compatible with the material basis of the economy.[11]

According to Sergio Risola, executive director of Centre for Innovation, Entrepreneurship and Technology (Cietec), in the last decade, companies began to see the relationship with the environment as a strategy to adapt to the scarcity of natural resources. This approach has increased consumer and industrial demand for innovation in the so–called green economy.[12]

**Corporate social responsibility in the adoption of sustainable practices: a constitutional determination**

As seen, the technological advances that mark Industry 4.0 have brought undeniable benefits, but also, many times in greater intensity, degradation and devastation of the environment, materialized by the pollution of seas, rivers and air, promoting scarcity of natural resources, greenhouse effect, acid rain, evidenced by the climate changes so discussed by nongovernmental organizations and environmental defense entities.[13]

It is a serious mistake to disassociate environment, productive system and consumption, since these aspects are umbilically linked, there is no way to produce without generating environmental impacts, in the same way that there is no way to consume without this impact being accentuated, either by the production of waste, or by the exploitation of human beings in work environments, or by the social inequality promoted by the capitalist and globalized system.

That is why there is no way to deal with sustainability in isolation, that is, the economic, social and environmental dimensions are interconnected.

Companies are responsible for complying with legal requirements for environmental protection and also for observing their social and solidarity function, which are important constitutions.

The property right guaranteed by the Brazilian Federal Constitution (FC) in Article 5, item XXII, is not absolute, i.e., it is limited by item XXIII of the same article: "property shall serve its social function". The FC mentions what is a social function, separating it in the social function of urban property from the social function of rural property. In order for urban property to achieve its social function, it will be necessary to meet the requirements of the city ordinance set forth in its master plan (Art. 182, §2). And the social function of rural property will be fulfilled when the following requirements are met: 1. rational and adequate use; 2. adequate use of available natural resources and preservation of the environment; 3. observing the regulations on labor relations; 4. exploitation favorable to the well–being of owners and workers (Art. 186)[14].

The company, as an asset resulting from the unfolding of the property right, an individual right protected by the FC, must also achieve a social function, a harmonizing or even moderating instrument of the exercise of the right to exploit the economic activity legitimized by the principle of free initiative.

As André Ramos Tavares teaches, the Constitution of the Republic attributes a double dimension to property: an individual right and a socioeconomic right, due to their delimitation by the social function, that is "to serve the purposes of the community and not only the individualistic purposes" (p. 567).[15] In this sense, not only the productive character must be performed by the property, but also the observance of national legislation, including employment contracts, constitutional principles and precepts, under penalty of being held responsible and subject to penalties. The consequences of noncompliance with the social function of the property are: The Government may demand adequacy, granting a deadline for this and/or imposing fines in pecuniary value and even expropriate the property (Articles 182, §4 and 184, CF).[14]

Companies have social and sustainable responsibility, that is, not only with society for the impacts that pollution may generate on human beings, but mainly with respect to environmental degradation, both in the production process so as not to cause exhaustion of natural resources, and in the destination of waste and garbage that their products and services generate.

Therefore, the need for a macroanalysis of sustainability contemplating some of its relevant influencing aspects in an attempt to point out mechanisms capable of controlling and even reducing environmental degradation from the development of biotechnological products and services was verified.

**Biotechnology as a springboard for sustainable development**
**Sustainable development**

Industry 4.0 unites technology, things and human beings, promoting the automation of industrial (and/or business) processes in a broad way through the internet, artificial intelligence, robotics, among other mechanisms and technologies, which make possible to increase production (with great speed), reduce costs, errors and risks, save energy, increase the value of products, providing greater profitability to the entrepreneur and also greater satisfaction, and convenience to the customer. However, this socioeconomic development should be associated with environmental development. But what is meant by sustainability?

The concept of sustainability was born at the United Nations Conference on the Human Environment (Unche, June 1972), in Stockholm

(Sweden). In 1987, the UN World Commission on Environment and Development (UNCED) produced the Brundtland report (named "Our Common Future"), which practically reaffirmed the definition of sustainable development of the 1972 Conference: "Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs". In 1992, the United Nations Conference on Environment and Development (UNCED), held in Rio de Janeiro, also known as Rio–92, Eco–92 or Earth Summit, demonstrated the world's interest in the future of the Earth; the participating countries reached consensus on the concept of sustainable development and began to shape actions with the aim of protecting the environment.[16]

Thus, sustainability is formed by a set of ideas, strategies and other attitudes ecologically correct, economically viable, socially fair and culturally diverse; it is the path to ensure the sustenance of the planet's natural resources, while at the same time allowing solution for ecological development of individuals and societies.[17] In Rio–92, it was concluded that the economic, environmental and social components must be aggregated, without which there is no way to achieve, or rather, ensure the sustainability of development[16].

It was evident that the definition of sustainability presupposes three pillars (economic, social and environmental), where economic sustainability refers to finance and involves financial resources, planning, organization and management of production, distribution and consumption of goods and services; aspect inherent to the country's economy, with variables in interest rates, credit lines for financing, GDP numbers, competitiveness in the international market, among other aspects relevant to the growth and development of the nation.[11]

Social sustainability, on the other hand, reveals aspects related to the human being and the community where it operates: labor force (labor force to companies), social inequality, education and violence, for example. A sustainable society presupposes that citizens have the minimum for a dignified life[11]. In short, social sustainability presupposes the eradication of poverty by promoting conditions of human equality and dignity for access to goods within an acceptable minimum limit.

Souza and Oliveira draw attention to the delicate and worrying issue of natural resources being finite assets and excessive exploitation causing extremely harmful results to society, most of the time these results are irreparable and irreversible. For this reason, the model of production and consumption should be compatible with the material basis of the economy. Every business activity entails impacts on the environment, with no exception, some more and some less.[11]

Environmental sustainability deals with the environment, natural resources, fauna and flora and their use; relevant factors that require care in the short, medium and long term. According to Elimar Pinheiro do Nascimento, sustainability in a simple way would be to produce and consume in a way that ensures that ecosystems can maintain their self–repair or resilience capacity.[18]

Companies should adopt sustainable practices and be concerned about the effect that their activities cause to the environment and adopt actions to reduce these harmful impacts, because, as already stated, all activity promotes negative environmental impacts, but at this time Biotechnology joins as a strong ally to sustainable development.

**Biotechnology and sustainable development: technology for environmental protection**

The pioneering in the capture, processing, understanding and use of large amounts of data puts Biotechnology in a privileged position, no doubt the convergence of innovations will be intensified with the use of the power of digitalization and the practices of Industry 4.0 in the industrial biotechnology sector[19]. An example of Industry 4.0 is digital fermentation, which has been tested and applied in the textile sector.

In this sense, Biotechnology will provide essential innovations to protect the environment, such as the sustainable consumption of diesel, the reduction of the impact of waste in agroindustry, the alteration of energy sources by other more sustainable in the pharmaceutical industry, food and cosmetics, are some examples. What would be the impact of human action on the environment? What legacy will the current generation leave behind? As can be seen, sustainable development presupposes actions in the medium and long term in the economic, social and environmental areas.

Malajovich, citing reports published in 2007 by the Intergovernmental Panel on Climate Change (IPCC), demonstrates humanity's responsibility for the future of the planet, where it has been proven, more than a decade ago, that actions to protect the environment are urgent.[2]

Biotechnology startups work on the decontamination of polluted water, developing products and techniques without the need for river and lagoon drainage. Biopolymers and paraffin plates embedded with bacteria–activated nano–minerals have been shown to be very efficient and cheaper).[20]

Modern biotechnology marked the beginning of a new phase of agriculture, highlighting molecular genetics. Advances in plant genetics reduced the excessive dependence of agriculture on mechanical and chemical innovations, which were the pillars of the green revolution". It is worth noting the increase in productivity, the reduction of production costs, the production of better–quality food and the development of less harmful practices to the environment.[21]

The use of environmental biotechnologies are ways to minimize and even try to reverse the action of human degradation and the consequent production of waste, residues and pollutants by the exercise of the most diverse economic activities from the development of controlled natural processes aiming to reverse the pollution of ecosystems and create biodegradable solutions. As example, the reuse of sugarcane bagasse (agricultural waste) and sewage (solid effluents) for the production of biofuels and energy.

It is possible to buy products whose raw material was obtained from the use of biological materials (bacteria, yeasts, fungi, among others), such as fabrics manufactured by cultivating living organisms that produce a type of (bacterial) cellulose.[22]

Scientists reproduced synthetic spider silk in the laboratory. The material is flexible, lightweight, biodegradable and more resistant than steel. It can be used by the automotive, pharmaceutical and safety industries. Silk threads woven by spiders are 30 times thinner than human hair and stronger than Kevlar, a synthetic fiber used in the manufacture of bulletproof vests.[23]

Biotechnology can also be applied in mining. Widely used for mining in Australia, South Africa and Canada, bioleaching is a technology that uses biotechnological routes for the recovery of metals present in oxidized and sulphide ores or for the pretreatment of ores.[24] Biolixiviation is used in more than 20% of the world's copper mines. It is estimated that 30% of the total volume of copper mined in Chile goes through this biotechnology process, while in China, Eldorado Gold recovers from 93 to 94% of gold from the Guizhou plant.[25]

Sustainable development presupposes production without degradation of the environment, a culture that should cover all levels of the organization. Companies need to adopt strategic plans or production projects that preserve the environment and its surroundings, and the use of technologies is an important instrument in fulfilling this responsibility. Many companies have already adopted sustainable development projects with the implementation of techniques such as: use and consumption of alternative energy sources such as solar, wind and geothermal; recycling of reusable materials; rational consumption of water and food and reduction of the use of harmful chemicals in food production.

Another example is the company Adidas, applying the Industry 4.0 model associated with sustainability, the company inaugurated in 2018 the first fully automated and 100% robotized factory in the United States, a speed factory (fast plant). The company has only 150 employees who direct the machines in production; additive manufacturing (3D printing) is used. Adidas states that this speed factory can make shoes and put them on the market three times faster than traditional production models.[26]

Asia's production moved to the USA, where most of the sneakers and sports products commercialized are concentrated, optimizing time and money, without having to travel between oceans, in addition, the production unit closest to the distribution centers brought savings with logistic resources (mainly in distribution and stock). This is a company strategy, but in addition to the positive results obtained by the enterprise, it is also possible to verify other benefits, included in the concept of sustainability, since the action, in addition to minimizing environmental impacts in relation to pollution, by reducing labor costs by transferring activities from Asia, which does not provide protection to workers (allows child labor and in exhaustive conditions, for example), has reduced the incidence of child labor and in conditions similar to slavery. The German company intends to produce a total of 1 million shoes per year by 2020 in its two speed factories. In addition, they develop technologies that allow consumers to create their own custom footwear.[26]

As can be seen, it is possible to unite innovation, technology, profitability and social responsibility, i.e., development must be sustainable at the economic, social and environmental interfaces.

Table 4 shows a few biotechnological alternatives to replace industrial processes and also to replace agricultural inputs demonstrating the role of Biotechnology in promoting sustainable development, providing that companies contribute significantly to reducing harmful impacts on the environment, fulfilling its social responsibility[2,27–30]:

| Alternatives | Main information | Application | Practical examples and results obtained |
|---|---|---|---|
| Enzyme Technology | Enzymes are non–toxic and biodegradable | Food, feed, detergent, textile, pulp and paper and leather industries. | Tanneries: 40% reduction in sulphur derivatives; improvement in leather quality; reduction in energy consumption. Bioplastic packaging based on polymers of bacterial or vegetable origin: Compostable in a few months (rapid degradation). |
| Biolixiviation | Use of bacteria, from Biotechnology and green chemistry, to extract metals. The bacteria feed on the sulfur present in the ore and produce sulfuric acid, responsible for separating the materials in the solution. | Mining industry for copper, silver and gold extraction. | During this process, the pile of ore, usually packed in large tanks (chemical reactors), coated with steel and with a special blanket to prevent leaks and soil contamination, receives continuous irrigation (solution containing the bacteria) that solubilizes the metals. |
| Biofertilizers | They contain living biological agents that promote plant development. | Agricultural sector: agroindustry/agrobusiness. | Rhizobium: Symbiotic bacteria found in the roots of legumes that fix atmospheric nitrogen; the industrial production of Rhizobia replaces chemical products derived from petroleum. |
| Biological agents for pest control | Use of entomopathogenic bacteria, fungi and viruses | Agricultural sector: agroindustry/agrobusiness. | *Metarhizium anisopliae* fungus: combat the sugarcane leafhopper (*Mahanarva posticata*). Bacteria *Bacillus thuringiensis* var israelensis: larvae of the *Aedes aegypti* mosquito and of the rubber bands (*Simulium* spp). |
| Biodegradation / aerobic biodigestion | Degradation of waste by the action of organic substances. | Transformation of organic waste into a compost for fertilizer, reforestation, soil filling and combating erosion. | The natural decomposition of waste in sanitary landfills produces biogas, released into the atmosphere contributes to the greenhouse effect and climate change. |
| Power generation: biofuels | Fuels of natural or biological origin – renewable energy source obtained by burning biomass or derivatives. | In many sectors of the economy; they can replace (totally or partially) fuels derived from oil and natural gas. | Genetic improvements (more resistant and more productive plants) and developing new planting systems to increase efficiency. Examples of biofuels are: ethanol, biodiesel, biogas and vegetable oil. Cellulosic ethanol is produced from sugarcane biomass (straw and bagasse). |
| Production of biopolymers (or bioplastics) | They are biodegradable, produced from renewable resources. | In countless sectors of the economy, such as for the manufacture of packaging and disposable materials; preparation of micro and nanocapsules (with the purpose of controlled release of drugs). | Lactic polyacid bioplastic (PLA) – by fermenting starchy vegetables, bacteria produce lactic acid. The products (PLA) are used in packaging (food, cosmetics and plastic bags), plates, pens, glasses, 3D printing filaments, among others. The estimated period for the degradation of PLA goes from six months to two years, being easily degraded by the action of water. |

**Table 4 –** Biotechnology alternatives: replacement of industrial processes and agricultural inputs.

Another point about the performance of Biotechnology in cultivars is transgenics. The cultivation of transgenics has been carried out by many countries for years. In 2001, genetically modified crops were planted on more than 52 million hectares of land around the world. This is approximately three times the total land cultivated in Germany. The cultivation of new varieties is undoubtedly not restricted to emerging and developing countries such as Argentina and China. Positive results have also been found in South Africa and Indonesia.[31]

The German Ministry of Consumer Protection, Food and Agriculture has estimated that 60 to 70% of all food in the country is affected by green Biotechnology. The European community supplies only 35% of its protein–rich food needs, for example, and must import the remaining 65% from the USA, Brazil, Argentina and other countries outside the European community. Large–scale international experiments with the cultivation of GM plants confirm that agricultural biotechnology is an innovative, effective method with several benefits such as abundant harvests, more resistant to external agents, promoting positive impacts and improvements on human quality of life and economic profitability to agribusiness.[31]

The genetically modified organism (GMO) is a living organism, which can colonize new environments. The new characteristics and activities acquired by the plant from the transgenesis may include quantitative or qualitative alteration in the production of molecules (RNA, proteins and metabolism), and may still remain active even after the destruction of the GMO.[32]

In this sense, some direct environmental impacts of GMO are:

1. Interaction with living organisms with a possible selection of organisms resistant to transgenicity and other consequences originating from the exogenous compound in the soil;
2. Change in persistence in the agricultural environment or invasiveness of the crop in natural environments;
3. Gene flow by pollinators in weeds and invasive plants.

On the other hand, the indirect impacts of changes in the environment may be:

1. Reduction in pest, disease or invasive plant control due to the development of tolerance to herbicides;
2. Effect on wild biodiversity – mass planting of monocultures that cause the reduction of genetic biodiversity and rupture of ecological niches, alteration in trophic chains due to impact on non–target organisms, with shortening or substitution of trophic chains or emergence of secondary pests;
3. Effect on water and soil – alteration in the use of herbicides and in patterns of cultivation and land use.[32]

The concern with safe products obtained by the performance of Biotechnology is a constant. The biosafety assessment of potential risks and their probabilities of occurrence should be judicious, with the widest possible coverage and rigorously executed in several aspects involved in human health, environment (ecosystems, fauna and flora) and, mainly, taking into account the unforeseen and potential changes in the long term – which has been unknown to researchers and scientists, because it is difficult to predict all risks, all changes (intentional or not; beneficial or not) that a GMO can trigger in the future. This seems to be an arduous and very difficult task.

## Conclusions

The concept of Biotechnology is still incipient, however, the definition provided by the UN has prevailed. Biotechnology has a multidisciplinary character, involving several disciplines and research in the areas of biology, chemistry, biochemistry, pharmacy, medicine, microbiology, information technology, robotics, law, among others, whose methods include procedures for modifying living organisms, from the simplest to the most complex such as genetic manipulation and nanotechnology.

The concept of sustainability was born at the United Nations Conference on the Human Environment in 1972, and its definition was reaffirmed with minor changes in other meetings and events held by the UN.

The exercise of the property right guaranteed by FC/88 (Art. 5, item XXII) is limited by the principle of social function, by which the owner must meet the legal requirements regarding the construction, rational and adequate use; respect for the determinations for the protection of the environment and the exploitation of labor (labor relations); the exercise of the right to use, enjoyment and disposal of the property is limited to collective interests, and must promote the well–being not only to its owner, but to society as well. The company, as an asset that generates property rights, must achieve this social function, under penalty of suffering penalties, from a simple notification for adequacy until the expropriation of the asset (Arts. 182, §4 and 184, CF).

Industry 4.0 is the fruit of the Fourth Industrial Revolution, whose basis is the digital revolution (internet, robotics, artificial intelligence, data storage and automatic learning). It is promoting surprising technological advances, such as genetic sequencing, nanotechnology, renewable energies and 3D printers. It will take a joint work between governments, companies and societies to face the consequences of the Fourth Industrial Revolution that breaks down all paradigms and barriers of distance and, until then, the "impossible". The industry needs to be reinvented and new professionals will be needed, with education to manipulate, execute, manage and create technologies.

Biotechnology can help in the sustainable development of the country, enabling more competitive, dynamic and technological companies, to stay "alive" in the market, in addition to constituting a relevant instrument at the disposal of Industry 4.0 in fulfilling its social responsibility.

Biotechnological studies and breeding provide many advantages and contribute to economic, social and environmental development, but also have drawbacks, which deserve further analysis and caution in application/use, such as GMOs – transgenics can alter the natural balance and compromise biodiversity. There are several uncertainties about the biosafety of these products, what the potential risks would be and their likelihood of occurrence to human and animal health, especially in the long term.

Biotechnology is an essential tool for minimizing the impacts on the environment, its achievements in research, development and technological innovation in the most diverse areas of its operation will be the springboard for sustainable development of the Fourth Industrial Revolution.

The defense of the environment is a duty of everybody, each of us with his own responsibility, and we should fight to guarantee this fundamental right, provided in the Federal Constitution, to present and future generations.

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# Biotechnology and Industry 4.0: The professionals of the future

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**Abstract: The process of developing technological research is being carried out beyond organizational boundaries, especially organizations that make intensive use of knowledge, such as Biotechnology. Considerable progress has been made in recent years in reducing costs and increasing the ease of gene sequencing and, ultimately, in activating or editing genes. In this context, discussions have been gaining prominence around Industry 4.0, in which new business models and intelligent processes for Biotechnology are evidenced. However, the challenges regarding the training and education of professionals are discussed, emphasizing the importance of a multidisciplinary education, unlike the more traditional nature of education in the areas of biology and sciences. These challenges can be partially transposed by strengthening the partnerships of universities and research centers with companies, in order to materialize in common projects, the demands of industries and the possibilities of transforming research projects into final products available to society. This paper presents a study on the impact of Industry 4.0 technologies on Biotechnology, and also presents the prospect of future professions with the influence of Industry 4.0. The results show that technologies such as artificial intelligence, robotics and 3D printing are promising in the development and advancement of Biotechnology. New automated laboratories are under development. Also, regulatory issues require a great deal of study, and business models will need to be more efficient to generate the results needed for the development of new drugs, food, and research of new products related to health.**

**Keywords: Biotechnology; Industry 4.0; Industry 4.0.**

## Introduction

The challenges faced by current organizations have fostered the formulation of alliances and partnerships among organizations. In this context, the technological research development process is being carried out beyond the organizational boundaries, especially organizations that make intensive use of knowledge, such as Biotechnology companies.[1] Also, in this context, discussions have been gaining prominence around the idea of the Fourth Industrial Revolution or Industry 4.0, in which new business models are verified, as well as accentuated remodeling in existing businesses around intelligent products, procedures and processes.[2]

Great changes and innovations arise in the field of Biotechnology. Considerable progress has been made in recent years in reducing costs and increasing the ease of gene sequencing and, ultimately, in activating or editing genes.[2] Advances in processing power meant that scientists no longer need to work with trial and error, making Biotechnology one of the areas that will be most impacted by advances in technology. According to the Brazilian National Confederation of Industry, Biotechnology is a disruptive technology and is intrinsically related to Industry 4.0. The introduction of new sensors, equipment and artificial intelligence applied to research ensure progress in Biotechnology; automation, big data, advanced process analysis and control and the internet of things (IoT) impact the way of work and communication in the industrial chain.[3]

The definition of Biotechnology is very broad. Trigueiro[4] states that modern Biotechnology emerges as a complex web of technical, social, economic, political, ethical and institutional relations, demanding an effort for its development. Modern Biotechnology presents itself as an area of knowledge, encompassing different natural sciences, which transform these sciences into an object of technology.

Biotechnology has two dimensions: scientific and technological. The scientific one consists of an articulated set of basic research programs (molecular biology, biochemistry, microbiology, genetics), being developed, fundamentally, in universities and academic institutions. The technological dimension comes as the study of the means of transforming these basic researches into industrial and commercial applications. These two dimensions coexist and complement each other.[4]

In practice, Biotechnology is a multidisciplinary science that integrates several areas of knowledge,[4] such as law and engineering, mainly chemistry, irreplaceable in the study of bioprocesses, in the pharmaceutical, food and oil industries.[5] Therefore, the investment in human capital focused on the area of Biotechnology is usually considered the main determinant for the development of the field.[6]

However, it is still necessary to focus on and support the increase of intellectual assets involved in the front line of biotechnological research, since these are fundamental for the exploitation of the potential of manufacturing and marketing of products of biotechnological origin.

Batalha et al. discussed the challenges of capacity-building and education of professionals for modern Biotechnology in Brazil, emphasizing the importance of a multidisciplinary education, unlike the more traditional approach of education in the areas of biology and health and agricultural sciences.[7]

These challenges can be partially transposed with the deepening of the partnerships of universities and research centers with companies, in order to materialize in common projects, the demands of industries and the possibilities of transforming research projects into final products available to society.[6]

The main objective of this work is to present the main impacts of Industry 4.0 in Biotechnology, the professions of the future involved in this area, and to shed light on the market perspectives and training for future professionals. Thus, it is a literature review research, using the methods of historical, analytical and deductive research, systematizing the obtained data, organizing them, to make possible, through these results, to draw specific conclusions on the proposed theme. Books, internet sites, and scientific articles located in databases such as: SciELO, Scopus, Emerald, Science Direct and Web of Science, with keywords (in Portuguese and English): Biotechnology, Industry 4.0 and future professions.

For the development of this work, first the industrial revolutions that

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have transformed the labor market will be explored, after the evolution of Biotechnology and labor market. The article also discusses the impact of Industry 4.0 technologies on Biotechnology, and describes the professions of the future impacted by these technologies.

**Evolution of industrial revolutions**

The word "revolution" refers to the idea of radical and abrupt changes. In industry as a whole, revolutions occur when new technologies encourage profound change in the economic and social aspects of societies.[2] Figure 1 shows the periods of the industrial revolution and the main technologies.

The First Industrial Revolution began with the invention of the steam engine, which made mass production possible in the 17th and 18th centuries. For example, in the textile industry, steam engines have made craftsmen's labor undervalued because the machines had greater production capacity.[9]

From 1811, the "Luddites" broke loom machines throughout England because they believed that this new equipment would cause mass unemployment. There was indeed a loss of employment; but the necessity of hiring unskilled labor was present, since the machines needed to be operated by the workforce. These unskilled workers who worked for a period of time became skilled and reached a relatively higher income level.[9]

Countries such as the United States, Japan and England, among others, have shown that industrialization has raised their citizens to a higher standard of living.[10]

Over the years, technology has evolved requiring the substantial presence of a more qualified workforce, generating wealth for countries and organizations and making countries understand the factors that change the economy and the future of work.[11]

From the 1870s onwards, the Second Industrial Revolution occurred after a series of events, such as the use of electricity, the surge of mass production and the method of division of labor. The third revolution emerged in the early 1970s as the Digital Revolution, and was driven by the use of the first information technologies that further developed the automation of the means of production.[2,12,13]

The Fourth Industrial Revolution began with the term Industry 4.0, which is derived from ***industrie 4.0***. It was created in Germany in 2011 by the Institute ***Fraunhofer–Gesellschaft*** and the German Federal Government as a collective term that defines the set of technologies for information flow, automation and manufacturing, as a high–tech strategy for 2020.[14] Politicians, universities and entrepreneurs have developed ideas to offer improvements in industrial processes involving: operation, engineering, production planning and control, logistics, and continuous analysis during the life cycle of products and.[15]

Since then, the academic, scientific, business and political interest in the subject has expanded rapidly, largely due to the fact that for the first time an industrial revolution is being observed before it concretely becomes a reality.[12]

According to Kagermann, Wahkster and Helbig, Industry 4.0 aims to optimize industrial processes that involve manufacturing, engineering, supply chain management and the life cycle of products. Three main concepts should be considered in the implementation of Industry 4.0,

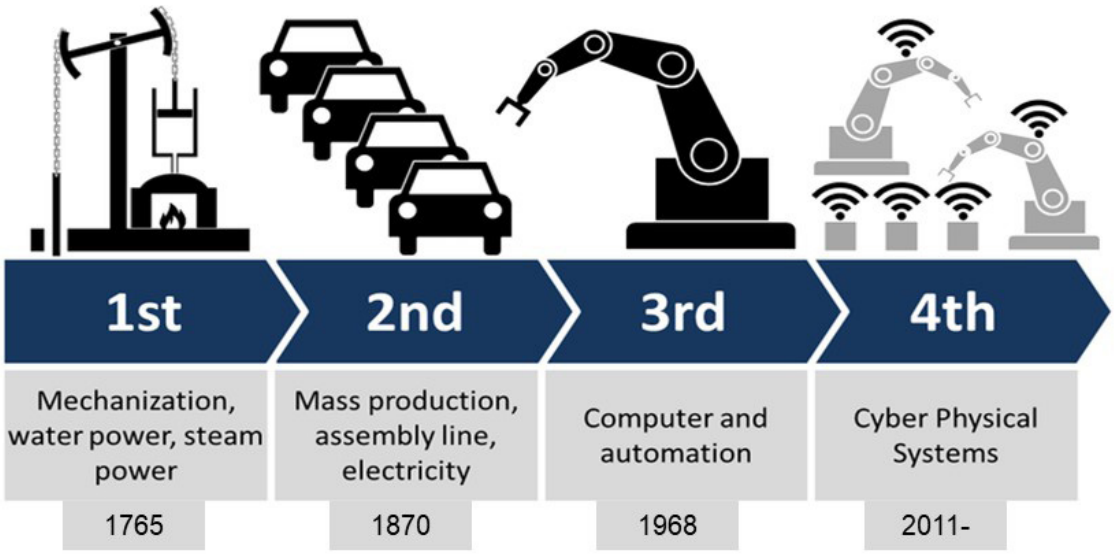


**Figure 1 –** Periods of industrial revolutions.**Source:** By Christoph Roser at AllAboutLean.com under the free CC–BY–SA 4.0 license.[8]

which are: horizontal and vertical integration, and end–to–end engineering. The authors also describe these concepts:[13]

- Vertical integration: corresponds to the implementation of a manufacturing system with characteristics of flexibility and reconfigurability through the complete integration of hierarchical systems of the internal environment of the company;
- Horizontal integration: create collaborations between companies to provide an integrated ecosystem where physical, intellectual, energy and financial resources can flow between many different companies;
- End–to–end engineering: encompasses the first two and aims to create a powerful chain of software tools, which enables mass customization through product modeling at each stage in the value chain.

By embracing new technologies such as developments in advanced robotics, artificial intelligence, nanotechnology, 3D printing and Biotechnology, this environment will be marked by a widespread disruption not only in business models, but also in labor markets in the coming years, with huge changes anticipated in the skill sets needed to prosper.[16] From then on, the main discussion that arises concerns the ways in which business, government and individuals will respond to these developments.

The future lies in the Fourth Industrial Revolution that depends on the integration of physical, digital and biological structures, sustained by the internet and the industrial value chain.[12,17]

**Understanding industry 4.0**

Industry 4.0 is the union of technologies applied to the production

environment. Among them are internet of things (IoT), cyber–physical systems (CPS), 3D printing, big data, autonomous vehicles, automated systems, artificial intelligence and new materials.[2]

The fourth industrial generation presents as main characteristics: data interconnection, integration and innovation.[18] Among the set of technologies are:

- *Cloud computing*: The delivery of faster and more flexible innovation services for data storage, including servers, software, database, analytics and intelligence over the internet ("the cloud");[18]
- *Virtualization or digital twins* (asset virtualization): Provides simulation of the process environment according to business requirements;[19]
- *Internet of things* (IoT): Makes possible to connect objects, transfer data without human intervention and interconnection between equipment through the internet in convergence with wireless technology;[20]
- *Big data analytics*: Due to the high volume, variety and speed of data generated by sensors and control systems, there is a need to collect, integrate, store, process and analyze data for Industry 4.0;[21]
- *Cyber–physical systems* (CPS): Complex systems that require specifications for the chronological control of processes, security modeling, rationalization of continuous events for mapping and sequencing in discrete events;[22]
- *Internet of services* (IoS): Characterized by a large number of services supported by software distributed over the internet, requiring new standards and integration architectures, as well as flexible and dynamic mechanisms of data security;[23]
- *Autonomous robots*: Robots with autonomous capacity used in production lines to perform complex activities;[18]
- *Vertical and horizontal integration*: Key concepts for the implementation of the Industry 4.0.[24] It integrates internal and intercompany activities in order to add value to the entire value chain;
- *Cybersecurity*: Consists of methods used to detect and prevent intruders. It represents the need to protect management systems and production lines with increasing connectivity; [25]
- *Additive manufacturing or 3D printing*: A manufacturing method that adds layer–by–layer material to produce an object. Industry 4.0 will enable the production of small custom batch volumes with the assistance of additive manufacturing.[26]

The Fourth Industrial Revolution will have major impacts on the economic scenario, such as consumption, GDP, employment, trade and inflation.[2] It is estimated that Germany alone should invest annually € 40 billion in Industry 4.0 by 2020, and that the figure could reach € 140 billion annually across Europe.[25] The United States should invest US$ 1.35 trillion in the next 15 years.[29]

In the industry, the implementation of cyber–physical systems will enable large gains in efficiency and flexibility, and incredible gains in productivity throughout the production chain.[13]

However, the main impacts of Industry 4.0 will occur on employability, because there will be the need for people to improve their skills to deal with all new technologies;[2,28]

According to a report issued by the World Economic Forum *The Future of Jobs – Employment, Skills and Workforce Strategy for the Fourth Industrial Revolution*, these impacts can lead to a cut of approximately 7.1 million jobs between 2015 and 2020 due to a series of redesigns that do and will take place from the factory floor.[16]

By introducing developments in artificial intelligence, robotics, new materials, 3D printing and Biotechnology, such an environment will be marked by "a widespread disruption not only in business models, but also in labor markets over the next five years, with huge changes anticipated in the skill sets needed to prosper".[16] That is why this work shows the Biotechnology and the possible professions of the future.

**Advances in biotechnology**

Biotechnology started to be pointed out as a high priority science recently, however, some biotechnological processes have been used since antiquity. The use of Biotechnology began long before the beginning of the Christian era, with the fermentative processes obtained from microorganisms. These fermentative processes were possible to manufacture alcoholic beverages from cereal grains; the Egyptians also used the fermentation process for the production of bread.[29]

In 1876, Louis Pasteur proved that fermentation is caused by microorganisms and that each type of fermentation was produced by a microorganism.[30]

With the discovery of penicillin by Alexander Fleming in 1928, it made the production of antibiotics a major industrial milestone. During the Second World War, antibiotics became part of biotechnological industrial processes.[30]

Biotechnology in the first half of the 20th century was based on enzymes, with the main objective of improving the quality of food.[29] Another landmark of modern Biotechnology was the chemical synthesis of DNA (deoxyribonucleic acid) performed by Kornberg in 1967, a fact he called "genetic revolution".[31]

Modern Biotechnology has a wide scope, multidisciplinary character and is linked to many and different applications in various segments of activities, such as: mining, health, fermentation, agriculture and livestock.[29]

In mining, the Biotechnology professional works with the improvement of metal concentration processes for the use of ores, biolixiviation mineral bacterial, hydrometallurgy and others.[30]

In the area of health, the Biotechnology professional works in the production of metabolic regulating proteins, interferon, human insulin, growth hormones, neuroactive, peptides, and others.[32] Still in the health area, we work in the production of vaccines with the objective of preventing several diseases.[29]

In agriculture, the Biotechnology professional develops research with genes, which have allowed the improvement of several cultures, such as coffee, sugar cane, soy, cotton, tomato, potato and many others. Other developments include the production of products for pest control, seed production, genetically modified foods.[33]

In livestock, the Biotechnology professional is present in the development of animal feeding, and in the control of reproduction that are available in embryo transfer techniques, such as artificial insemination, *in vitro* experiments, cloning, genetic mapping and molecular markers.[34]

Biotechnology is a powerful tool that can replace a large number of current processes in the near future and create innovative and sophisticated solutions to a wide range of problems.

Several developments that happened with the advances in Biotechnology can be cited:[33]

- New biological therapies;
- Discovery of new energy sources based on biotechnological re–search;
- Structuring of analytical tools;
- Expansion of nanobiotechnology;
- Proliferation of transgenic technology;
- Development of tools in bioinformatics;
- Expansion of biomass to biofuel conversion technologies;
- Development of research based on sustainability;

- Biotechnology law, intellectual property, patents and biotechnological ethics;
- The development of business models, processes and management of Biotechnology companies.

The multidisciplinary nature of Biotechnology has allowed researchers and professionals to seek new research and studies. According to studies by Deloitte consultancy, Biotechnology today represents about 27% of the global market, and the expectation is that by 2024 this number will increase to 31%.[35,34]

In Brazil, Biotechnology has increasingly acquired great importance in the economic and social sectors. One of the reasons is the population size, because the greater the number of inhabitants of a country, the greater the consumption of food, medicines, vaccines, among other products.[34]

**Biotechnology and industry 4.0**

What is understood by the integration of Biotechnology and the Industry 4.0 is the incorporation of digital systems and technologies (big data, IoT, cloud computing, advanced robotics, virtual simulation, artificial intelligence, 3D printing) to Biotechnology activities, in order to allow the integration of physical systems with virtual systems (cyber–physical systems). It has been used by large Biotechnology companies, and partly also by academic laboratories. But compared to manufacturing and service industries, Biotechnology needs to evolve in the automation of research laboratories for productivity and quality to improve exponentially.[36]

A fully–automated laboratory uses robots or other networked computer devices to monitor experiments and gather more accurate data. This would increase productivity, reproducibility and research accuracy.[36]

A recent research published by Nature indicates that Science is actually experiencing a crisis of reproducibility, as more than 70% of the 1576 researchers interviewed tried unsuccessfully to reproduce the experiences of another scientist, following the methodology described by the latter in books or articles.[37] Another study published in Plos Biology estimated the amount of US$ 28 billion in nonreproducible pre–clinical research in the US alone.[38]

Companies have developed laboratory automation with software and hardware, developing organizations for efficient production of compounds according to the customer's request. There are many companies where their genetic engineering steps are automated, including high–performance analysis.[30]

In the laboratory of the future, there will be autonomous communication of task execution processes, with automated process flows; and professionals for the design and implementation of these laboratories will be necessary, i.e., a new modality of work will be created.[39]

According to STEQ the German national innovation network aims to facilitate the integration between intelligent laboratories and Industry 4.0. The goal is to drive the development and standardization of innovative technologies, and the intended results "include simplified process flows, better quality, greater efficiency and greater process reliability".[39]

Another initiative in Germany in Stuttgart is the development of the Innovation Center for Laboratory Automation, with the aim of creating the intelligent laboratory of the future. This laboratory is already providing some initial impulses and generating many ideas, one of which is intelligent tracking, where it automatically documents and analyzes hand movements using 3D image analysis. The system accurately captures and records every step of the process, and consequently saves time, reduces workloads for employees and provides better results.[39]

In parallel to the initiatives developed in Germany, Japan is also developing a two–arm robot for use in pharmaceutical laboratories. The project leaders are the company Yaksawa and the National Institute of Advanced Industrial Science and Technology of Japan. The robot named "Mahoro" can perform laboratory work and conduct crops more quickly, accurately and efficiently. As the robots have no immune system, they are particularly ideal for working with biological hazards, such as radioactive materials, and conducting clinical trials.[40]

In Austria, researchers at the University of Technology in Vienna recently used a high–resolution 3D printing process to produce live cells. The technology uses a special "biological ink" that allows the team to incorporate cells into a 3D matrix.[41]

According to the Brazilian Association of Bioinnovation (ABBI), innovative Biotechnology solutions also provide a vital contribution to the transition from current unsustainable economic practices to renewable industrial systems – the circular and bio–based economy – combining innovation and sustainability to solve major global challenges. The main benefits of these innovative solutions are:[42]

- Improved productivity and industrial competitiveness by 40%, reducing the use of natural resources, fossil raw materials, and the number of processes;
- Accelerates the transition from a nonrenewable base industry to a circular, restorative and regenerative economy, avoiding emissions of up to 2.5 billion tons of $CO_2$ per year;
- It contributes significantly to a food–secure and low–carbon future by reducing the amount of land needed to replace 10% of the world's gasoline with advanced biofuels by 60%;
- It replaces traditional chemicals in food and beverage production, causing less impact on natural flavors and colors.

**The professions of the future**

Changes in labor markets have been driven by interconnected forces throughout the globalized world: technological advances and innovations, constant changes in organizations and the market, competition in local, national and international markets at the same time, and the need to sustainably manage waste disposal and energy use to avoid harmful climate change. These forces together are potentially driving significant changes in socioeconomic systems throughout the world. Trends that can be identified will shape the work of the future. Globalization, population aging, and social, technological and business trends will create opportunities for many professions, with names that often do not exist today.[43]

According to the UN, with the advancement of technology and the insertion of Industry 4.0, many impacts on employability will occur.[44] Still according to the UN, in 2035 the global unemployment rate will reach 20% and by the middle of this century, 40% of jobs that exist today will cease to exist. [44] A consultancy carried out by McKinsey shows that 50% of current jobs in Brazil could be automated, and about 60% of professions could have at least 30% of their activities automated.[45]

Schwab also identifies as the most prone professions to automation: telemarketing; insurance appraisers, executive secretary, receptionists, real estate agent, among others. That is, activities marked by a high degree of repetition of tasks and low complexity. Still according to the researcher, the professions with lower propensity for automation would be: mental health professionals, choreographers, doctors, psychologists, human resources managers.[2]

This dynamic, however, would be accompanied by a growing and significant work opportunity to be created, especially related to computing and mathematics, for analysts and data scientists, specialists in artificial intelligence and automation, marking a separation between humans, machines and algorithms, and their respective performances in the productive and social chain. It is also at this point that it is necessary to mention the discussion on the emergence of hybrid jobs, combining skills from previous functions in a new role.[35]

**The future of work – World Economic Forum**

In 2016, leading entrepreneurs and world leaders met at the World Economic Forum in Davos, Switzerland, to debate the future of the

economy and business worldwide. One theme has received special attention: the enormous revolution in the profile of professions in recent years and what to expect from the future of work in the coming decades. The report *The Future of Jobs and Skills* published by the World Economic Forum, interviewed entrepreneurs, human resources executives and professionals specialized in business strategy from 15 developed or developing regions or countries, including Brazil, United States, Germany, United Kingdom, France, Mexico and Persian Gulf countries.[16]

The mechanisms for changing the working environment envisaged by the study are many and all linked to the concept of Industry 4.0. Figure 2 shows the main technologies that should drive changes in the future of work.[16]

It is possible to observe in Fig. 2 that the technological mechanisms that will transform the work environment are related to the cyber–physical systems, i.e., machines, robots and vehicles will be connected to the internet, and through a large data storage (big data) the artificial intelligence will control the decisions and actions to be taken, integrating the physical systems with the virtual ones.

The study also showed the demographic and socioeconomic factors of change in jobs according to the World Economic Forum, and are represented in Figure 3:[16]

The big change in jobs is also related to the work environment (technology insertion) and flexible working arrangements. Companies and employees need to adapt, as there are and there will be major changes in geographical barriers and greater consumer interest in ethical and privacy issues.

In Brazil, the drivers of change in work are identified in Figure 4 according to the World Economic Forum:[16]

- By 2020, there will be a drop of more than 7.1 million jobs due to changes in the market;
- Of these, 2/3 are concentrated in office functions and administrative areas.

An example of a job that should become obsolete is in the consumer service area – because of the growth in the use of mobile technology as a customer relationship channel.

On the other hand, there should be a gain of 2 million jobs in areas related to computing, mathematics, engineering, architecture and Biotechnology.

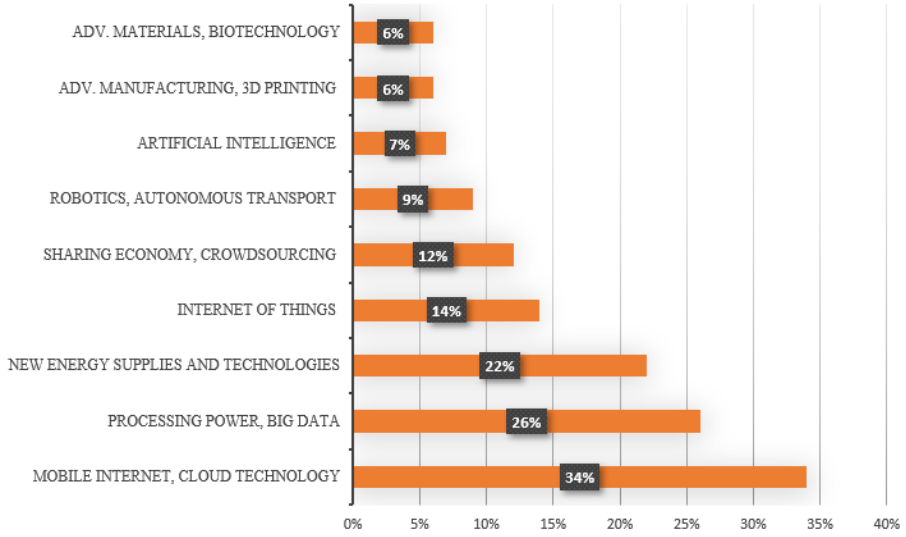


**Figure 2 –** Technologies that drive changes in the future of work. **Source:** Adapted from the Global Challenge Insight Report (World Economic Forum 2016).[16]

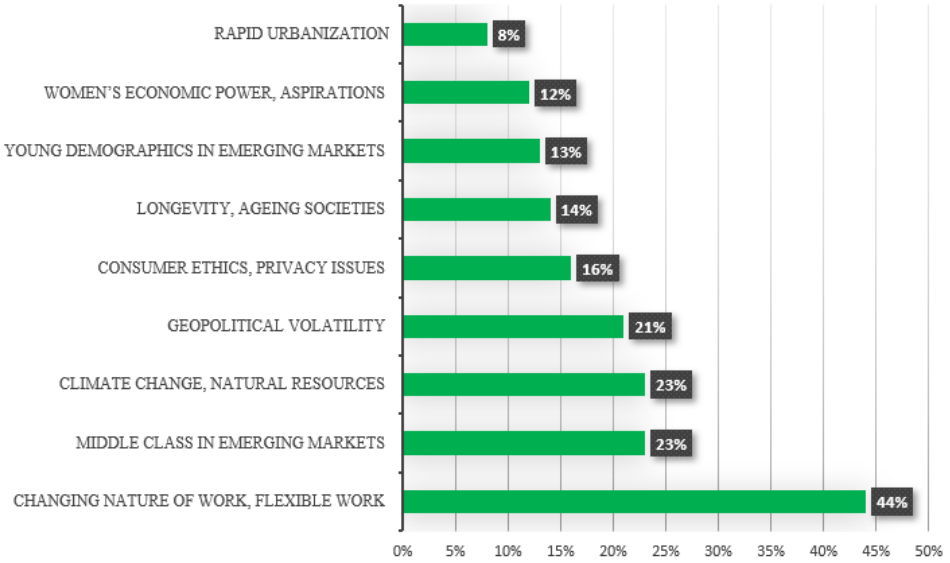


**Figure 3 –** Factors of changes in employment. **Source:** Adapted from the Global Challenge Insight Report (World Economic Forum 2016).[16]

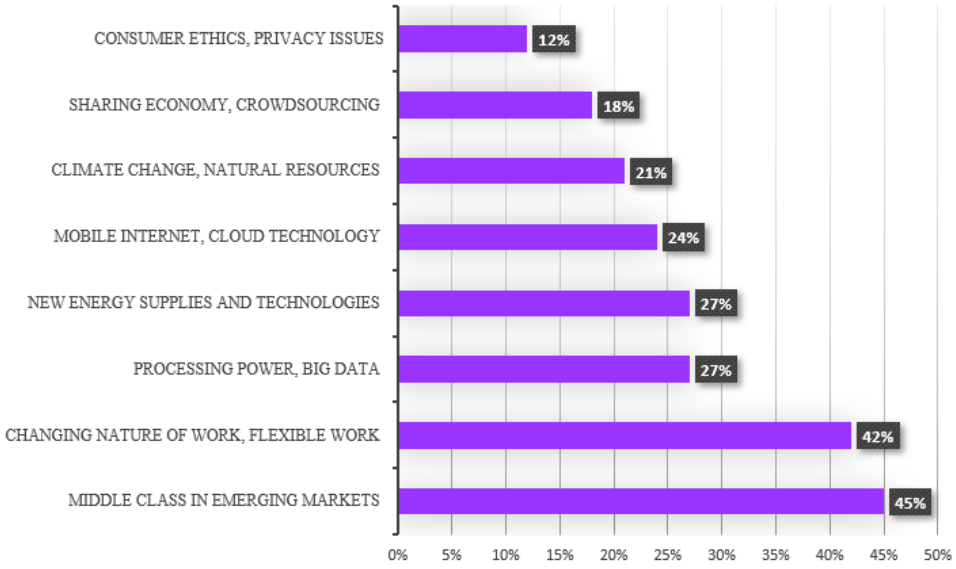


**Figure 4 –** Drivers of change in labor in Brazil. **Source:** Adapted from the Global Challenge Insight Report (World Economic Forum 2016).[16]

**The future of work – Por Luís Rasquilha**

According to Rasquilha, it is essential to know the innovations that are emerging in the market to know which path to follow and thus ensure professional success. Currently, the demand for professionals with skills in Industry 4.0 begins to emerge, and it tends to increase. The professional who is able to practice any of the professions of the future will be ahead of his peers, ensuring the best positions in the market and higher revenues. The reason is that, from the moment a certain occupation is valued, the search for professionals who are qualified to exercise it increases.[46] Some occupations are listed below:

- Waste manager
- Environmental engineer
- Oil and gas engineer
- Hospital engineer
- Bioinformacionist
- Telemedicine technician
- Retirement adviser
- Quality of life manager
- Cloud specialist
- Big data manager
- Corporate lawyer
- Tax lawyer
- Uranium recycler
- Mechatronic engineer
- Personal robot mechanic
- Geomicrobiologist
- Experimental therapist
- Drone driver
- Personal food shopping advisor
- Productivity adviser showroom manager.

**The future of work in Biotechnology**

The study developed by the World Economic Forum presents Biotechnology as one of the main technologies driving the future of work, i.e., professionals in the field need to observe the changes and professionalize.[16]

The power of digitalization has enabled Biotechnology to develop new Biotechnology products and processes from the processing and understanding of genetic information of microorganisms. With the use of Industry 4.0 practices, the convergence of innovations will be intensified.[47]

Rasquilha also presents current and future possibilities of professions in the field of Biotechnology:[46]

- Quality control and material analysis: Use of the automated laboratories for high efficiency analysis, but for this it is necessary to build these laboratories needing professionals from Industry 4.0 and also from Biotechnology to support the new research;
- Regulatory affairs: Biotechnology also lacks professionals who can deal with documents, bureaucracy, deadlines from regulatory agencies, read and interpret legislation. Regulation of new transgenics and biopharmaceuticals. To operate in the application of the main regulatory laws in new biotechnological products or services in food, chemical, veterinary or pharmaceutical industries;
- Scientific consultant: To conduct consultancies for companies interested in performing or optimizing processes, for example DNA cloning and fermentation, or interested in interpreting genetic data. Their role is to transfer scientific knowledge about a particular medicine to physicians for everyday use;
- Waste management: The production of waste by industry in line with government policies generates demand for this type of service. The correct direction of waste and the transformation of waste into a source of income are the primary activities of professionals in this area.
- Environmental engineering: The concern with the impact on the environment, both in the civil construction sector and in the industrial sector makes professionals with knowledge in the environmental area necessary, since sustainability and the environment are pressing issues today and in the future.
- Bioinformation: It is a scientific area that works with genetic

information bridging clinical techniques and drug development. It is a profession linked to innovation and also to the aging macro trend of the population.

- Telemedicine: The search for innovation and increased life expectancy of the population will highlight telemedicine professionals. It is a person who is part of a team that offers diagnosis and treatment for the inhabitants of more remote areas. An alternative to the lack of health professionals in more remote areas of Brazil, telemedicine allows people to have access to diagnosis without being in the same place as the medical team.
- Uranium recycling: Through sustainable techniques it converts uranium waste into energy that can be used for cities and infrastructures.
- Geomicrobiology: Bacteria and microorganisms have characteristics that can help in research in areas such as medicine, food or health and well–being, incorporating these microcharacteristics into everyday industry and research.

## Conclusions

New technologies have impacted on employability, especially those related to process automation and information digitization, and have also influenced the relationship of the labor market and the new professions that will occupy the old and new functions.

According to the United Nations, the global unemployment rate in 2035 will reach 20% with the advancement of technology and the insertion of Industry 4.0, and by the middle of this century, 40% of the jobs that exist today will cease to exist. McKinsey showed that 50% of current jobs in Brazil could be automated, and about 60% of professions could have at least 30% of their activities automated. Some professions are more prone to automation: telemarketing, insurance assessors, executive secretary, receptionists, real estate agent, among others. [2]

In this scenario, there will be many challenges in the labor market, professionals will have to continuously develop skills and be attentive to the evolution of technologies that will generate demands for future professions.

Biotechnology is one of the driving technologies of the future of work, i.e., professionals working in this area need to observe changes and professionalize. The power of digitalization has enabled Biotechnology to develop new Biotechnology products and processes from the processing and understanding of genetic information of microorganisms.

Artificial intelligence, robotics, 3D printing are technologies that will impact Biotechnology on a large scale. In the future, it will be required the production of new drugs for health treatment, food with greater amount of protein and vitamins, cultivation of seeds with greater resistance to pests and climatic conditions, but for this to happen there will be a demand for professionals who can develop enabling technologies such as algorithms, software and hardware, with biology and Biotechnology knowledge.

The analysis leads to the conclusion that, with economic growth, Biotechnology innovation and sufficient investment, the creation of new jobs can counterbalance the impact of automation, even if some economies may need additional investment to reduce the risk of job scarcity. An even greater challenge will be to ensure that workers have the skills and support needed to complete the transition to new jobs. In this sense, it is necessary to invest in education, research and studies to develop and implement means for transition.

However, it must be recognized that this is a process of reflection, adaptation and innovation in education systems. In addition, the need for companies to work in collaboration with educational institutions and governments is reiterated, in order to find the necessary skills and abilities for the professionals of the future.

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# Biotechnology and vaccines

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**Abstract: Biotechnology has demonstrated its importance for health development, especially in the discovery of new drugs and production of vaccines. On account of the occurrence of many diseases that have killed millions of people in the world, vaccines were developed to control infections and prevent diseases caused by viruses, bacteria, protozoans and fungi, and even eradicate them, as is the case of smallpox. Vaccines can be of first, second and third generation. Currently, vaccine manufacturing can be directed to the use of DNA containing the gene that encodes an antigenic protein. The present work is a literature review, with the objective to present the first-, second- and third-generation vaccines, as well as to make an analysis of the use of these vaccines in Brazil.**

**Keywords: Biotechnology; Medicine; Immunology; Vaccines.**

## Introduction

Biotechnology, according to the Office of Technology Assessment (OTA),[1] can be defined as any technique that uses living organisms (or their parts) to obtain or modify products, improve plants and animals, or develop microorganisms for specific uses. Modern Biotechnology has a wide application in the health industry, attracting interest from scientists for production of drugs and vaccines, thus contributing to the economic development and, especially, to growth of pharmaceutical industry.[2]

Science and technology complement each other, integrating basic and applied sciences with other technologies such as molecular biology, cellular biology, immunological techniques, biochemistry and microbial fermentation processes.[3]

Advances in the industrial sector has brought many benefits to society, but it is worth saying that, among several consequences, massive migration of the population from countryside to urban centers has occurred, diminishing quality of life due to lack of basic sanitation and, consequently, causing more diseases for people and animals. In the 19th century, 80% of children died of various diseases before their 10th birthday.[3] In addition, millions of deaths have been recorded as a result of human contamination by viruses and bacteria, causes of diseases such as yellow fever, pertussis, smallpox, tetanus, diphtheria, measles, polio, rubella, hepatitis, bubonic plague, cholera, rabies, chickenpox, and mumps, which are among the most well-known diseases.

In order to control and prevent infections and diseases, scientists discovered vaccines, which were based on the principle that the individual's contact with the antigen produced an immune response.[4] As a result, several studies have been conducted to develop more effective vaccines. Genetic manipulation established by modern Biotechnology has modified in different ways research and development of these vaccines, which have been classified as first second and third generation.[5]

First generation or traditional vaccines are produced with live attenuated microorganisms, i.e., the weakened pathogen itself is inoculated into the patient. Vaccines against smallpox, polio, measles, rubella, adenovirus and tuberculosis can be included in this type. Second-generation vaccines arose from the idea that vaccines can be obtained by toxins produced by microorganisms that cause diseases. These toxins, called toxoids, can be inactivated and become recognized by the host's immune system. They can also be produced with purified polysaccharides. The recombinant proteins are used as a source for the antigens to be incorporated into the formulations.[5] In the 1990s, third-generation vaccines emerged, in which the material inoculated into the patient is not the attenuated or dead pathogen, but its DNA containing the gene that encodes an antigenic protein. However, it is necessary to have an agent that introduces DNA into the patient's cells, which are called DNA vectors, for the vaccine to present any effect.[6]

The history of vaccine, from its discovery by Edward Jenner in the late 18th century[7] until the present day, has provided the world's population with greater safety, health, and has undoubtedly saved billions of people from various diseases. Vaccine production in Brazil deserves special consideration, as the country is a global reference due to the efforts of the National Immunization Program, Butantan and Fiocruz Institutes.[8,9]

It is important to remember that the first vaccine reported was for smallpox, discovered in 1789. This vaccine arrived in Brazil almost one century later, in 1887, and, only in 1922, the Oswaldo Cruz Institute began to produce it. Smallpox eradication became a goal by the World Health Organization from the 1950s onwards, but it was eradicated only in the 1970s. The BCG vaccine was discovered in 1909 and only began to be used in Brazil in 1925. Production and use of the vaccine against yellow fever started to be produced in 1937 in Brazil. Currently, Fiocruz is the largest producer of this vaccine. The vaccine against poliomyelitis, discovered in 1949 by Jonas Salk, was later developed for oral application by Albert Sabin and started to be used in 1961. The discovery of the vaccine against rubella occurred in 1969. In 1963, the vaccine against measles was developed. In Brazil, this vaccine was effectively implemented in 1973 with the National Immunization Plan (NIP), but only in 1992 the fight against this disease was defined as a public health policy priority. The second dose of the triple viral vaccine (measles, rubella and mumps) was introduced in 2004.[10]

In addition, Brazil has adopted a successful vaccination strategy.[8] Brazil's National Immunization Program (NIP) is considered one of the most comprehensive among developing countries. For example, Brazil received the poliomyelitis eradication certificate in 1994 and also pioneered the introduction of rotavirus, conjugate pneumococcal, meningococcal meningitis, and conjugate C serogroup vaccines in 2007, and vaccination against the H1N1 pandemic influenza in the second half of 2010.[9] Tables 1, 2, 3 and 4 show the vaccination calendar in Brazil for children, teenagers, pregnant women, and travelers, respectively. Table 5 shows the calendar of national vaccination campaigns.

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| Child | | | |
|---|---|---|---|
| **Age** | **Vaccine** | **Avoidable diseases** | **Dose** |
| At birth | BCG–ID | Prevents severe forms of tuberculosis (miliary and meningeal) | Single dose |
| | Hepatitis B | | Single dose |
| 2 months | Pentavalent (DTP + Hib + Hep. B) | Prevents diphtheria, tetanus, pertussis, hepatitis B, meningitis and Hib infections | 1st dose |
| | Inactivated polio (VIP) | Prevents polio (infantile paralysis) | |
| | Pneumococcal 10–valent (conjugated) | Prevents pneumonia, otitis, meningitis and other diseases caused by Pneumococcus | |
| | Human rotavirus (VORH) | Prevents rotavirus diarrhea | |
| 3 months | Meningococcal C | Prevents pneumonia and meningococcemia (generalized infection) | 1st dose |
| 4 months | Pentavalent (DTP + Hib + Hep. B) | Prevents diphtheria, tetanus, pertussis, hepatitis B, meningitis and Hib infections | 2nd dose |
| | Inactivated polio (VIP) | Prevents polio (infantile paralysis) | |
| | Pneumococcal 10–valent (conjugated) | Prevents pneumonia, otitis, meningitis and other diseases caused by Pneumococcus | |
| | Human rotavirus (VORH) | Prevents rotavirus diarrhea | |
| 5 months | Meningococcal C | Prevents pneumonia and meningococcemia (generalized infection) | 2nd dose |
| 6 months | Pentavalent (DTP + Hib + Hep. B) | Prevents diphtheria, tetanus, pertussis, hepatitis B, meningitis and Hib infections | 3rd dose |
| | Inactivated polio (VIP) | Prevents polio (infantile paralysis) | 3rd dose |
| 12 months | Triple Viral (SCR) | Prevents measles, mumps and rubella | 1st dose |
| | Meningococcal C | Prevents pneumonia and meningococcemia (generalized infection) | Reinforcement |
| | Pneumococcal 10–valent | Prevents pneumonia, otitis, meningitis and other diseases caused by Pneumococcus | Reinforcement |
| 15 months | Triple Bacterial (DTP) | Prevents diphtheria, tetanus and whooping cough | 1st Reinforcement |
| | Oral poliomyelitis (OPV) | Prevents polio (infantile paralysis) | 1st Reinforcement |
| | Hepatitis A | Prevents hepatitis A | Single dose |
| | Tetraviral (SCRV) | Prevents measles, mumps, rubella and chickenpox | Single dose |
| 4 years | Triple Bacterial (DTP) | Prevents diphtheria, tetanus and whooping cough | 2nd Reinforcement |
| | Oral poliomyelitis (OPV) | Prevents polio (infantile paralysis) | 2nd Reinforcement |
| 9 years* | Human Papillomavirus (HPV) | Prevents papilloma, human virus that causes cancers and genital warts | Two doses in a period of six months |

* It may be applied up to 14 years 11 months and 29 days

**Table 1 –** Vaccination calendar for children.Source: Adapted from the Brazilian Ministry of Health's website.[11]

| Adolescent | | | |
|---|---|---|---|
| **Age** | **Vaccine** | **Avoidable diseases** | **Dose** |
| 11 to 14 years (boys) | Human Papillomavirus (HPV) | Prevents papilloma, human virus that causes cancers and genital warts | Two doses six months apart |
| 11 to 14 years (boys and girls) | Meningococcal C | Prevents meningitis and meningococcemia (generalized infection) | A booster or single dose |
| 11 to 19 years | Hepatitis B (recombinant) | Prevents hepatitis B | Three doses** |
| | Adult double bacterial (dT) | Prevents diphtheria and tetanus | One dose every ten years** |
| | Triple viral (SCR) | Prevents measles, mumps and rubella | Two doses** |

** According to the vaccination situation

**Table 2 –** Vaccination calendar for teenagers.Source: Adapted from the Brazilian Ministry of Health's website.[11]

| Pregnant | | |
|---|---|---|
| **Vaccine** | **Avoidable diseases** | **Dose** |
| Hepatitis B (recombinant) | Prevents hepatitis B | Three doses** |
| Adult double bacterial (dT) | Prevents diphtheria and tetanus | Two doses** |
| Triple acellular bacterial (dTpa) | Prevents diphtheria, tetanus and whooping cough | One dose with each pregnancy (from the 20th gestational week) |

** According to the vaccination situation

**Table 3 –** Vaccination calendar for pregnant women.Source: Adapted from the Brazilian Ministry of Health's website.[11]

| Traveler | | |
|---|---|---|
| **Vaccine** | **Avoidable diseases** | **Dose** |
| Hepatitis B (recombinant) | Prevents hepatitis B | Three doses** |
| Adult double bacterial (dT) | Prevents diphtheria and tetanus | Two doses** |
| Yellow fever | Prevents yellow fever | Single dose (9 months to 59 years of age)*** |
| Triple viral (SCR) | Prevents measles, mumps and rubella | Two doses (1 to 29 years) and one dose (30 to 49 of age)** |

** According to the vaccination situation
*** Only applied to residents and/or travelers to areas with vaccination recommendation (ACRV)

**Table 4 –** Vaccination calendar for travelers.Source: Adapted from the Brazilian Ministry of Health's website.[11]

| Traveler | | |
|---|---|---|
| Vaccine | Priority Groups | Period |
| Influenza | Children (6 months to under 6 years of age) | April 10 to May 31 |
| | Elderly people (60 years of age or older), those who have recently given birth (up to 45 days after birth), health workers, teachers in public and private schools, indigenous peoples, groups with chronic noncommunicable diseases and other special clinical conditions, adolescents and young people between 12 and 21 years of age under social and educational measures, people deprived of their liberty and prison system employees | April 22 to May 31.<br>D" day of mobilization: May 4th |

**Table 5 –** Calendar of the vaccination campaign. Source: Adapted from the Brazilian Ministry of Health's website.[11].

**Objectives**

The objectives of this work are to present the first–, second– and third–generation vaccines as well as to make an analysis of the use of these vaccines in Brazil.

**Methodology**

For this review work, a survey of the articles with the highest number of accesses and easy acquisition within databases such as Google Scholar and PubMed was carried out. The method of bibliographic research related to the subject was used and then the most relevant data of the main themes related to Biotechnology and vaccines were collected. The keywords used were: biotechnology, medicine, immunology and vaccines.

**Development**

**Biotechnology**

Biotechnology has contributed to a number of significant changes in the area of biology and health, especially in the field of genetics. Gene manipulation techniques, such as gene sequencing, have altered scientific research for the health sector in different ways. Some areas to which research is currently focused are: synthetic biology (creation of "customized" organisms), three–dimensional bioprinting and uses and applications in the area of neurotechnology.[12]

The insertion of Biotechnology in the pharmaceutical industry has enabled an increase in the number of therapeutic compounds, greater knowledge of the processes causing diseases, more accurate diagnoses and more efficient delivery mechanisms, such as recombinant DNA for therapeutic protein production, hybridization for monoclonal antibody production and techniques for cloning and manipulation of stem cells.[2]

Another area of health that has benefited from technological advances is the area of vaccines. Biotechnology has brought changes in the way vaccines are produced, such as the discovery of new antigens, adjuvants, vectors or delivery systems. The production process of a drug or vaccine can be briefly divided into three stages: a) production of the active ingredient, which is the substance responsible for the therapeutic action; b) formulation, a phase in which the active ingredient is mixed with other substances (adjuvants) in order to adapt characteristics of the final product, such as solubility, effect duration, stability, and absorption and elimination by the body; and c) packaging, i.e., the packaging of the finished product in quantities for final consumption.[5]

Many vaccines are still administered by methods created in the past centuries. It is expected from biotechnological advances that safer and more effective vaccines will be produced and made available to an increasing number of people.[5]

The development of the third–generation vaccines (called DNA vaccines) was possible due to technological advances in Biotechnology. Although their use in humans is not yet approved, such vaccines have been applied against diseases such as cancer.[4]

**Vaccine concepts**

Vaccination or immunization is the process of developing the body's immunity or defense against infections with antigens (substances that stimulate the body to produce antibodies against the infectious agent – viruses, bacteria or parasites). Vaccines can be produced from dead or attenuated microorganisms or from their components (first generation). However, in some cases, the disease may occur due to a toxic substance produced by the microorganism. Then, the vaccine needs to neutralize this toxin (second generation). In other cases, however, the problem is not the virus or the bacteria, but the quantity of them inside the host. Therefore, its multiplication must be controlled.[13] Figure 1 shows the three generations of vaccines.[14]

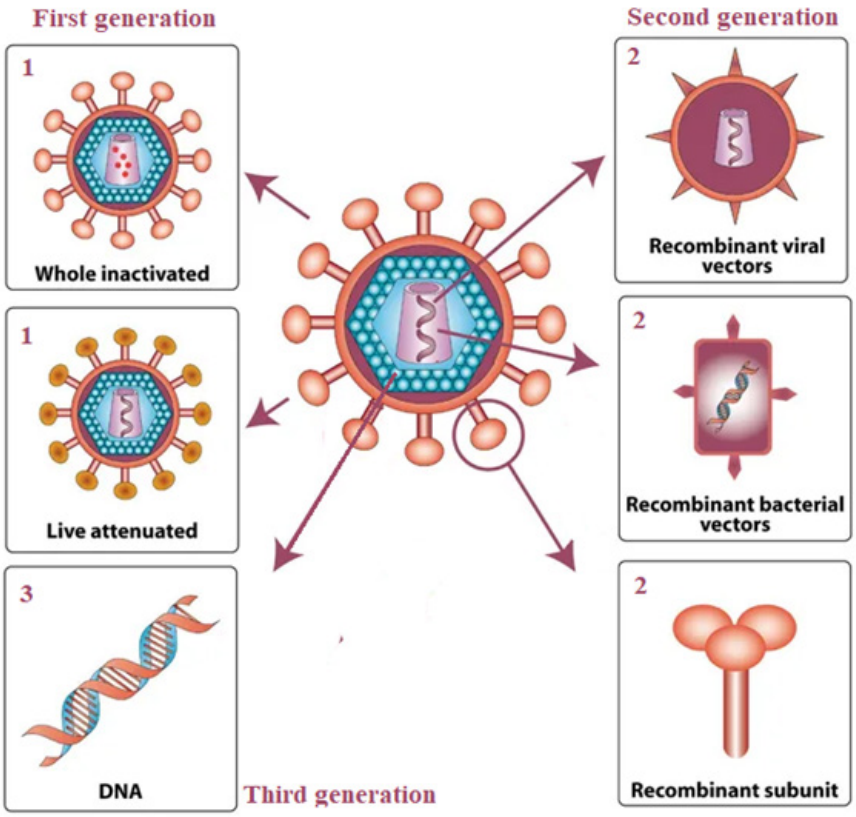


**Figure 1 –** The three generations of vaccines. **Source:** Adapted from Gorry et al. (2017).

**The three generations of vaccine**

**First generation**

First–generation vaccines are produced with antigens of only one infectious agent, which produces protective antibodies only for that agent, such as the diphtheria vaccines. They can also be combined, which are those that have two or more agents, such as diphtheria–tetanus or triple viral (against measles, mumps and rubella). Vaccines can also be conjugated, i.e. bacterial antigens are bound to protein carriers (polysaccharides) generating a long–lasting antibody response, such as pneumococcal vaccines.[13]

One of the first–generation vaccine production techniques is the inactivation of microorganisms (dead and inactive), such as antipertussis, typhoid fever, bubonic plague, Salk (injectable poliomyelitis), hepatitis A, influenza, rabies, whooping cough, anthrax and cholera vaccines. First–generation vaccines can also be produced with live attenuated microorganisms, such as BCG against tuberculosis.[15,16]

Smallpox is a disease that has devastated populations for many years, but is no longer considered a concern for humanity. Its eradication was registered in 1977. Vaccine against smallpox is considered the basis of immunology, suggesting that virus infection conferred specific immunity to the disease.[17] Vaccine against smallpox is not available to the public and the virus no longer exists in nature. The vaccine is produced from the attenuated ***vaccinia virus***, a poxvirus similar to smallpox virus, but less harmful.[18]

Vaccine against yellow fever, produced in Brazil since 1937 and internationally recognized, also consists of attenuated viruses, more specifically from the attenuated strain of the 17D virus of the ***Flavivirus*** genus.[20]

Another disease against which an attenuated virus vaccine is used is measles. This is a highly contagious viral disease that occurs throughout the world. The infection is characterized by fever, malaise, cough, coryza and conjunctivitis, followed by exanthema.[20] Humans are the sole host.[21] In Brazil, the vaccine was introduced in 1960. It is contained in the triple viral (measles–mumps–rubella), which contains weakened measles, rubella and mumps live virus and is currently recommended for children aged 12 months, with application of the second dose between four and six years of age or in any consultation after 12 months of life, with a minimum interval of four weeks. The Ministry of Health reports that Brazil was awarded a certificate of measles elimination by the Pan–American Health Organization in 2016, but states that the country has struggled to maintain this certificate, since two outbreaks had already been identified in 2018.[22]

Rubella is a disease caused by viruses of the ***Rubivirus*** genus which is transmitted by direct contact through tiny drops of saliva released into the air when the infected person coughs, sneezes or talks, or from the mother to the fetus through blood circulation. The vaccine, which is part of the triple viral (measles–mumps–rubella), is a combination of live attenuated viruses.[23]

Poliomyelitis, also called infantile paralysis, is an acute contagious disease caused by poliovirus, which can infect children and adults through direct contact with feces or with secretions eliminated by the mouth of sick people and can cause paralysis. In Brazil, the last case of infection by the wild poliovirus occurred in 1989 in the city of Souza, in the state of Paraíba. The strategy adopted for the virus elimination in the country was centered on conducting mass vaccination campaigns with oral polio vaccine (OPV). Sabin attenuated virus strains types I, II and III was used.[19]

Another immunization obtained by an attenuated strain is the vaccine against tuberculosis. The BCG vaccine (Calmette and Guérin bacillus) originates from avirulent strains of ***Mycobacterium bovis,*** after genetic mutation and with immunogenic protective properties against tuberculosis. In Brazil, BCG was applied in 1925 and was orally used until 1973, when it started to be intradermally administered, as determined by the Ministry of Health. There is a consensus in the literature that intradermal BCG is effective against severe forms of tuberculosis, such as meningoencephalic and miliary.[25]

The third part of the triple bacterial vaccine was the result of immunizers developed in 1942 by Louis Sauer, Pearl Kendrick and Grace Eldering against pertussis. Sauer et al. found that the vaccine was most effective in uniting diphtheric and tetanic toxoids.[26] Thus, the triple

bacterial vaccine emerged. According to the Ministry of Health, pertussis is a respiratory infection, transmissible and caused by the bacteria ***Bordetella pertussis***, a Gram–negative and aerobic coccobacillus. Pertussis transmission occurs mainly by direct contact of the patient with an unvaccinated person through droplets eliminated through coughing, sneezing or talking. Pertussis is produced with dead and inactivated microorganisms.[27]

**Second generation**

After the emergence of attenuated and inactive vaccines, second–generation recombinant vaccines emerged, proving that for individuals to be protected against an infectious agent, it is not necessary for him/her to produce antibodies against all the antigens of the microorganism. Recombinant vaccines are obtained by genetic engineering, by inserting a gene that produces an immunogenic protein in a microorganism.[28] The identification of one or two proteins crucial for immune protection is sufficient to create a second–generation vaccine in which the immune response is induced by a specific isolate antigen. The antitetanic, antidiphtheric, antihepatitis B vaccines and those for the control of meningococcal meningitis and pneumonia are found in this group.[5]

The first approved recombinant vaccine was the vaccine against hepatitis B, which only began to be produced in cell culture systems in 1986.[29] Among the second–generation vaccines there is the vaccine against meningococcal meningitis, caused by the bacteria ***Neisseria meningitidis***. The Cuban vaccine, the first in the world with proven efficacy against the disease caused by meningococci B, is based on proteins from the external membrane of this microorganisms capable of inducing antibodies against all tested ***Neisseria meningitidis*** pathogenic groups. To combat meningococcal meningitis, a conjugate vaccine is used, which is a differentiated type of vaccine because it was specially developed to combat encapsulated bacteria that have a protective membrane around their cell structure. These capsules are made of polysaccharides, which ensure a great resistance of the bacteria against the human immune defense system. Because of this, when producing a vaccine against encapsulated bacteria, it is necessary to wrap the protective capsule with a protein. So, the antibodies can penetrate the bacteria destroying and guaranteeing health for the vaccinated individual.[30]

Other second–generation vaccines that act against toxins are the antitetanic and antidiphtheric ones.[4] Diphtheria is a transmissible disease caused by bacteria that affect tonsils, pharynx, larynx, nose and occasionally other parts of the body such as skin and mucous membranes. The main form of prevention against the disease is through the pentavalent vaccine (diphtheria, ***tetanus***, pertussis, hepatitis B and ***Haemophilus influenzae*** type B). Since the 1990s, Brazil has shown an important reduction of the incidence of cases by expanding the vaccine coverage. In that decade, the incidence reached 0.45/100,000 inhabitants, decreasing as the coverage increased. Between 2008 and 2017, only 10 deaths caused by the disease occurred in Brazil[31]. The Butantan Institute reports that the antidiphtheric serum is the only effective drug for neutralization of toxins produced by the ***diphtheria*** bacillus (***Corynebacterium diphtheriae***). Serum, when applied to a patient diagnosed with diphtheria, acts by neutralizing the toxin produced by the diphtheria bacillus. By applying the antidiphtheric serum, the antibodies contained in the serum specifically bind to the toxin and thus neutralize its toxic actions in the body.[32]

According to the Butantan Institute, the antitetanic serum has the function of neutralizing the toxins produced by the tetanus bacillus (***Clostridium tetani***) and is intended for the treatment of patients with accidental or neonatal tetanus, for the prevention of tetanus in patients with injuries, not vaccinated, with uncertain vaccination or with less than three doses, or vaccinated with three doses, the last dose being for more than 10 years. The specific immunoglobulins (antibodies) generated by the immunization of horses produce the purified antitetanic serum that acts by neutralizing the tetanic toxins that are in the blood circulation.[32] ***Tetanus*** is a noncontagious acute infectious disease caused by fixation in the nervous system of exotoxins of ***Clostridium tetani***. Clinically, tetanus manifests by hypertonia of the jaw and neck muscles, and may reach progressive muscle stiffness and generalized muscle contracture, affecting the rectum–abdominal muscles and the diaphragm, leading to respiratory failure.[33]

**Third generation**

Gene (or third–generation) vaccines are so called because they are composed of DNA–coding plasma proteins of pathogens, allergens and tumors and encode potentially immunogenic antigens using gene fragments.[34]

DNA vaccines are so called because the antigen is synthesized ***in vivo*** after the direct introduction of their encoding sequences. They present a unique method of immunization that can solve many of the shortcomings of traditional vaccines. Other advantages are low cost, relative facility of manufacture, thermal stability, possibility of obtaining multivalent vaccines and rapid development of new vaccines in response to new strains of pathogens.[35] These vaccines are being evaluated as prophylactic and therapeutic treatments for infectious diseases, allergies and cancer. Plasmids that encode normal human proteins are also being tested as vaccines and treatments for autoimmune diseases.[36] Since cells do not accept receiving DNA from different cells, a vector playing this role is required, as is the case of DNA vectors. This must occur for DNA to encode an antigenic protein. The easiest method to produce and characterize gene transferring is the direct application of pure plasmid DNA. It is often applied by intramuscular injection, although it can be administered by several other routes such as oral mucosa, vaginal and epidermal layer by "gene guns".[35]

Although rodent research shows efficient immunogenicity. the lower efficacy of DNA vaccines in experiments on big animals, including humans, is due to the inefficient delivery of DNA plasmid to cells.[37] Ulmer et al. found it was sufficient to transplant stably transfected myoblasts expressing the influenza nucleoprotein to induce cell–mediated protective immunity. The mice used in the test produced high titer antibodies and cytotoxic T lymphocytes, which protected them from a lethal cross–strain test with influenza A virus. The authors concluded that the expression of the antigen by muscle cells is sufficient to confer protective immunity mediated by cells.[38]

Plasmid DNA vaccine has also been widely explored for immunization against tuberculosis. Zhang et al. produced a plasmid DNA vaccine that encodes Ag85A and GM–CSF genes and provides immunological protection against the ***Mycobacterium tuberculosis*** bacillus in mice tests. The authors showed that the use of electroporation allows a single intramuscular injection of DNA to be as effective as repeated injections of DNA in activating Ag85A–specific T–cells.[39] Until 2015, four DNA vaccines have been licensed for veterinary uses. They are vaccines against West Nile virus in horses, against infectious hematopoietic necrosis virus in salmon and for the treatment of melanoma in dogs (malignant neoplasia).[40]

The biotechnological advance of vaccines, which were previously manufactured with inactivated, live attenuated and sub–unit (first and second generation) micro– organisms, is evident. By 2020, other technologies such as a better comprehension of the innate immune system and the development of new adjuvants should contribute to the improvement of existing vaccines and the development of new vaccines to prevent and treat many types of infections.[41]

While development brings benefits to society such as increased employment opportunities and a warming economy, human health is the first to suffer the malefic consequences of reduced quality of life due to a lack of basic sanitation in a scenario of mass migration to urban centers as a result of industrialization. Advances in Biotechnology have made it possible to develop new technological products, including vaccines, which are increasingly effective in protecting health.

Brazil is one of the countries with the highest coverage of infantile and adult vaccination, as it has presented successful vaccination strategies. The national immunization program is considered an example for the whole world.

Immunization by the attenuated pathogens discovered by Edward

Jenner has given the world's population more security, health and a long–life expectancy. Smallpox, poliomyelitis, rubella, measles, diphtheria and pertussis are examples of diseases that were, at some point in human history, pandemics or epidemics that ravaged continents for decades or even centuries, and today are eradicated or with regional foci and very low mortality rates. However, diseases such as tuberculosis, AIDS, Ebola, influenza, yellow fever and cholera continue to affect millions (if not billions) of people because first– and second–generation vaccine immunization methods are ineffective against the microorganisms that cause such diseases.

One of the areas in which Biotechnology has made a great progress is health. New technologies, such as gene sequencing, have made it possible to discover new routes to eradicate viruses and bacteria that are harmful to human health. Manipulation of DNA has led to an evolution in treatment and prevention of diseases, despite ethical questions that may be raised. DNA or third–generation vaccines, although currently ineffective, are promising in the fight against diseases such as cancer and AIDS. Many researches have been carried out in the area of Biotechnology with the objective of bringing more safety and efficacy in the combat and eradication of diseases considered today without cure.

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# The Fourth Industrial Revolution and the disruptive technologies: implications in work relations

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**Abstract:** This research covers an analysis of the essential aspects involving the Fourth Industrial Revolution, disruptive technologies and their consequences on work relations. Using literature reviews and analyzing cases of companies that failed and others that remodeled themselves to survive the yearnings of the technological age it was possible to obtain important results. The research was conducted in three parts: (i) historical contextualization guiding the reader on the main aspects and peculiarities of the Fourth Industrial Revolution; (ii) definition, application and some examples of disruptive technologies; (iii) confirmation of the impact of these technologies on work relations. The methods used were: hermeneutics, privileging theoretical studies and analysis of documents and texts and the deductive method, starting from existing laws and theories for the development of a logical reasoning to explain the central problem. Negative impacts of mass unemployment due to the replacement of human labor by highly technological machines cannot be estimated. These machines are part of what has been called disruptive technology, i.e., an innovative product that destabilizes competition, overcoming it in such a way that it promotes the rupture of existing models, ruining them. Professionals will be called to fill new jobs, with skills and competencies for Industry 4.0, whose interaction between man and machine will be essential. Use of big data in quality control, robots, fully automated vehicles, 3D printers in production lines, among other activities are examples of work demands.

**Keywords:** Fourth Industrial Revolution; Disruptive technologies; Work relationships.

## Introduction

The technological revolution and the new business dynamics imposed by capitalism, with a view to free trade and the Fourth Industrial Revolution have affected work relations, business models have been created, labor rights have been mitigated, but the great concern is about jobs and the future of workers, because the impacts will be even greater as there will be elimination of traditional jobs and, consequently, significant increase in unemployment rates.

It is well known that the Fourth Industrial Revolution is happening and, allied to it, paradigm shifts and the implementation of the so-called disruptive technologies, directly affecting the productive process, the routine of companies, promoting such a restructuring that it is already being talked about replacing human work with machines in high percentages and, in many activities, the replacement will be so accentuated that it will cause the extinction of professions, leaving a large number of unemployed people.

There is no doubt that the technological revolution and the new entrepreneurial dynamic imposed by capitalism towards free trade and the Fourth Industrial Revolution have affected work relations. However, there are positive aspects to be considered, such as greater convenience of society, innovation with research, scientific and technological development never seen before, but there are also extremely harmful points to be analyzed, involving government, human conduct (interpersonal and practice of offenses), environmental degradation, and increasing inequality and poverty. In short, revolution is synonymous of transformation and it is not always for good, so it is necessary to discuss the subject of this work, with a view to overcoming the original barriers of Revolution 4.0, considering what humanity intends to reach, what values should be protected and what measures should be taken to minimize the detrimental impacts on human development itself and the maintenance of our own essence: humanity.

## Objectives

In general terms, the objectives are to analyze the relevant aspects of the Fourth Industrial Revolution, defining disruptive technologies, verifying the impacts caused on work relations by technology, robotics and automation and studying some work modalities based on technological advances. In this way, this work demonstrates the relevant aspects of the Fourth Industrial Revolution and the impacts caused on work relations by technology, robotics and automation.

But what is the repercussion of all this? How to prepare for this scenario? What is the role of the governments? These and other questions were addressed in this work, envisioning the adoption of means and public policies which minimize the negative and harmful impacts on human development.

## Methodology

It is intended, by a literature review, to analyze doctrinal studies, articles, current laws and other materials that may be necessary for the pursuit of the objectives outlined in this research. The methods used are: hermeneutics, privileging theoretical studies and analysis of documents and texts and the deductive method, starting from existing laws and theories for the development of a logical reasoning to explain the central problem.

## Historical Contextualization

The Fourth Industrial Revolution began at the end of the 20th century, based on the digital revolution, which is characterized for uninterrupted access to the Internet through mobile devices (cell phones and notebooks, for example), smaller size, greater processing capacity and mass production of hardware that has become cheaper and for artificial intelligence and automatic learning. The so-called Revolution 4.0 is much more complex and goes far beyond real-time connected intelligent sys-

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tems and machines. Other areas, such as genetic sequencing, nanotechnology, renewable energies and quantum computing are also relevant.[1]

Drawing a historical overview of all the industrial revolutions, as to their main characteristics and the time of transition from one to another, it is possible to state that the Fourth Revolution develops much faster, being considered by researchers and historians the largest revolution in industry since the First Industrial Revolution, occurred in the 18th century (Table 1).

| INDUSTRIAL REVOLUTIONS | | | | |
|---|---|---|---|---|
| | Period | Milestone | Main features | Main impacts |
| 1st | Half of the 18th century – from 1760 to 1860, began in England. | Invention of the steam engine and its application in textile production; Invention of new steam transport systems, such as locomotives. | Coal mining as a new source of energy; Increasing and accelerating production (large-scale production mode); Replacement of human labor. | Replacement of human labor (unemployment) and cheapening of the labor force; Rural exodus, growth of cities; Endemic diseases; Increase in production, in the number of factories and in competitiveness. |
| 2nd | Half of the 19th century – from 1860 to 1960. | Electricity and the discovery of oil as a source of energy. | Faster technological development; Development of the chemical and steel industries; Industrial organization and production models developed by Frederick Taylor and Henry Ford; Innovations: automotive, telephone, radio and aircraft. | Faster progress in the industry than in agriculture; machine-made and factory-mounted products; Capital-controlled industrial production; Workers' movements for rights (wages, reduced hours, health and safety at work); Verticalization of labor relations (separation: intellectual and manual labor); The US was elected as the world's main industrial center with the emergence of Ford's revolutionary production techniques. |
| 3rd | After the Second World War – from 1960 to 1990 (20th Century). | Nuclear power discovery. | Emergence of electronic equipment, telecommunications and computers; Space exploration (man reached the moon); Research in the field of Biotechnology; Invention of robots and automata; Toyotism production mode, created by Taiichi Ohno, a Toyota employee. | The changes outweigh the industrial transformations. In this phase, technological processes are born from the interaction between science and production, also known as the techno-scientific revolution. |
| 4th | Late 20th century – from 1990 to the present day. | Interconnection of all stages of production, based on the scanning of information – storage and use of data. | Artificial intelligence: machines "learn" and perform activities without human interference; High data and information storage capacity; Power generation with lower pollutant index; Use of nanotechnology for scientific innovation, creation of materials and improvements in health – widely used in information technology, communication, medicine and pharmaceuticals. | This phase has barely begun and the impacts can be seen in all sectors of society (economic, political, social and environmental). Cutting-edge technology, the emergence of virtual currencies (bitcoin), 3D printing, big data (data set on the internet motivated by the increased speed of the internet), robotics, augmented reality, artificial intelligence and nanotechnology are examples of this revolutionary historical phase. |

**Table 1** – Relevant aspects of Industrial Revolutions. **Source**: Adapted from Penprase, 2018.[2]

As can be seen, technology and digitalization have only started to influence the production system in general. The evolution and achievements of the Fourth Industrial Revolution continue at a surprising rate.

According to Anderson,[3] the last decade has been about discovering new ways to create, invent and collaborate on the internet. The next decade will aim to apply that learning to the real world. Disruptive technologies have contributed to this result. Briefly, disruptive technology is an innovative product or service (creates a new market) that destabilizes the previously dominant competition. Disruptive, because it comes from a rupture, these products or services transform the market in such a way that they promote a true rupture, the ruin of the previously existing models.[4] The services provided by Uber, Apple, Netflix and Google are examples that revolutionized ways of locomotion, listening to music, watching movies and searching for information on the internet, respectively.

Technologies considered disruptive, for example artificial intelligence, natural language processing and computer vision, are turning from revolutionary ideas into essential business tools, and this is just the beginning.[5]

In addition to the positive impacts from the use of disruptive technologies, there will be extremely negative impacts, harmful to society and the state itself in terms of work relations, which cannot be prevented, because the global economic scenario denotes high competitiveness and whoever goes ahead will lead the ranking of the most powerful companies.

Schwab states that the Fourth Industrial Revolution will have a tremendous impact on the world economy, will be vast and multifaceted and will not be able to separate one effect from the other.[1]

Therefore, the analysis of the impacts on labor relations from the disruptive technologies in the Fourth Industrial Revolution is of paramount importance and general interest, mainly with the intention of creating mechanisms for governments to fulfill its role in the protection of fundamental social rights, guaranteeing the constitutional principles and precepts, among them the right to work and a decent life.

**Definition, application and examples of disruptive technologies**

The name "disruptive technology" is strange at first, but it is responsible for breaking current paradigms, for the serious impacts on the economy, politics, economy and society – people already use disruptive technologies in daily life, companies are investing more and more in the search for the "new that implicate", that is, the disruptive technologies are a reality of this century and make history in the Fourth Industrial Revolution; there is no stopping them; studies, development of strategies and public policies to remedy and prevent their negative impacts are essential.

The word "disruptive" means something that causes or can cause disruption; ultimately it interrupts the regular follow-up of a process; it has the ability to break or alter.[6] Therefore, disruptive technology is that which breaks standards, models or technologies established in the market. Instead of being an evolution of a product or service, it is a disruption.[7] Table 2 shows the main disruptive technologies.

| DISRUPTIVE TECHNOLOGIES | |
|---|---|
| Analytics | Allows to creation of relationships between variables and business data directing the entrepreneur (or managers) to make decisions in sales, logistics and finance. |
| Cryptocurrencies and blockchain | Cryptocurrencies are currencies and digital transactions.<br>Blockchain is a decentralized and secure technology. It would be a kind of "accounting book" (it registers transactions, being mirrored by numerous PCs, for auditing by anyone, but cannot be copied or altered by them). |
| Shared economy | Especially for travel and journeys through private car sharing – reduction in the sale of goods (such as cars and houses), it will not be necessary to "have" to "use". |
| 3D printing or rapid prototyping | Additive manufacturing technology from a three-dimensional model, created by printing on successive layers of material; used for the construction of buildings, consumer products such as household appliances, footwear and human organs. |
| Artificial intelligence | Robotic devices, some with the ability to learn from their experiences. |
| Internet of things (IoT) | Connection of machines via internet for data sharing. |
| Virtual and augmented reality | Computational interface techniques, there are three-dimensional interactive technologies (3D) that allow simulating product characteristics, perform training of people, application in games and use in e-commerce. |

**Table 2 –** Main disruptive technologies: Industry 4.0. **Source:** Adapted from Schwab[6].

Technological innovations have brought about major and relevant changes around the world, but that doesn't stop there. It is estimated that, in the next 20 years, the technology will be hundreds of thousands of times greater compared to today.[8]

For Schwab, technology and digitalization will revolutionize everything. To try to explain why current disruptions and innovations are so significant, Schwartz states that the speed of innovation has been faster than ever in terms of development and disruption. The existing disruptors such as Airbnb, Uber, and Alibaba, which are now benchmarks for companies in their industries, were relatively unknown a few years ago. The iPhone was launched in 2007 alone, and by 2015 there were already about 2 billion devices.[6] In 2010, Google announced its first fully autonomous car, these vehicles will become a common reality on the streets.[1]

The advancement of technology, the form and speed in which it has presented itself in recent times and the advances observed since the twentieth century are immense and extremely rapid compared to previous centuries. Technology is evolving faster than human capacity.[9] Current mobile phones are more "powerful" than the NASA computer that took Apollo 11 to the moon on July 16, 1969.[8]

Today's smartphones are compact, lightweight mobile phones with extremely powerful processors, high data storage capacity (up to 256 GB), extensible by attaching memory cards, high–resolution cameras, the possibility of recording videos in 4K, in short, a multitude of possibilities and functions that can be carried in pockets. Today's mobile phones are more "powerful" than the NASA computer that took Apollo 11 to the moon on July 16, 1969 (Figs. 1, 2 and 3).[10–14]

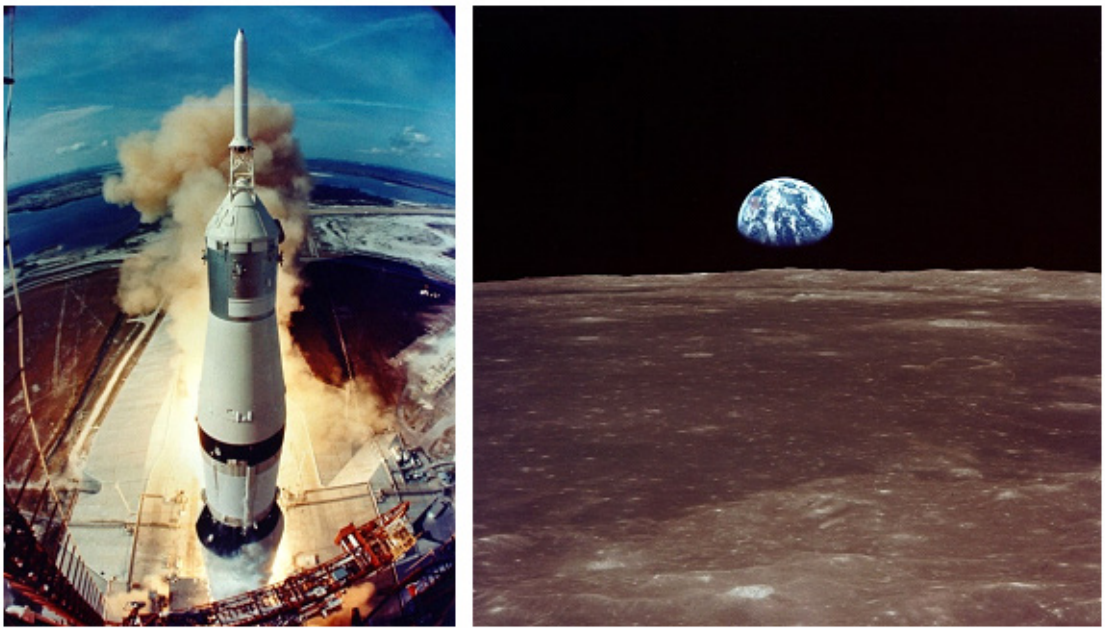
Companies that do not project and invest in technology will be doomed to failure and, of course, will close their doors. Like Kodak, a large company, consolidated in the photographic market in the 2000s. In the 1970s, Kodak held 80% of the market for cameras and 90% of photographic films. In that same decade, the company invented the digital camera, but "turned off" the technology by assuming that this new product would hurt its film sales. In 2002, the cameras with film lost space for the digital ones, the photographic market changed substantially, with a decline in the development of printed photographs, which became files able to be homemade printed by their users from the connection in USB cables in computers and, later, sent by signal, Bluetooth or the internet.[15]

In 2008, Kodak's largest share of revenues came from patent licensing rather than from the sale of its products and services. In January 2012, the company went into bankruptcy and participated in the government's program to protect companies for financial recovery. Apple, Google and other brands were major competitors, which led to several Kodak patents. It is reported that Kodak currently works in the business–oriented printing market and also in the packaging market, besides keeping contracts with movie studios to supply films. In 2016, the company tried to launch a smartphone called Ektra, relaunched a Super 8 camera and an action cam called PixPro, but did not have good reception in the market.[15]

With the Fourth Industrial Revolution, even digital camera manufacturers faced problems and fired many employees. Smartphones have increasingly technological cameras, some with up to three cameras, bringing convenience and high technology to users.

Another example is Atari, a Silicon Valley company that weakened in the 1980s, and even had to bury thousands of unsold videogames and take the loss. When the market recovered, other more innovative companies had taken the lead, such as Nintendo. Atari went bankrupt and was sold in 2008 with the intention of keeping the valuable brand alive.[16]

These examples reflect on the society and economic landscape of countries, especially those that do not project themselves and prepare to face the impacts and possible losses. How many unemployed people will be launched inday to meet the demands of this new, dynamic and demanding reality of full–time connected consumers, thirsty for the latest technological releases. The answers should be sought from a careful analysis of each sector of the economy, to practice actions to minimize the losses and how to remedy them.



Left: Saturn V rocket takes off on the morning of July 20, 1969. Right: "Earthrise" seen from the moon.

**Figure 1 –** Man's arrival on the moon in 1969. **Source:** NASA. The use of these images is regulated under the Creative Commons license [10,11]

Left: ENIAC (Electrical Numerical Integrator and Calculator), the world's first computer (1946). (This image is a work of the U.S. federal government and is in the public domain).[12] Right: IBM 5100, one of the first personal computers to be commercialized (1975) (the use of these images is regulated under the Creative Commons license).[13]

**Figure 2 –** The world's first computers.

Built by IBM and Nvidia for the U.S. Department of Energy's Oak Ridge National Laboratory, the Summit is a 200 petaflops machine (can do 20 quadrillion calculations per second). (the use of these images is regulated under the Creative Commons license).[9,14]

**Figure 3** – Summit: world's most powerful computer.

**Impacts of disruptive technologies on work relations**

Industry 4.0 will promote even more impacts, especially in work relations regarding employability and the need to improve skills for handling new technologies based on the qualification of the professional.

Tessarini Junior and Saltorato,[17] after studying the impacts of Industry 4.0 on the organization of work, categorizing four main impacts that are distinct, but interrelated and interdependent:

- Increased technological unemployment in return for the creation and/or increase of more complex and skilled jobs;
- Need to develop new skills and abilities;
- Greater interaction between man and machine;
- Changes in socioprofessional relations.

Although there are doubts about job opportunities and jobs that will be extinguished, the fact is that there will be replacement of human labor by technology, whether by robotics or artificial intelligence, triggering the phenomenon of technological unemployment; consequently, there will be an increase in social inequality and reflections on the economy, politics and society itself, such as an increase in depression and violence. Therefore, it is time for governments to rethink their structures and develop regulatory and social policies to contingent the evils arising from technology.

It is already possible to draw an overview of the transformations in work relations, the reduction or even extinction of some professions, the creation of new jobs (Table 3).[17]

Some professions are more prone to automation, whose jobs will be reduced or even extinguished: telemarketing operators, people responsible for tax calculations, insurance appraisers, sports referees, legal secretaries, real estate brokers, agricultural workers, couriers and messengers.

Speed in development and technological innovation is exponential. According to Schwab, the world is multifaceted and deeply interconnected; new technologies generate even better and more technological products; the impact is systemic, entire systems will be transformed (among countries and within them, companies, industries, government and society); there is no time to wait, action is inevitable.[1]

Artificial intelligence and robotics are gaining space and even conquering people, since they achieve more and more accurate results, with low probability of errors, in the short term, that is, the service improves and becomes faster.

In the medical area, it is already common to use robots in surgeries, screening care and diagnostics of some diseases. A study called *What doctor? Why AI and robotics will define new health,* conducted by Price Waterhouse Coopers, showed that most of the interviewees have no objections in receiving robot care (clarification of doubts, testing, diagnosis and treatment of diseases until small surgeries). About 11,000 people from 12 countries in Europe, Africa and the Middle East were interviewed, 55% of the participants said they were willing to be assisted by robots with artificial intelligence for faster access to health services (36%), speed and accuracy of diagnoses (33%), thus improving care and treatment; it was clear that confidence in technology is increasing. Lack of confidence in the robots' ability to make decisions (47%) and lack of human contact (41%) were cited by those who are not willing to undergo a robot–controlled treatment.[18]

It will be necessary to find a way to transform artificial intelligence into artificial assistants to help workers, train people, rethink professional training to meet this new reality. In addition to these aspects, other concerns have already arisen and have become daily accentuated. The use of artificial intelligence for illicit purposes such as scams (frauds), improper capture of personal data, attacks, cyber–attacks, fake news, industrial espionage, in short, the technology has brought benefits and criminals are also reinventing themselves with them.

The market will need qualified, competent professionals, not only capable of planning, executing and managing the new technologies, but also creative, proactive and innovative, and companies will need to provide new work environments with adequate structures, both in logistics and telecommunications.

Stakeholders in global society must come together to understand emerging trends, seeking solutions to problems arising from technological advances, among them, to update and develop survival mechanisms, because, as can be seen, disruptive technologies are available to various segments and business sectors that range from the operational area to strategic decision–making by company managers. Their use can optimize operations, reduce (or even avoid) expenses, eliminate error margins due to lack of information, reduce time in the production and execution of products and services, promoting faster processes, improving the purchasing experience and consumer satisfaction and, associated with all this, mass unemployment, the extinction of professions and the annihilation of companies that do not reinvent themselves based on this new reality and their perspectives.

| TRANSFORMATION | JOB REDUCTION | JOB CREATION |
|---|---|---|
| Use of big data in quality inspection | Specialists in quality inspection | Industrial data analysts |
| Use of robots, autonomous vehicles and 3D printers in production lines | Production, assembly and packaging operators Logistics personnel | Robot coordinators Engineers and specialists in research and development |
| Supply networks and autonomous and intelligent production lines | Specialists in production planning | Specialists in data modeling and interpretation |
| Automated predictive maintenance | Traditional maintenance technicians | Data, systems and IT analysts |

**Table 3 –** Transformations versus jobs. **Source:** Adapted from Tessarini Junior and Saltorato.[17]

## Conclusions

The Fourth Industrial Revolution (or Revolution 4.0) began at the end of the 20th century, whose milestone is the interconnection of all stages of production, based on the digitalization of information, storage and use of data.

Artificial intelligence, high capacity for storing data and information and the speed of the internet, energy generation with lower levels of pollutants (clean or renewable energies), the use of nanotechnology, the emergence of virtual currencies, 3D printing, and augmented reality are examples of this revolutionary, new and highly technological era, which has promoted unprecedented changes in the economy, business, society and individuals, including their way of being, seeing and understanding the world. It is a total breakdown of paradigms, a historical phase that is just beginning.

The negative impacts of mass unemployment due to the replacement of human labor by highly technological machines cannot yet be estimated, which has been called disruptive technology, i.e., a product or innovative servant destabilizes competition, overcoming it in such a way that it promotes the rupture of existing models, ruining them. There are positive aspects such as technological advancement, comfort, ease and greater convenience for users (of society in general), but also harmful aspects, especially in work relations – all will be affected (companies, society and the governments). The world needs to reflect and prepare itself. The governments will lose revenue from tax collection; innovation in criminology with cybercrime and the use of AI for illicit practices; there will be an increase in poverty and in the amount of waste, due to the incentive to consumerism with the turnover of technological products launched and put on the market very quickly. Companies will need to adapt to this new reality, technological innovation is the watchword to maintain competitiveness.

Professionals will be needed to fill new jobs, with skills and competencies for Industry 4.0, whose interaction between man and machine is essential; use of big data in quality control, robots, fully automated vehicles, 3D printers in production lines, among other activities are examples of labor demands.

Undoubtedly, there are many challenges, but we must be prepared and overcome them, remodeling the industrial processes (production, consumption, transportation, logistics, among others) and work relations based on the demands of the technological revolution that has transformed humanity.

Revolution is synonymous of transformation and radical changes, but such changes and paradigm breakers should focus on the human beings and their well-being, safety and protection; everything that harms life, in its broadest concept (psychophysical integrity and dignity) should be seen sparingly. In this sense, the academic community must mobilize and assist the State in the adoption of policies capable of minimizing such impacts and ensuring work and dignity to the nation.

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# The importance of Biotechnology in the health area: historical overview and future perspectives

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**Abstract: Biotechnology, in a broad sense, comprises the manipulation of microorganisms, plants and animals, with the proposal to obtain processes and products of interest to society. Although the term appeared at the beginning of the 20th century, biotechnological processes have been present since ancient times in the production of fermented food and beverages. Biotechnology has also been present since the beginning of Medicine. Inhaling powder from sun-dried human smallpox wounds or using secretion of the udders of cows contaminated with bovine smallpox are examples of ancient techniques used as methods of immunization of the disease. In this sense, many treatments have emerged over time, such as vaccines, the production of penicillin, the first antibiotic and the production of insulin by recombinant DNA, which have revolutionized Medicine and enabled a better quality of life for humans. Many products and materials from Biotechnology are still emerging, such as scaffolds in Regenerative Medicine for bone tissue recovery, bacterial cellulose tubes used as artificial blood vessels, induced pluripotent stem cells and microphysiological systems, which promise to further improve modern Medicine and provide better techniques and therapies for various treatments.**

**Keywords: Biotechnology; Vaccines; Scaffolds; Regenerative Medicine.**

## Introduction

Biotechnology, a word derived from the Greek ***bios*** (βίος) life; ***technos*** (τεχνης) practice, and ***logos*** (λόγος) thought or study, is conceptually recognized as the union of Biology and Technology. However, there is no consensus about its definition and various ways of defining it can be found. According to Faleiro and Andrade,[1] "in its broader meaning, Biotechnology is defined as the use of living beings, their parts or products for the production of goods and services". According to the Brazilian Ministry of the Environment,[2] "Biotechnologies, in its broadest sense, include manipulation of microorganisms, plants and animals, with a view to obtaining processes and products of interest to society".

There is also a discussion on what is meant by classical and modern biotechnology. According to Brazilian Ministry of Industry, Foreign Trade and Services,[3] classical Biotechnology refers to traditional processes such as fermentation, genetic improvement by selection, the use of living beings or their parts to produce goods and services. Modern Biotechnology, according to Faleiro and Andrade[1] started with the discovery of DNA that enabled a revolution in the areas of Genetics and Molecular Biology.

Although the term Biotechnology appeared at the beginning of the 20th century, the techniques of classical Biotechnology have been used for quite some time. It is estimated that fermentation processes for the production of food such as cheese and wine date back to 6000 BC by the Assyrians and Babylonians.[1,4–6]

Classical Biotechnology techniques were also used in antiquity for therapeutic purposes. The Chinese practice of inhaling dust from sun-dried smallpox wounds and the use of bovine smallpox wound secretions to prevent human smallpox are good examples of these techniques.[7,8]

## Objectives

The objectives of the present work focus on presenting a brief historical overview of the contributions of Biotechnology to human health and pointing out some future perspectives that may further improve Medicine and possibly provide new technologies and therapies for various treatments.

## Methodology

A brief bibliographic survey was conducted in databases, books and scientific articles related to Biotechnology, its definitions and applications in the area of Medicine. In addition, numerical and statistical data cited in the present study were taken from sources and reports of national and international entities, as well as from scientific articles and websites present in the literature.

## Literature Review

### Variolation And The Origin Of Vaccines

The history of the emergence of vaccines is directly related to a very old disease, today practically eradicated: smallpox. There are ancient Egyptian, Indian and Chinese writings that already described the symptoms of the disease. In 1898 the mummy of Pharaoh Ramses V was discovered (dated 1157 BC) with signs of skin eruptions quite characteristic of people with this disease.[9] Smallpox was responsible for the death of millions of people around the world. It is estimated that the disease prevailed in China and India around 1000 BC. However, the first recorded epidemic occurred in Arabia during the sixth century. The disease is believed to have spread throughout North Africa and Europe during the sixth, seventh and eighth centuries[9] as a result of Arab invasions. According to Behbehani,[9] the disease was reintroduced in Europe due to the Crusades, which occurred between the years 1096 and 1291, and stayed there. During the 18th century, smallpox was estimated to have been responsible for the deaths of approximately 400,000 people a year in Europe and caused about a third of all blindness on the continent.

Variolation was one of the first techniques of smallpox immunization. The method was practiced in different ways by different people. However, in short, the practice consisted of the same principles, which promoted the contact of healthy people with parts of wounds or pustules

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of contaminated people in the hope of developing a milder form of the disease, and so becoming immune.[10] More than 500 years ago in China, sun–dried smallpox wounds were used to prevent the disease. The method was based on the exposure of pathogens to solar radiation that weakened them. Thus, when inhaling the powder obtained, the body would come into contact with a more harmless version of the virus and become immune.[7,9] Variolation has been reported in Persia where dry pustules powder was taken as a form of immunization. In Greece, Turkey, Arabia, North Africa and the Caspian Sea region, the practice consisted of rubbing pustules from wounds on the scratched skin of children.[9]

It was only after the emergence of a safer and more efficient method of smallpox prevention that variolation was left aside. During his studies, around 1780, Edward Jenner observed that bovine smallpox could immunize people from the human version of the disease. According to Behbehani,[9] Jenner heard from a dairy patient who treated pustular wounds on her skin that her disease, although similar, could not be smallpox because she had already been contaminated with bovine smallpox. This was a very popular knowledge among local farmers. Thus, Edward Jenner discovered, by performing several tests, the efficiency of using wound secretions present in udders of cows contaminated with a specific type of bovine smallpox (because he observed other variations attributed to the same disease in cattle) in the immunization of human smallpox. Therefore, he developed a method very similar to variolation, but using secretions from cows, and the first vaccine appeared, a term derived from the Latin *vaccinus* which means "derived from the cow".[9,11,12]

During the 19th century, vaccination against smallpox became a worldwide practice, especially in Europe and North America, but the principles learned from Jenner's results remained dormant for more than a century and a half, during which no new vaccines appeared. Despite being shown to be effective in immunization, Jenner's vaccine suffered protests and objections from the population and the church, as the possibility of death of the inoculated was recognized (from two to three deaths out of every 100 inoculated individuals). In addition, there was the fear on part of the population that this operation consisting in the introduction of material extracted from cows would transfer bovine characteristics, such as bovine features, birth of horns and mooing, to the "vaccinated". Thus, it was necessary to increase the reliability of vaccines and eliminate the idea that diseases would be "divine punishment".[11,13,14] Figure 1 shows a James Gillray's cartoon drawn at 1802 which demonstrates the population's concern about Jenner's vaccines.[15]

Much of this disbelief was due to ignorance of the cause of the diseases. Even at that time, experiments were being conducted to obtain

**Figure 1 –** English charge from the 19th century showing people acquiring bovine characteristics after immunization. **Source:** The British Museum[15]. Image in the public domain.

evidence that would discredit the theory of spontaneous generation in favor of the germinal theory of the disease. Louis Pasteur and Robert Koch, however, developed research that could prove that infectious diseases were caused by microorganisms.[11,13,14,16]

Pasteur, by studying the metabolism of microorganisms, has discovered ways of transforming pathogens to produce vaccines and other new ways of preventing and treating infections. Thus, techniques such as the attenuation or inactivation of pathogens that enabled the immunization of several diseases, such as rabies, typhoid fever, cholera and black plague (bubonic plague)[13] have emerged.

**Penicillin discovery**

Another great contribution of Biotechnology to modern Medicine is the production of penicillin, the first antibiotic. The world's population, especially in Europe and Asia, has suffered several cases of pandemic in the pre–antibiotic era. It is estimated that the black plague alone, caused by the bacteria *Yersinia pestis*, was responsible for at least three pandemics in history, causing almost 100 million deaths in Europe and the outbreak between 1895 and 1930, with about 12 million victims, mainly in India.[16]

For thousands of years, when nothing was known about the cause of infections, prevention methods, antibiotics, and vaccination, mankind was tortured by massive epidemics such as syphilis, smallpox, malaria, typhoid, yellow fever, leprosy, tuberculosis, Spanish flu, cholera, and black plague, to name only a few examples.[16]

The accidental discovery of penicillin in 1928 by the English physician Alexander Fleming[17] inaugurated a new era in Medicine, since infectious diseases that would normally lead to death could be cured. Penicillin is a substance secreted by the fungus *Pennicilium* and has proven to be more efficient in controlling bacterial proliferation compared to chemical

substances used at the time, such as salvarsan (used in the treatment of syphilis), proflavine (widely used in World War II, mainly in deep wounds) and sulfas or sulfonamides (prontosil).[18] However, the antibiotic was only introduced as a therapeutic agent in the 1940s. After the process of industrialization of penicillin, especially as a consequence of World War II, a rapid growth was observed in the discovery and development of new antibiotics.[18]

**Diabetes and the production of human insulin by recombinant DNA**

The production of synthetic insulin by transgenic microorganisms was also a major milestone in biotechnology in the field of Medicine for the treatment of diabetes. Diabetes mellitus is a very old disease. There are records of his symptoms dating from 1500 BC, such as frequent and abundant diuresis, uncontrollable thirst and marked weight loss as its main clinical manifestations. Aretaeus, a Roman physician, created the term diabetes which means "going through" because of excessive diuresis, one of its most evident symptoms.[19,20]

It was only in 1889 that Joseph von Mering and Oskar Minkowsky, during their research on fat digestion, observed that removing the pancreas from dogs triggered very similar symptoms of the disease. In 1908, the scientist Georg Zuelzer developed the first injectable pancreatic extract that suppressed glycosuria, but this therapeutic modality did not evolve due to its adverse effects.[19,20]

Insulin was isolated from pancreatic extract in 1921 by Frederick Banting and Charles Best in the laboratory of physiologist John Macleod. The first test for decreased glycosuria and ketonuria was performed in 1922 by injecting 15 mL of pancreatic extract in a 14–year–old patient. However, the effect obtained was lower than expected, in addition to causing abscesses at the place of application. Only after purification of the extract by biochemist James Bertram Collip, which had the desired effect immediately. The discovery of the relationship between pancreatic secretion and diabetes mellitus earned the Nobel in Medicine and Physiology due to this therapeutic achievement.[19,20]

The first commercially produced insulin, the regular insulin or insulin R, extracted from animals, has a rapid mechanism of action, which required several applications throughout the day yielding several complaints from patients. Thus, other types of insulins with longer action time were produced, such as NPH insulin (neutral protamine Hagedorn), with addition of protamine basic protein, and PZI insulin (protamine zinc insulin) with addition of zinc in its composition. However, in clinical practice, different complications of the use of these insulins were observed, such as allergic conditions, lipodystrophy at the sites of application and, most importantly, immunological resistance to insulin.[19,20]

With the advancement of Molecular Biology, the era of human biosynthetic insulins began. Its principle is based on the genetic modification of bacteria to induce the production of the hormone used in the treatment of diabetes. The technique has five general procedures: to fractionate the DNA in specific locations using restriction enzymes; to unite DNA fragments in a covalent way (DNA ligase); to select a DNA for cloning vector, that is, that can self–replicate; to transfer the DNA to a host cell; and to select the cells with the recombinant DNA. The bacteria *Escherichia coli* was the first organism used for rDNA work and is still the most common host cell.[21]

**Biotechnology perspectives for the medicine of the future**
**Regenerative Medicine**

With the development of techniques and new biotechnological materials, the prospects for health are very promising. In particular, the use of bacterial cellulose in the production of scaffolds (three–dimensional matrices for cell growth) with potential application in Regenerative Medicine stands out.

Regenerative Medicine is a branch of Medicine that proposes to restore or replace cells, tissues and organs to recover their functionality. According to Porcellini,[22] it is a very recent area, it appeared in 1997 when Whitman et al.[23] proposed the integration of platelet–rich plasma adhered to fibrin for possible maxillary bone regeneration. One year later, Marx et al.[24] *there was also a greater bone density in grafts in which platelet–rich plasma was added (74.0% ± 11%* demonstrated that platelet rich plasma is capable of inducing mandibular bone regeneration.[24]

Bone regeneration consists of the treatment of conditions such as trauma, osteosarcoma (primary bone tumor), osteomyelitis (severe infection of bone tissue) and osteitis (inflammation of bone tissue), and has been a major challenge in surgical practice. Many therapeutic approaches such as allografts (transplantation between different individuals of the same species), xenografts (surgical transplantation between individuals of different species), autografts (transplantation taken from the individual himself) and artificial grafts have been employed to restore bone function. However, such approaches have limitations, such as the low amount of bones donated or removed from the patient, risk of immune system response, possible infections and death of tissues that hinder their application. In addition, synthetic prostheses are hardly capable of mimicking true bones and interacting well with surrounding bone tissues, which can cause some fatigue.[25] Therefore, Biotechnology applied to bone regeneration has proven to be very promising, especially scaffolds in Tissue Engineering. The scaffolds can promote different types of mechanisms for bone regeneration such as osteoconduction (ability to stimulate the growth of new tissues on the surface and pores of the implant, promoting adherence and proliferation of cells to form new vascularized bone tissue), osteoinduction (ability to attract immature cells and induce them to transform into bone tissue forming cells; in general, induce cells to bone formation in ectopic medium by biomolecular signaling mechanisms) and osseointegration (integration between bone tissue cells and the implant occurs by adhesion of cells on the surface of the implanted material).[25]

The bone has a very complex biomechanical system, which requires from the scaffolds specific properties that to promote this restoration such as biocompatibility, mechanical properties similar to bone structure, adequate porosity that allows the vascularization of bone tissue (between 200 and 350 μm) and bioabsorbability/biodegradability. Thus, scaffolds that use bacterial cellulose have shown to be very promising for bone regeneration, since they have the ideal properties required for such application.[25]

Torgbo and Sukyai's review paper[25] cites several materials based on bacterial cellulose with potential application in bone regeneration, such as a Huang et al. work,[26] which produced a scaffold bacterial cellulose/hydroxyapatite agarose gel that was able to promote adhesion, proliferation and cell viability in the osteogenic process of human bone marrow stem cells.

Another area of Regenerative Medicine that will benefit from new biotechnological materials is the production of artificial blood vessels. According to data from the World Health Organization (WHO), cardiovascular disease is the leading cause of mortality worldwide. It is estimated that in 2016 alone they caused the death of approximately 17.9 million people worldwide, about 30% of all deaths.[27] According to the study and Lee and Park,[28] about 600 thousand surgical procedures are performed worldwide every year.

Such a demand, according to Schumann et al.,[29] caused the widespread use of artificial grafts with those of polyethylene terephthalate and expanded polytetrafluoroethylene (PTFE). However, in small diameter grafts (< 6 mm) the occlusion rate and consequent thrombosis was 40% of the cases in less than six months of implantation. The occlusion of artificial vessels is associated with blood interactions with synthetic materials that cause obstruction. In addition, artificial materials increase the risk of bacterial infections and can cause widespread rejections and inflammation.

Considering the natural grafts, implants have been performed using the saphenous vein in revascularization processes, such as the internal mammary vein in autologous graft. However, the procedure is not totally adequate, since, because it is small, the graft may present varicosity. Thus, the search for new implants of small diameter, biocompatible and

in which inclusion does not occur is of great importance.

Bacterial cellulose presents characteristics such as mechanical properties (Young 134 GPa module and tensile strength of 2 GPa per fiber), high purity, microstructure that allows the adhesion and proliferation of cells and biocompatibility relevant for the production of artificial blood vessels.

The work of Schumann et al.[29] proposes bacterial cellulose blood vessel transplants ***in vivo*** as a replacement for carotid arteries in five rats, with a 1 mm inner cavity, which were allowed to grow for one year. They were also implanted in eight pigs, weighing between 35–40 kg, in which they were administered in carotid arteries that had free access to food and were allowed to grow for three months. The animals were sacrificed and the implants were evaluated for resistance to blood pressure of 70 mmHg and vessel obstruction.

In the rats, it was observed that at the contact site between the prosthesis and the newly formed blood vessel, the presence of active fibroblasts that produce collagen, necessary for the integration and adequacy of synthetic vessels to the body of the animal, was verified. Furthermore, in all one–year–old animals, there was no obstruction of any synthetic artery. It is estimated that the high permeability rate may be favored by the targeted nature of BASYC fibers, which facilitate blood flow.

In pigs, on the other hand, the rate of permeability of the grafts was 87.5% (1 out of 8 grafts was found obstructed). It is believed that the obstruction may have occurred due to the circundant tissue that did not adhere correctly to the graft. However, no type of narrowing of the artificial arteries (stenosis) and no type of anastomosis (network of channels that bifurcate) were identified. It is also noteworthy that there were no apparent changes in any of the grafts, including dilation (aneurysm), dehiscence (natural fissure) and fistulas (inappropriate connections of distinct tissues and organs).

**Induced pluripotent stem cells**

Modern biotechnology techniques are also emerging and have great potential for various areas of Medicine. One such procedure is the production of induced pluripotent stem cells (iPSC).

The interest in pluripotent cells consists in the treatment of tissues, organs injured or destroyed, so they can be a route for the treatment of Parkinson's disease, spinal cord injuries and diabetes.[31]

Currently, stem cell treatments suffer a major ethical impact from the use of human embryos in the process. Moreover, there is a concern that immunological rejection could occur in treatments. Stem cells can be classified into two main types: embryonic and nonembryonic. Embryonic stem cells have the characteristic of being pluripotent, that is, they can form any type of cell in the three germ layers (endoderm, mesoderm and ectoderm), while nonembryonic stem cells are capable of forming several types of tissues, that is, they are multipotent and can be extracted from some tissues or organs such as the placenta, umbilical cord, bone marrow, peripheral blood.

Initially obtained in the work of Takahashi and Yamanaka[30] using cultures of embryonic and adult fibroblasts of mouse, iPSCs were produced ***in vitro*** by gene reprogramming of somatic cells through four transcription factors that induce them to acquire properties similar to embryonic stem cells. The hypothesis was based on nuclear reprogramming based on oocyte genes (oocytes II), which maintain embryonic pluripotent property for a long time. Through retroviral vectors, exogenous genes are introduced into somatic cells and induce endogenous expression of pluripotency. In addition, exogenous factors that have key roles in cell differentiation are silenced and the pluripotent state becomes completely dependent on the endogenous transcriptional circuit.[30,31]

The induced pluripotent stem cells produced in the work of Takahashi and Yamanaka[30] were subcutaneously transplanted in immunodeficient mice; tumors with cells of the three types of embryonic tissues were found, indicating that the induction of somatic cells to obtain pluripotent properties was effective. The work won the authors a Nobel Prize.

According to Reis et al.,[31] gene reprogramming has been performed using human skin fibroblasts with the use of viral vectors. However, due to the difficulty in obtaining epithelial tissue due to the need for biopsy, some recent studies indicate that other human cells are being used for the production of iPSC, such as adipose tissue stem cells, hepatocytes, peripheral blood lymphocytes, among others, which can enable various treatments and avoid rejection reactions by using the patient's own cells.[33]

**Microphysiological systems**

Still thinking about iPSC applications and the use of 3D supports for cell culture (scaffolds), the work of Edington et al.[33] addresses the production of microphysiological systems (MPSs) that mimic physiological functions ***in vivo*** through specialized culture microenvironments to perform drug testing ***in vitro***.

Known as "human body in chip" or "organs in tablets", the microphysiological systems are microenvironments of specialized cell cultures, which in 3D matrices (scaffolds) interconnected by microperfusion channels seek to simulate the physiological functioning of the human body. They are being developed to supply the demand in pre–clinical pharmacology for more effective models for toxicity testing and drug safety. Currently, animal models used delay the development of treatments and are not very safe in predicting the safety and efficacy of drugs in humans, causing billions of dollars lost each year.[33]

However, the production of multifunctional microphysiological systems is still under development and presents some technical challenges to be overcome, as described by Edington et al.:[33]

> (i) creation and maintenance of MPSs that exhibit sufficiently representative and robust physiological function in periods of prolonged culture, typically requiring intensive procurement and preparation of primary cells or pluripotent stem cells (PSCs) to achieve functional maturity in specialized microen–vironments;
>
> (ii) Design and manufacture of platform–shaped equipment that can accommodate and sustain the relevant MPS – in–cluding off–platform transfer to the platform in the case of MPS requiring disparate maturation times and complex means of maturation – by fluidically linking them in a manner that is permissive to quantitative analysis of biological phe–nomena involving the fate of drugs or disease phenomena;
> (iii) selection of an average composition compatible with the different MPS on the platform;
>
> (iv) a variety of other practical and translational aspects, in–cluding flow separation, flow rates, physiological.

Figure 7 is a schematic representation of the functioning of micro–physiological systems.[33]

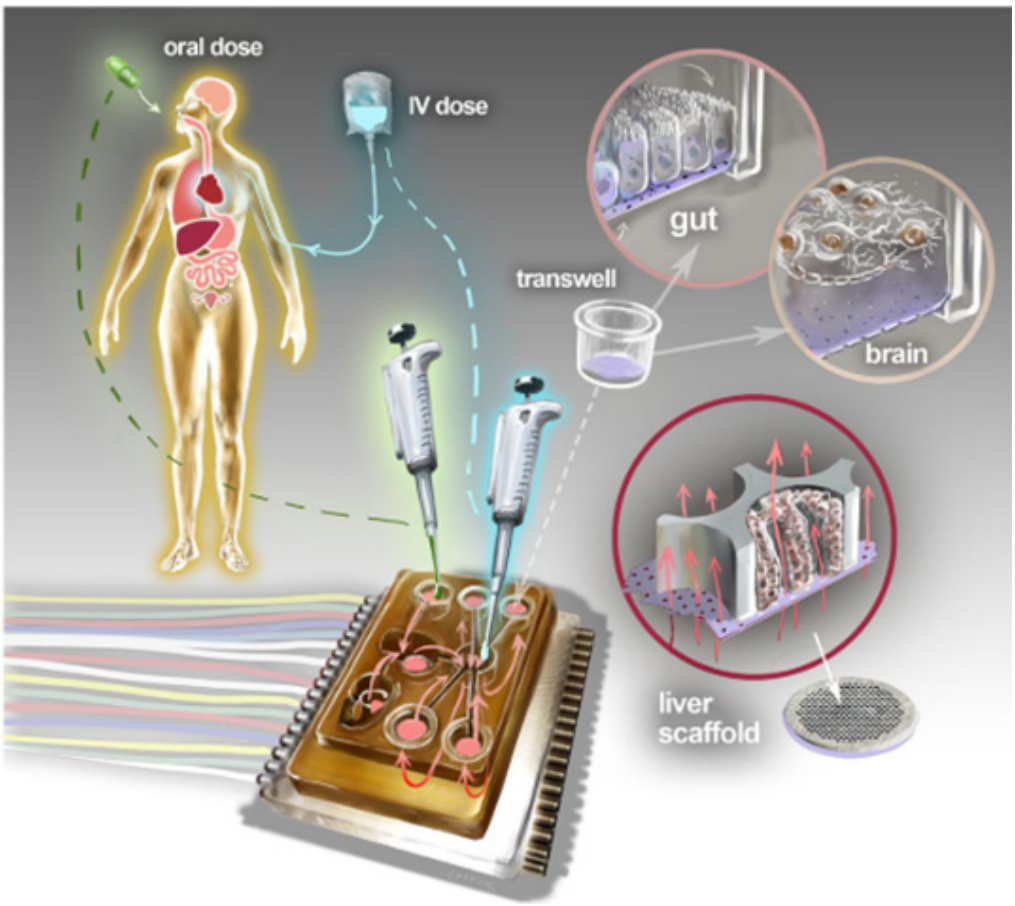


**Figure 7 –** Schematic representation of the functioning of microphysiological systems applied in pre–clinical pharmacological tests. Source: Edington et al. (2018). Image licensed under a Creative Commons Attribution 4.0 International License.

## Conclusions

Biotechnology has long contributed to the maintenance of human health. From the production of forms for mass immunization, such as vaccines, to the production of revolutionary drugs, such as penicillin, to the production of human insulin for the treatment of diabetes, Biotechnology has enabled an increase in the quality of life of man and helped in the development of the society we know. Even today, biotechnological processes and materials have been developed with the objective of meeting the demand for new products and services to further improve the procedures applied in modern Medicine. The production of scaffolds applied to the regeneration of tissues and organs, induced pluripotent stem cells and microphysiological systems, are just some of the technologies that can further improve Medicine and provide better technologies and therapies for several treatments.

Therefore, the importance of Biotechnology for contemporary society is undeniable, since it would be difficult to progress without the contribution of this important area.

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# Encontro de Polímeros Naturais

## EPNAT

5 e 6 de Agosto de 2019

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# VISIBLE AND INFRARED EMISSION FROM LUMINESCENT HYBRID FILMS BASED ON GELLAM GUM AND KARAYA CONTAINING $GbNbO_4:Er^{3+},Yb^{3+}$ PHOSPHOR

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Area: ( ) Food and Agriculture ( ) Medical and Pharmaceutical ( x ) Multifunctional Applications

**Abstract:** The study of hybrid materials has been driven by its versatility and potential application several areas. These compounds demonstrate distinct properties due to the inherent characteristics of the polymeric matrix, such as easy processability, flexibility, mechanical strength and the inorganic particles characteristics, which have adjustable luminescent properties, high photoluminescence, high quantum yield, physical–chemical stability, as well as resistance to photochemical and metabolic degradation. Thus, the resulting composites exhibit interesting properties than starting components. In this work, luminescent composites films were obtained by the incorporation of 1.0 and 3.0% in weight of $GdNbO_4:Er^{3+},Yb^{3+}$ (molar ratio of $Er^{3+}/Yb^{3+}$ = 1:4) particles in the Gellan gum and Karaya. The inorganic phosphor was prepared by the Non–Hydrolytic Sol Gel process and the composite films were obtained by the casting technique. The results reveal that after the incorporation in the polymers, the luminescent properties were preserved. Photoluminescence results showed emission bands at 1005 and 1535 nm assigned the transitions $^2F_{5/2} \rightarrow {}^2F_{7/2}$ and $^4I_{13/2} \rightarrow {}^4I_{15/2}$ of the ions $Yb^{3+}$ and $Er^{3+}$, respectively. The results of luminescence by upconversion energy showed emission bands in the visible region at 520, 550 and 655 nm, which were attributed to the electronic transitions of $Er^{3+}$ $^2H_{11/2} \rightarrow {}^4I_{15/2}$, $^4S_{3/2} \rightarrow {}^4I_{15/2}$ and $^4F_{9/2} \rightarrow {}^4I_{15/2}$, respectively, in addition to indicating a process by absorption of 2 photons. Finally, the increase in the concentration of the phosphors did not cause macroscopic changes in the hybrid materials, however, an increase of the emission intensity was observed for all of the materials.

**Keywords:** *Down– and upconversion; Sol–gel and casting.*


# EVALUATING THE EFFECT OF PROANTHOCYANIDINS AND GLUTARALDEHYDE IN THE PHYSIOMECHANICAL PROPERTIES OF DENSE LAMELLAR SCAFFOLD FOR REVERSE CARDIAC REMODELING

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**Abstract:** The regenerative medicine is an emerging field that aim is healing damaged tissue. Collagen type I is the main component of the extracellular matrix (ECM) and constitutes about 25% of the total protein in the body. The formation of natural cross–links in the biological tissues provides strength and makes it more resistant to degradation. Proanthocyanidins (PA) have the ability to crosslink collagen. The choice of crosslinking agent is one of the most important require for the development of 3D scaffolds devices. This study aimed to investigate the effects of proanthocyanidins (PA) and glutaraldehyde (GA) associated with plastic compression method on the properties of the dense lamellar scaffold with a stiffness above of the range of the heart muscle. The scaffolds are composed by collagen type I, silk fibroin, hyaluronic acid, and chitosan. The physiomechanical, antioxidant activity (by DPPH method), and viability and proliferation cellular (by MTT and imaging cytometer – H9c2 cells) were evaluated. The crosslinking agents modified the physiomechanical properties but did not modify the mucoadhesion properties. PA–scaffold and GA–scaffold showed, respectively, 44% and 17% of antioxidant activity. Both crosslinking agents did not influence the viability and proliferation of H9c2 cells. Considering the physiomechanical properties, cellular compatibility, and protective action against reactive oxygen species, this study may provide a way to improve the inverse remodulation of heart tissue, after infarct acute of the myocardium.

**Keywords:** Dense Lamellar Scaffold; Collagen; Proanthocyanidin; Glutaraldehyde; Plastic Compression.


# EXTRACTION AND YIELD OF DNA–BASED HYDROGEL EXTRACTED FROM ORANGE (*Citrus sinensis L.*)

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**Abstract:** Hydrogels are polymers that can be used in regenerative medicine as biomaterials to encapsulate and carry cells, aiming at tissue regeneration. The important challenge when developing materials for this purpose is to ensure that cells remain viable in their interior, must be biodegradable, non–toxic and biocompatible. However, existing materials do not have all these characteristics. Due to its properties, DNA based hydrogels have been gaining prominence. DNA–based hydrogels are able to maintain viable cells, carry them and release them to their sites of action. DNA extraction of the orange (*Citrus sinensis L.*) was carried out using the cationic hexadecyl trimethyl ammonium bromide detergent (CTAB) and determined the yield. Then, the structural characterization of the DNA was performed using the Fourier transform infrared spectroscopy (FTIR) method, the tests were performed on the Spectrum 100 (Perkin–Elmer) equipped with an attenuated total reflectance (ATR) accessory and spectra were obtained in the range of 4000–650 $cm^{-1}$, after 16 runs per spectrum, with a resolution of 4 $cm^{-1}$. The yield of the sample from DNA extraction was 0.1% wt. The structural characterization indicated the presence of bands typical of the clusters that make up the DNA. Thus, it is concluded that there is a perspective that the use of orange DNA for the preparation of hydrogels may be an alternative with potential for use in regenerative medicine. The research is still in progress.

**Keywords**: Biomaterial; Hydrogel; DNA.


# INTEGRATING CELLULOSE FIBERS FROM PALM INTO PHBV COMPOSITES AND APPLICATION IN 3D PRINTING

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**Abstract:** Tissue engineering requires new rapid solutions to replace synthetic materials in the production of prostheses for bone regeneration. Rapid prototyping through 3D printing is configured as one of the most efficient tools for producing scaffolds that function as a cell growth carrier. Thus, the objective of this work was to develop PHBV filaments reinforced with cellulose fibers from the Australian royal palm tree for 3D printing. The filaments were obtained in a mini–extruder in different proportions of cellulose fibers (1 to 10% wt / wt). The filaments were characterized by scanning electron microscopy (SEM), stereoscopic microscopy (MO) and thermogravimetry (TGA). The 3D–printed poly(3–hydroxybutyrate–co–3–hydroxyvalerate)/cellulose fibers (PHBV/cellulose) scaffolds were developed by using fused deposition modelling (FDM) technique. The results showed that the insertion of the cellulose fibers into the PHBV to obtain the filaments altered the coloration, increased surface roughness, opacity and increased thermal stability. From the SEM, fiber agglomerations were observed in the fracture region of the composites as the percentage of cellulose fiber increased. The developed filament was suitable for the production of three– dimensional structures using a 3D priting, which is promising for the development of biomaterials.

**Keywords:** Polymer–matrix composites (PMCs); Thermogravimetric analysis (TGA); Scanning electron microscopy (SEM); Additive manufacturing (Fused deposition modelling – 3D printing).


# MULTILAYER FILMS STRUCTURED WITH NATURAL POLYMERS AND ZEOLITES AS A NEW FERTILIZER DELIVERY VEHICLE

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**Abstract:** As a strategy to increase food production and reduce environmental damage, the scientific community has intensified studies on obtaining Enhanced Efficiency Fertilizers (EEFs). These are attractive because they decrease the release rate of nutrients compared to conventional fertilization. EEFs have mechanisms to release plant nutritional synchrony, in this way, promote nutrient reduction by leaching and volatilization. The objective of this work was to prepare and characterize EEFs based on zeolites (Ze) adsorbed with macro and micronutrients, carboxymethylcellulose (CMC) and chitosan (Ch). First we evaluated the sorption capacity of Ze in relation to the nutrients potassium ($KNO_3$), copper ($CuSO_4{\cdot}5H_2O$), manganese ($MnSO_4{\cdot}H_2O$), zinc ($ZnSO_4{\cdot}7H_2O$) and iron ($FeSO_4{\cdot}H_2O$). After the sorption process, Ze enriched by the nutrients was incorporated into the CMC solution to obtain mono and multielement films by casting. Additionally, multilayer films containing CMC–Ze–macro in the inner layer and Ch or CMC–Ze–micro in the outer layers were prepared. Ze presents higher selectivity for the $Cu^{2+}$ and $Zn^{2+}$ ions in detriment of the $Fe^{2+}$, $Mn^{2+}$ and $K^{+}$ ions corroborant with the physical–chemical properties of the ions. The films were evaluated for their ability to release nutrients in the water. Monoelemental films (CMC–Ze–nutrients) release slower than Ze–nutrients. In addition, multilayer films of CMC–Ze–macro/CMC–Ze– micro multielement significantly decreased the speed for all the nutrients used. Structural and morphological results showed that physical interactions occur between the constituents of the films. The material has the potential for commercial application due to the low cost, simplicity of production, environmentally friendly and high value–added contributing to more sustainable agricultural practices.

**Keywords:** Agriculture; Green Polymers; Sustainability.


# MICROSPHERES OF STARCH AS ENHANCED EFFICIENCY FERTILIZER MATERIALS

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**Abstract:** Encouraging the development of sustainable agriculture is very important to reduce the negative impacts of the overuse of land. The use of intelligent materials that release nutrients on a long–term basis, together with sustainable policies, can contribute to sustainable agriculture. To achieve this goal is important to use materials with interest properties like biodegradation, natural source, and abundance. Starch polymer fits all these characteristics. The aim of this work was developed microspheres of starch and micronutrients by spray drying technique. For this purpose, starch gelatinization and atomization conditions were evaluated to establish the best experimental parameters. For starch gelatinization, time (10, 15 e 30 min), concentration (3, 5, 6, and 7 %), and temperature (57 and 97 °C) were tested under oil bath and magnetic stirring. For starch atomization, temperature (130 and 185 °C), concentration (3 and 6 %), and aspiration rate (10, 20 and 30 %), were evaluated. The best conditions for starch gelatinization (30 min, 6 %, and 97 °C) were established by the maximum concentration of solids possible, keeping the viscosity and homogeneity of the solution ideals for atomization. The best atomization parameters (6 %, 130 °C, and the aspiration rate of 10 %) were chosen by yield (dry mass obtained). It was possible to establish the best experimental conditions to obtain a yield of 47 % of starch microspheres.

**Keywords:** Natural Polymer; Spray Drying; Starch.


# VIABILITY OF CARRAGEENAN AND ALGINATE TO OBTAIN POLYMER FILMS FOR AGRICULTURAL APPLICATION

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**Abstract:** The global technological development is linked to the needs of society and, given the exponential population increase, one factor becomes essential: supplying food demand. Designing materials with effective properties that aid agricultural development have gained prominence in the last decades mainly inputs destined for the quantified and prolonged release of nutrients used in the diverse cultures of Brazil. In view of this demand, films consisted of marine algae matrices, such as carrageenan and alginate, are attractive due to the environmentally favorable properties as biodegradability and vehicle for releasing the nutrients in a programmed way. We hypothesized the casting films can be an interesting vehicle to sustain the nutrients and prolonged their release. Therefore, we evaluated and optimized the concentration and homogeneity of the polymer dispersions to improve the mixture and drying of the films by casting prior to nutrient addition. In this way, the study of new materials with high added value from sustainable, renewable and abundant sources is strategic not only from the technological/economic point of view but also from the environmental, due to the decrease in the damages caused by the excessive use of fertilizers in the Brazilian crops.

**Keywords:** Polymers; Film Casting; Agriculture.


# SCREEN PRINTED FLEXIBLE DEVICES FOR SENSING AND BIOSENSING APPLICATION

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**Abstract:** Printing technologies are widely used in electronic industry and for manufacture chemical sensors and biosensors. The screen–printed technique occupies a privileged place, because is an efficient and robust method with which can be produce low cost devices, also is possible operate in large scale capacity with facile and friendly operation. With rapid progress in the nanomaterials sciences new materials have been developed, that can be used as components in the manufacture of conductive inks, substrates and biomolecule stabilizers. The manufacture of screen printed electrodes and electrodes array on flexible commercial substrates and bacterial cellulose membranes obtained from biotechnological processes are presented in this research. In addition is also shown the advances obtained in the development of conductive inks derived from renewable sources and recycled polymers. The developed devices have been used in the manufacture of wearable sensors for the determination of cystic fibrosis in sweat and as electrochemical platform for composites using electrochemically reduced graphene/carbon black nanoparticles and its subsequent application in the detection of neurotransmitters (epinephrine, dopamine) and drugs (paracetamol).

**Keywords:** Screen Printed; Sensor; Biosensor; Flexibles Devices; Wearable Devices.


# DEVELOPMENT OF LOW COST BIOPRINTER AND BIOINKS BASED ON GELLAN GUN – LAPONITE FOR BIOMEDICAL APPLICATIONS

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**Abstract:** Three–dimensional (3D) bioprinting is a fusion technology that has recently gained significant attention in the biomedical field. However, commercially available bioprinting platforms can be prohibitively expensive for small research facilities, especially in an academic setting. Microextrusion is a simple and relatively inexpensive technique that presents sufficient resolution and excellent viability potential to design the printing platform, learning space for polymers for biomedical application. Gellan Gum (GG) is a microbial polysaccharide generated from the bacterium Pseudomonas elodea where it has properties like biodegradability and biocompatibility, being the most used biopolymer with gelling properties, while Laponite (LAP) is a synthetic clay frequently used to improve performance and properties of the products as rheological modifiers. In the present work was to develop a new bioink with GG / LAP gum and an economical benchtop bioprinter using microextrusion technology, modeling of depository financing model (FDM) so that it can use the bioink, serving as results for its validation of the bioprinter. The projected bioprinter was able to print hydrogels with spatial precision along the X, Y and Z axes of 0.2 mm. Hydrogels were characterized by techniques such as Scanning Electron Microscopy (SEM), thermogravimetric analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR) and Rheology, were tested where they produced prototypes that could be applied as biomaterial in regenerative medicine. Our results demonstrated that both the projected bioprinter and the bioink compound of GG / LAP have excellent properties for applications in additive manufacturing and biomedical applications.

**Keywords:** Gellan Gum; Laponite, Bioprinting; Bioink.


# FUNCTIONAL BIONANOCOMPOSITES BASED ON NATURAL POLYMERS AND SEPIOLITE CLAY

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**Abstract:** The design of novel materials in biomedical science has experienced a rapid growth in the last decades. Within this field, nanocarriers based on hybrid materials for pharmaceutical nanotechnology purposes are becoming particularly attractive due to the possibility of manipulation of structures at the nanometer scale, providing unique properties, such as high interfacial area, efficient drug loading, and high biocompatibility and bioavailability, which can contribute to minimize the required dose of medicines. Silk fibroin (SF) is a natural polymer extracted from cocoons of the silkworm (Bombyx mori). This protein is biocompatible, biodegradable, it has amino acids that act as cell receptors and mediate important interac–tions between mammalian cells and extra cellular matrix (ECM) facilitating cell adhesion and growth and it presents antimicrobial activity. Sepiolite is a fibrous hydrated magnesium silicate which shows an alternation of blocks and tunnels that grow up in the fiber direction. In the biomedical area, current investigation has demonstrated this argilomineral do not affect the cell viability, and in some cases can show anti– inflammatory properties, which support their effective use in the health sector. In this perspective, sepiolite and silk fibroin films have been used to prepared new bionano–composites by casting process aiming the use of this material as a possible biomolecule carrier for application in biomedical area. The character–ization these materials has been done with Thermogravimetric Analysis (TG/DTA), Fourier–transform infrared spectroscopy (FTIR), X–ray Diffraction (DRX), Transmission Electron Microscopy (TEM), Mercury intrusion porosimetry (MIP) and Helium Pycnometry.

**Keywords:** Silk Fibroin; Sepiolite; Bionanocomposite.


# ORGANIC–INORGANIC NANOCOMPOSITES BASED ON BACTERIAL CELLULOSE NANOCRYSTALS MODIFIED WITH POLYSILOXANES

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**Abstract:** In recent years, natural biopolymers have increased researcher´s interest in using biomedical devices and materials, including drug delivery. Bacterial cellulose nanocrystals (BCN) stand out as biomedical material due to remarkable characteristics such as great crystalline structure, high stiffness and low density, as well as excellent biological properties such as biocompatibility, biodegradability and low toxicity. Polysiloxanes are elastomers which may be considered good modifying agents because of their interesting properties like very low surface energy, excellent gas and humidity permeability, good thermal stability, low temperature flexibility, biocompatibility and low toxicity. In this work, organic–inorganic nanocomposites of bacterial cellulose nanocrystals modified with polysiloxanes have been prepared, aimed at controlled drug release. The surfaces of bacterial cellulose nanocrystals have been modified via sol–gel process using aminopropyltriethoxysilane (APTS) and glycidyloxypropyltrimethoxysilane (GPTMS), which give impart different functionalities to the bacterial cellulose nanocrystals. The experimental results expected will be possible prototypes that will describe the structure–property relationship of modified bacterial cellulose nanocrystals and the evaluation in relation to the controlled release of drugs.

**Keywords:** Bacterial Cellulose; Nanocrystals; Polysiloxanes, Drug Delivery.


# ONION FILMS FOR USE IN EDIBLE PACKAGING

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Area: ( ) Food and Agriculture ( ) Medical and Pharmaceutical ( x ) Multifunctional Applications

**Abstract:** The great need for replacing the traditional food packaging for their sustainable and environmentally correct counterparts has been recommended due to severe damage caused to the environment. In this context, biodegradable films based on materials from renewable sources have been thoroughly searched around the world and those likely to be ingested feature strongly innovative character and high potential of industrialization. In the present work, are presented methods for getting and characterization of self–supporting films obtained by the casting process, from the processing of pulp of onion (*Allium cepa* L.). The films were obtained using three routes: a) from the raw onion pulp washed; b) hydrothermally treated pulp washed and, c) hydrothermally treated pulp not washed. The films were evaluated by Thermogravimetry (TG), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), Contact Angle Measurements, High–Performance Liquid Chromatography (HPLC) and Fourier–transform infrared spectroscopy (FTIR). The TG/DTG curves for washed pulp films show higher thermal stability with two decomposition steps and one single decomposition step for non–washed. The DSC curves indicate glass transition (Tg) between 63°C and 81°C with higher temperature for the unwashed sample. By MEV the unwashed pulp films exhibit irregular surfaces, but continuous; the films of washings pulp have surfaced with layer and cracks. The Contact Angle Measurements suggest unwashed films are more hydrophilic that washed pulp films. The films of unwashed pulp present soluble carbohydrates and washed insoluble carbohydrates. By infrared, it is possible to indicate the chemical groups contained in films hydrothermally treated of pulp before and after washing. The washed and unwashed pulp films absorb 10% and 25% respectively of humidity in the climatic chamber. In this sense, were propose different protocols for the processing of onion pulp raw, and hydrothermally treated, aiming at obtaining the self– supporting films for application as edible packaging or medical applications.

**Keywords:** Package; Onion; Biodegradable; Biopolymer.


# BIO–CURATIVOS OF THE COMPLEXES CURCUMIN WITH BACTERIAL CELLULOSE: DEVELOPMENT AND CHARACTERIZATION

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**Abstract:** Bacterial cellulose was produced by Komagateibacter rhaeticus. Bacterial cellulose is an interesting material for using as a wound dressing. However, bacterial cellulose itself has no antimicrobial and leishmanicide activity to be used in treatment of different types of wound, such as cutaneous leishmaniasis. Curcumin is a natural compound which exhibits leishimanicidal and antimicrobial activity, but its low solubility makes it difficult to use in pharmaceutical formulations and preparing curcumin complexed with high amylose may increase its solubility in aqueous medium. Curcumin complexed were impregnated into bacterial cellulose by immersing bacterial cellulose in complexed curcumin solution. Several methodologies were tested for the incorporation of the curcumin complex in the bacterial cellulose and after selecting the most promising the morphology, thermal stability and curcumin content of the bio–curatives were examined by scanning electron microscope (SEM), differencial scanning calorimetry (DSC) and quantification by UV–Vis spectroscopy, respectively. The selected methodology resulted in the incorporation between 37.69% and 48.61% of complexed curcumin in bacterial cellulose. SEM results demonstrated the presence of the curcumin complexes on the surface and also covering the bacterial cellulose nanofibre , indicating the presence of the formulation incorporated by all extension of the bacterial cellulose membrane. In DSC analyses were observed a shift of the endothermic events of the complexes when associated with bacterial cellulose, indicating an increase in the thermal stability of complexed curcumin when associated bacterial cellulose membranes. Bio–curatives have shown promising results. Other tests will be performed to evaluate its effectiveness for wounds, especially in cases of cutaneous leishmaniasis

**Keywords:** Curcumin; Bacterial Cellulose; Bio–curatives.


# BACTERIAL CELLULOSE/ CHITOSAN/CIPROFLOXACIN BIOCURATIVES: IN VIVO STUDY ON THE CICATRICIAL PROCESS IN RATS

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**Abstract:** In research for dressings with most cost effective, the biopolymers gain prominence, especially bacterial cellulose and chitosan, which have proven efficacy in the treatment of lesions. Bacterial cellulose has high tensile strength, flexibility, water retention capacity and is non–toxic. In addition, its porosity allows the introduction and release of antimicrobial agents, drugs and other biofunctional materials. Chitosan, a biopolymer produced from the deacetylation of chitin, contains antibacterial effectiveness, emulsifying, and non – toxic, biocompatible and biodegradable properties. The present study aims at analyzing the cytotoxic, mutagenic and cicatricle characteristics of a bio–curative produced by bacterial cel–lulose (BC) and chitosan (QTS) associated with a ciprofloxacin (BC/QTS/CIP) and comparing it to pure BC. All samples showed no cytotoxicity or mutagenicity. Through the in vivo tests, it was possible to analyze the capacity of maintenance of moisture in the interface curative / injury, acting as barrier for microorganisms, toxicity and absence of any sign of irritability in the lesion for both analyzed biocuratives. Regarding the area of healing, until the 7th day, the percentage of reduction of the lesion area was higher for the BC/ QTS/CIP biocurative, however, on the 14th day, reepithelization was superior for the animals treated with BC and with formation of more mature tissue. On the 21st day, 100% healing of the injured area it observed in both cases. Finally, it concluded that the pristine BC membrane, obtained with little difference superior results regarding the reduction of the lesion area, and both did not demonstrate cytotoxicity and mutagenicity.

**Keywords:** Bacterial Cellulose; Biocurative, Biopolymer


## EVALUATION OF BACTERIAL CELLULOSE FORMULATIONS IN THE TREATMENT OF PRESSURE INJURIES: CLINICAL STUDY

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**Abstract:** Pressure interruptions (PL) occur due to tissue pressure and, consequently, pressure levels by the pathologies at the periphery of the site. They are characterized by necrotic areas that affect muscles, adipose tissue, bones and skin. The treatment of lesions with the use of bacterial cellulose (BC) is promising, since this material is non–toxic, biocompatible and stimulates tissue remodeling by maintaining energy levels and activating growth factors. Studies have shown that a. It was like a mechanical unit and adjuvant in the processing of ulcerative operations and surgical wounds. In addition, cellulose is one of the most abundant polymers, of low cost and of greater use worldwide. BC dressings are marketed by means of two formulations: the BC membrane contains silver and hydrogel with alginate. The objective of this study is to quantitatively evaluate the healing of PL with the use of bacterial cellulose– based formulations. The research will be carried out with a series of PL diagnoses, randomly distributed, in the Hospitalization Units, in the emergency department and in the Intensive Care Unit of the Santa Casa de Misericórdia Hospital of Araraquara. The patients are divided into 5 distinct groups, with 8 patients each, receiving formulations with different BC, such as: BC membrane, BC/silver nanoparticles membranes and a pristine group control. This study aims to accelerate the healing process, reduce the risk of patients, reduce the incidence of side effects, reduce treatment capacity and reduce the work of the multidisciplinary team.

**Keywords:** Pressure Lesions; Bacterial Cellulose; Polymers.


# INFLUENCE OF PROCESSING ON THE STABILITY OF BIOACTIVE COMPOUNDS PRESENT IN PROPOLIS: MICROENCAPSULATION X CASTING

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**Abstract:** Bioactive compounds have occupied a prominent place in the scientific environment, due to it antimicrobial and antioxidant functions. However, the difficulty of using such compounds is often linked to the unpleasant taste and its difficult solubilization. An alternative to these problems is their incorporation into oral disintegrating films (ODF) and their microencapsulation, which allows a "controlled" release system. Therefore, this study aimed to evaluate the influence of the process on the stability of the bioactive components present in the propolis by using two techniques: (1) the production of films by casting and (2) microencapsulation by spray–dryer. For this, a solution was produced using 10g macromolecule / 100g solution in three different formulations (gelatin:starch ratio 30:70, 50:50 and 70:30) and 20g sorbitol/ 100g of macromolecule. For the production of microparticles 200g of propolis ethanolic extract / 100g of macromolecule was used. The ODF were characterized in terms of water content, soluble matter, color, mechanical properties, morphology, infrared spectroscopy, and total phenolic and flavonoid content; and microcapsules in relation to water content, cold water solubility, color, wettability, particle size, morphology, infrared spectroscopy, and total phenolic and flavonoid content. The formulation with higher gelatin concentration produced ODF with better mechanical properties, as well as lower humidity. Similar, microcapsules with higher gelatin content, showed a distribution of unimodal particles, demonstrating a good encapsulation, homogeneous color parameters, low humidity and solubility in medium cold water, and a more spherical shape. Furthermore, ODF and microcapsules were stable, in relation to the total phenolic compounds, and flavonoids, for approximately nine months of storage at 25° C. Therefore, it could be concluded that the use of ODF and microcapsules, can represent an excellent alternative to transport the active compounds, mainly phenolic compounds, present in the propolis extract.

**Keywords:** Microparticles; Orally disintegrating films; Material.


# EFFECT OF ADDITION OF HYDROXYAPATITE AND POMEGRANATE PEEL EXTRACT ON COLLAGEN SCAFOLDS

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**Abstract:** Collagen type I (C), a bioabsorbable fibrous protein, hydroxyapatite (HA), a bioceramic that exhibits osteoinductive and osteoconductive properties and a pomegranate peel extract (R), abundant in flavonoids that have antioxidant and anti– inflammatory properties were used to obtain a biomaterial with possible use in reconstruction of bone tissue. Anionic collagen gel was obtained by alkaline hydrolysis of porcine serosa, and it was mixed with a suspension of synthetic hydroxyapatite and pomegranate peel extract solution to form scaffolds named as C, CHA (16.7% HA), CR (14, 4% R) and CHAR (14.6% HA and 12.2% R). These were characterized by differential scanning calorimetry (DSC), collagenase degradation assays and immersion porosity assays in ethanol. It was observed by DSC that the flavonoids present in the pomegranate extract act as a collagen crosslinking agent, and the denaturing temperature (Td) of the scaffolds without extract are 47.6°C (C) and 47.9°C (CHA) and for CR and CHAR scaffolds is 52.8°C. The collagenase degradation assay shows that in a period of 2h, C and CR degraded 6.3±2.9% and 4.4±1.7%, respectively, showing no significant difference, however the addition of HA to the scaffolds significantly increases the percentage of degradation, being 22.7±3.0% for CHA and 17.0±1.3% for CHAR. This may indicate that HA destabilizes the structure of the scaffold and the difference of 5.7% between CHA and CHAR may be due to the crosslinking of the collagen caused by the extract. Porosity tests show that there is no difference between the porosities of C, CR and CHA, being about 92%, however with the addition of extract and HA a reduction in porosity is observed (64.7±7.9%). It can be concluded that the addition of extract crosslinks collagen, the addition of HA increases the degradation and the addition of HA and extract reduces the porosity in approximately 30%.

**Keywords:** Collagen; Hydroxyapatite; Pomegranate Peel Extract.


# INFLUENCE OF SYNTHESIS METHODOLOGY ON THE PROPERTIES OF COLLAGEN:CHITOSAN:CALCIUM NANO PHOSPHATE SCAFFOLDS

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**Abstract:** Scaffolds of calcium nano–phosphate (CNP) and collagen have been developed aiming applications in the regeneration of bone tissue, due to properties as cellular adhesion, biocompatibility, porosity and capacity of drugs incorporation in the three–dimensional structure. However, one of the major challenges to be overcome is the heterogeneous distribution of calcium phosphate in the collagen matrix. This issue can be solved with the use of calcium phosphate crystals at the nanometer scale and the use of collagen associated with other biopolymers, such as chitosan. Thus, the aim of this work was to evaluate how different methodologies of CNP synthesis could affect the structural and thermal properties of collagen/chitosan scaffolds. The methodologies developed were: 1) CC–CNPM1: $H_3PO_4$ solution was added to a 1% chitosan gel and $Ca(OH)_2$ solution was dripped in the mixture. The mixture (pH 9.0) was homogenized in the collagen gel (1:1); 2) CC–CNPM2: CNP crystals were synthesized within a pectin matrix, precipitated, calcinated and incorporated to a 1% collagen gel; chitosan powder was then added to the mixture (1:1), which pH was also raised to 9.0. After, 5% (w/w) of ciprofloxacin was added in both methodologies. DSC thermal analysis revealed that the addition of CNP increased the collagen denaturation temperature in both cases. Photomicrographs by SEM revealed scaffolds with porous surfaces containing CNP crystals internally distributed. X–ray diffractograms confirmed the presence of CNP in the scaffolds. The CNP synthesis methodologies led to significant differences in scaffolds porosity, CC–CNPM1 being 30% most porous. Ciprofloxacin release increased rapidly in both cases and stabilized after 1 hour, CC–CNPM2 releasing about 33% less antibiotic than CC– CNPM1. Thus, it can be stated that both methodologies successfully generated CNP, better stabilizing the triple helix of the collagen and affecting the porosity of the scaffolds, as well as their drugs releasing capacity.

**Keywords:** Chitosan; Collagen; Calcium Nano–Phospate.


# DEVELOPMENT OF CHITOSAN–CELLULOSE FILM AS MATRICES FOR CONTROLLED RELEASE FERTILIZER

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**Abstract:** Controlled release fertilizers (CRF) are a technological alternative capable of avoiding the excessive application of fertilizers and, consequently, their harmful environmental and economic consequences. The use of polymer matrices composed of green and renewable source materials has been a widely exploited innovation for the development of new fertilizer materials. We developed a film based on chitosan/cellulose/fertilizer to use as an efficient CRF system. Cellulose fibers were extracted from sugarcane bagasse using an alkaline treatment (NaOH 4% m/v, 70 °C, 5 min, twice). A multielement solution with 10 mg $L^{-1}$ of N, P and K nutrients were sorption on fibers. Then, chitosan acid solutions (1% w / v) were prepared and 1 g of mixed NPK–type fertilizer (1: 1: 1) was added to the solutions. After obtaining a homogeneous mixture, 0.1, 0.5 and 1.0 g of sorbed fibers were added to the systems. The mixtures were oven dried at 30 °C for 24 h. The release property of the films was characterized by photometry by the quantification of the potassium ion. For the release tests, films with standardized mass were placed in tea bags and sequentially dipped in 50 mL of distilled water. At each pre–set time interval, the entire volume of water was exchanged, calculating the cumulative mass, in grams, of the available potassium ion in the medium. From preliminary results, it was found that polymer films composed of fibers are able to reduce the release of the potassium nutrient in water, indicating the feasibility of using these materials as fertilizer release systems.

**Keywords:** Sugarcane Bagasse; Npk; Film Casting.


# ORGANIC–INORGANIC NANOCOMPOSITES BASED ON *ALLIUM* CEPA BIOPOLYMER CONTAINING HYDROXYAPATITE / SIMVASTATIN FOR APPLICATION IN DENTISTRY

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**Abstract:** Bone regeneration has become a space of great interest in medical specialties, especially in the dental area. When teeth are extracted there is a concern on the part of the professional when it comes to the process of bone repair, since the regenerated bone can serve as a support for oral rehabilitation in that place. Thus, the bone volume in the alveolar region is essential for successful dental treatment. In view of the need for the bone regeneration procedure, many studies were conducted in this direction with the creation of biomaterials such as polymers and the formation of natural composites because these biopolymers have remarkable physical properties, special surface chemistry, sustainability, biosafety and excellent properties such as biocompatibility, biodegradability and low toxicity. In the present Project nanocomposite films will be obtained through the casting process from the onion pulp (Allium cepa L.) associated with hydroxyapatite and simvastatin. The pristine material, as well as all nanocomposites, will be evaluated for physico–chemical properties by means of thermogravimetric (TG), Vibrational Spectroscopy in the Infrared Region (FT–IR), X–ray Diffraction and Scanning Electron Microscopy), 3 specimens being analyzed for each
technique. In vitro assays for cell viability, cell adhesion and proliferation will also be conducted in addition to animal studies where mice from the laboratory of the University of Araraquara UNIARA will be used. At the end it is desired to obtain a material with properties for it to be used in guided bone regeneration in dentistry.

**Keywords:** Bone regeneration; Biomaterials; Membranes.


# CHITOSAN/RHAMNOLIPID NANOPARTICLES AS AN EFFICIENT ANTIMICROBIAL AGENT AGAINST Staphylococcus aureus

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**Abstract:** In recent years, the antimicrobial resistance has led to serious health and food problems. Nanomaterials have been identified as a new approach to deal with this problem because of their unique physical and chemical properties. In this study, the antimicrobial activity of chitosan solution (C), rhamnolipid nanoparticles (RL–NPs), chitosan nanoparticles (C–NPs) and chitosan/rhamnolipid nanoparticles (C/RL–NPs) was evaluated against Staphylococcus aureus ATCC 25923. Chitosan (MW $1.63 \pm 0.03 \times 10^3$ g $mol^{-1}$, degree of deacetylation 82.6%) was extracted from squid pens by deproteinization, deacetylation and depolymerization. A 0.5 mg $mL^{-1}$ chitosan solution was prepared in 0.5% acetic acid. RL–NPs were obtained by dissolution of 0.5 mg $mL^{-1}$ commercial rhamnolipid (25% Rhamnolipid Inc.) in water. C/RL–NPs were prepared by mixing chitosan and rhamnolipid at 1:1 (v/v) ratio with addition of sodium tripolyphosphate (TPP) aqueous solution (0.5 mg $mL^{-1}$) under constant stirring. The minimum inhibitory concentration (MIC) was determined using the microbroth dilution technique and minimum bactericidal concentration (MBC) was also evaluated. Chitosan solution inhibited bacterial growth showing a MIC of 14 µg $mL^{-1}$ and MBC of 116 µg $mL^{-1}$ whereas, the MIC and MBC values of RL–NPs were 37 µg $mL^{-1}$ and 75 µg $mL^{-1}$, respectively. The bacterium was resistant to C–NPs at the concentrations tested. C/RL–NPs showed a MIC of 7/9 µg $mL^{-1}$ and MBC of 29/37 µg $mL^{-1}$. In conclusion, the MIC and MBC values were lower than that obtained for isolated molecules, suggesting a synergistic effect and offering a promising strategy to design non–toxic functionalized NPs for applications in several areas.

**Keywords:** Nanoparticles; Chitosan; Rhamnolipid; Antimicrobial Activity.


# INCORPORATION OF DRIED CAMU–CAMU EXTRACT IN STARCH/GELATIN ORALLY DESINTEGRATING FILMS

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**Abstract:** Oral Desintegrating Films (ODF) is an efficient way to administration of several compounds by the oral mucosa. ODF have been highlighted by the incorporation of active compounds obtained from natural sources. The aim of this work was to development Starch/Gelatin ODF with the addition of camu–camu powder (CCP) obtained by spray dryer. ODF was produced by casting, without extract (control) and with the addition of CCP (4 g/100g of filmogenic solution), maintaining constant the concentration of macromolecules and polymer (2 g/100 g and 20 g/100g of filmogenic solution, respectively), varying the ratio of starch (S) and gelatin (G) (30S:70G; 50S:50G; 70S:30G). ODF were characterized in relation to thickness, contact angle, surface pH and disintegration time. All ODF, showed homogeneity, absence of insoluble particles and film forming capacity regardless of the formulation. ODF presented thickness between 0.068 and 0.074 nm, without significant difference. A reduction of the contact angle (~84.6 ° to ~62.3 °) and surface pH of ODF (~6.9 to ~5.1) was observed after incorporation CCP, possibly due to the presence of hydrophilic compounds and more acidic characteristics of the fruit. No significant differences were observed in relation to the increase of the starch concentration for the contact angle and surface pH for the ODF control and with addition of extract. All ODF presented disintegration time between 12–20 s. Formulations with the highest concentration of starch, with and without the addition of CCP, disintegrated faster. In this way, it can be concluded that the ODF of starch and gelatin with CCP incorporation presented a short disintegration time (<18 seconds) and surface pH near the buccal, confirming its potential as an innovative dosage form for the incorporation of extracts obtained from natural sources such as camu–camu powder.

**Keywords:** Natural extract; Active compounds; *Myrciaria dubia.*


## ACTIVE PACKAGING SYSTEM BASED ON CHITOSAN FILM CONTAINING LEMONGRASS ESSENTIAL OIL

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**Abstract:** New packaging materials that are economical and easily degraded have gained prominence. Active and biodegradable materials could maintain the quality, promoting safety and prolonging the shelf life of food. Chitosan is a natural polymer derived from chitin, found in the exoskeleton of insects and crustaceans. Chitosan films have advantages such as flexibility, oxygen barrier, biodegradability, antimicrobial property and low toxicity. The incorporation of natural antioxidants in chitosan film matrix forms an active packaging system, as an alternative for the application in food products susceptible to lipid oxidation. The objective of this work was to develop a new and sustainable active packaging material from chitosan films, incorporating essential oil of lemongrass. The formulation was defined from Factorial Design $2^2$ + central points, with the independent variables: concentration of chitosan ($C_{chi}$, 1.0, 1.5 and 2.0%, w/w) and concentration of lemon grass oil ($C_{oil}$, 0.5, 1.5 and 2.5%, v/w). The highest antioxidant capacity (DPPH and ABTS) was verified in formulation containing $C_{chi}$ = 1.0% and $C_{oil}$ = 2,5%, as well as the higher phenolic content (5.94 mgAGE / g). The variables $C_{chi}$ and $C_{oil}$ promoted positive and significative effect in color parameters a* and b*, tending to more yellowish films. Chitosan active films containing lemongrass present potential to apply as antioxidant material for food packaging.

**Keywords:** Chitosan; Antioxidant Film; Sustainable; Food Packaging.


## INFLUENCE OF STERILIZATION ON COLLAGEN, ELASTIN AND JATOBA RESIN SCAFFOLDS

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**Abstract:** In the field of tissue engineering, a common challenge is to identify a sterilization method for biopolymers scaffolds that does not affect the structural or biochemical properties of the material. Some methods used for the sterilization of biopolymers are ionizing irradiation, ultraviolet radiation and gas sterilization by ethylene oxide. The aim of this study was to evaluate the influence of different types of sterilization in collagen and collagen/elastin scaffolds with or without Jatoba resin. Collagen was extracted in acetic acid pH 3.5 from bovine tendons after an alkaline hydrolysis treatment (72 h at 25°C). Elastin was obtained from bovine auricular cartilage by alkaline hydrolysis (24 h at 45°C). The Jatoba resin was purified and solubilized in ethanolic solution in the proportion of 1:20 (w/w). Scaffolds of collagen (C), collagen/elastin (CE), collagen/resin (CJ) and collagen/elastin/resin (CEJ) were prepared and sterilized by: ethylene oxide (Sterilization Center Com. Ind. Ltda.), ultraviolet radiation (20 minutes with a 30 W UV lamp) and gamma radiation (15 and 25 kGy doses by $^{60}$Co source) in the Multipurpose Irradiator type compact, with a dose rate of 5 kGy/h at the Radiation Technology Center of the Nuclear and Energy Research Institute. SEM and DSC were made to analyze the scaffold morphology and integrity of collagen triple helix. Photomicrographs showed no changes in the morphology of the scaffolds after sterilization procedures. DSC curves showed that the sterilization procedures do not modify collagen triple helix, with the exception of gamma irradiation at the dose of 25 kGy. Gamma irradiation was introduced as the simplest and most efficient sterilization procedure without toxic residues. Doses between 5 and 25 kGy are currently reported as sufficient to sterilize collagen materials. Therefore, the 15 kGy gamma irradiation is the most indicated to sterilize the scaffolds obtained in this study.

**Keywords:** Collagen; Elastin; Gamma Irradiation.


# MECHANICAL PROPERTIES OF PVA/ANIONIC COLLAGEN MEMBRANES CONTAINING ANTIBIOTICS DESIGNED AS THERAPEUTIC CORNEAL DEVICES

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**Abstract:** Collagen has been used extensively as a biomaterial to enhance tissue regeneration. In ophthalmology, collagen–based devices such as corneal shields and keratoprostheses are used to stimulate corneal regeneration. Although frequently collagen has not the adequate mechanical properties to the required application and some strategies, such as crosslinking or blending, are necessary to enhance its performance. Blends of collagen and PVA are relative new biomaterials with excellent mechanical and film forming properties that have not been applicated in ophthalmology. In this study, the tensile properties of membranes of PVA/anionic collagen containing antibiotics were assessed to evaluate their affordability for ophthalmological application. The mechanical compatibility between the two polymers was verified in the dry membranes. In the swelling state, the tensile properties of the blends were superior to the tensile properties of the commercial soft contact lenses. The results indicated that the PVA/anionic collagen membranes are mechanically stable systems with potential use in corneal regenerative medicine.

**Keywords:** Collagen; PVA; Blending; Corneal Regeneration.


# INFLUENCE OF ZEOLITE ZK406H ON THE SWELLING AND STRUCTURAL PROPERTIES OF THE POLYSACCHARIDE NANO–COMPOSITE HYDROGELS

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**Abstract:** The new nanocomposite hydrogels based on methylcellulose (MC) supported in poly(methacrylic acid)–co–polyacrylamide (PMAA–co–PAAm) networks were developed with the goal to reduce the environment impact caused by the indiscriminate use of agrochemical. In this work, the hydrophilic and structural properties of these nanocomposites prepared from 0.5, 1.0 and 1.5% w/v of zeolite, was studied by swelling degree and X–ray diffraction (XDR) technique, respectively. The swelling degree results showed that the addition of zeolite into hydrogel matrix reduced its water absorption. This factor is probably related to physical crosslinking caused by interactions between the zeolite and the hydrophilic groups of the hydrogel chain. XRD nanocomposites diffractograms showed the zeolite crystalline peaks at 2θ= 9.77°, 22.30°, 26.53° and 29.93° with d = 0.91, 0.40, 0.34 and 0.30 nm, respectively. These results indicate an increase in the crystallinity of the polymeric chains, and it is corroborating with hydrophilic properties. In addition, both results confirmed that the zeolite remained into hydrogel after dialysis process. In this way, the presence of zeolite in the hydrogel matrix can improve other important properties such as sorption and desorption properties that may qualify these materials for future use in agriculture, such as carrier vehicles for controlled release of agricultural inputs.

**Keywords:** Hydrogel; Swelling; XRD; Zeolite And Agriculture.

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# BACTERIAL CELLULOSE–BASED PHOTOACTIVE MULTIFUCNTIONAL HYBRID MEMBRANES

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**Abstract:** Photoactive films with photocatalytic, self–cleaning and antibacterial properties formed on flexible and biocompatible substrates are important due to their potential application in the design of self– cleaning and antibacterial surfaces, filters and facemasks. In this research study, we report flexible and multifunctional organic–inorganic hybrid membranes (BC–$SiO_2$–$TiO_2$/Ag) based on bacterial cellulose (BC) that contain photoactive ($TiO_2$) and antibacterial (Ag) components, rendering them photocatalytic, self–cleaning and antibacterial properties. The $SiO_2$ and $TiO_2$ particles were obtained from the hydrolysis–polycondensation of the respective alkoxide precursors and the amorphous $TiO_2$ obtained after sol–gel coating was selectively crystallized in anatase phase using a soft hydrothermal treatment at 130 °C as confirmed by XRD and Raman spectroscopic analysis. The resulting hybrid membranes were characterized by SEM, EDX, XRF, XRD, dynamo mechanical analysis, FTIR spectroscopy, Raman spectroscopy and UV–visible spectroscopy. The $TiO_2$ coating exhibits a typical film–like smooth surface at low Ti/Si ratio but undergo morphological changes with the formation of a rougher surface consisting of $TiO_2$ nanoparticle of around 170±35 nm, as observed by SEM analysis. The prepared BC–$SiO_2$–$TiO_2$ membranes showed good photocatalytic and self–cleaning activity under UV irradiation which increases with increase in Ti/Si ratio or $TiO_2$ loading of the hybrid membranes, as evaluated by the photo–bleaching of a crystal violet over–layer deposited on the surface of the hybrid membranes. The hybrid membranes containing Ag were also tested for their antimicrobial activities against the selected bacterial strain and the samples showed good antibacterial activity in dark. The prepared membranes have the potential to be used in facemask which could be easily sterilized under UV irradiation and safely discarded after use.

**Keywords:** Bacterial Cellulose; $TiO_2$; Self–cleaning; Photocatalysis.


# DELIVERY SYSTEM OF CASHEW GUM LOADED WITH AN ANTIOXIDANT OIL

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**Abstract:** Nanoparticles technology is developed in order to minimize the side effects and keep acceptable the delivery system, improving the bioavailability of the compound. This system can be developed from synthetic and natural polymers. Cashew gum (CG) is a natural polymer, which can be an efficient alternative to encapsulate antioxidant oils, once that the oils have limited application due to their lipophilicity, oxygen and light sensibility, and bioavailability. Thus, the aim of this study is to develop a nanocapsule to protect the antioxidant oil in order to obtain a control delivery system. The samples were prepared by nanoprecipitation technique with antioxidant oil (10, 25 and 50 %, w/v). After developed, the mean particle diameter (Z– average), polydispersity index (PDI) and zeta potential were determined by dynamic light scattering (DLS). *In vitro* release study was performed in triplicates during 24 h and analyzed by UV–visible spectrophotometer at 292 nm. Particle size analysis showed diameters ranges 300 to 600 nm and PDI 0.1. The smallest size, 370.97 ± 55.80, was observed with the lowest concentration of antioxidant oil, what suggest which more oil concentration, bigger is the particle. Z–potential showed a negative charge for pure CG, –2.98mV, and the oil applied was already reported in the literature with a positive charge and the complex range –23.9 to –25.8, the opposite charges can suggest that the oil was coated by the gum and homogeneous solution and moderate stability. *In vitro* release study of CGNPs+10 was 16.2 % while the pure oil was 11.1 % after 24 hours. Therewith, besides protecting the oil oxidation the particles also present a higher oil release if compared with pure oil. Thus, these results reinforce the capacity of CG encapsulate an antioxidant oil and that can be used as a delivery system for different purposes.

**Keywords:** Cashew gum; nanocapsule; *In vitro* release.


# DEVELOPMENT AND CHARACTERIZATION OF MICROSPHERES OF SILK FIBROIN AND CARBOXYMETHILCELLULOSE

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**Abstract:** In the last few decades, the use of polymers in several areas had been increased exponentially due to their versatility and multiple applicability. When related to polymers, it is possible to found the natural and the synthetic polymers. Moreover, the biopolymers had a special highlight because the proprieties found in this biomaterial had a huge interest in health care, food and drug applications, and so on. The biopolymer carboxymethilcellulose is a hydro–soluble derivate from cellulose and it is found in huge amounts in the nature. Another biopolymer is the silk fibroin that is biopolymer of easy obtainment from silkworm's cocoon (*Bombyx mori)*. Both compounds had several reports about their applicability in health studies, especially related to obtainment of hydrogels. For this reason, our objective was to develop and to characterize microspheres of carboxymethilcellulose (CMC) and silk fibroin (SF) potentially applicable for medical sciences. The hydrogel SF+CMC was obtained using a solution of 1%SF with 2%CMC (w/w) in 25mL of ultrapure water. Then the hydrogel was submitted to a syringe pump to drip the hydrogel in a solution of aluminum chloride 5% (w/v) for reticulation. After, it was washed with ultrapure water for obtaining the microspheres cleared of reticulation agent. The characterization was made by swelling in water studies, thermogravimetry and differential scanning calorimetry, The results suggest that a silk fibroin and carboxymethilcellulose composite was obtained by physical reticulation with $Al^{3+}$ ions. Thus, the obtained microspheres will be used for a next step of applicability in drug release.

**Keywords:** Silk Fibroin; Carboxymethilcellulose; Microspheres.


# MODIFICATION OF SURGICAL SILK SUTURES WITH CHLORHEXIDINE AS ANTIMICROBIAL AGENT

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**Abstract:** Silk is a natural polymer fiber composed of an organic protein called fibroin. This polymer is derived from *Bombyx mori L* silkworm species. The silk suture is a sterile and non–absorbable surgical suture very used in Oral Surgery. It can induce infections by bacterial accumulation. For this reason some attempts have been made to develop sutures with antibacterial properties. Thus, the objective of this work was the incorporation of chlorhexidine gluconate (CHX), as antimicrobial agent, in surgical silk sutures. Chlorhexidine is a chemical antiseptic with fungicide, bactericidal and bacteriostatic action, inhibiting bacterial proliferation. It was incorporated by impregnation at ambient temperature by using 2% and 4% (v/v) water solutions. Sutures were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA). Also, in vitro drug release studies were performed in water. The FTIR results revealed the CHX incorporation by drug characteristic bands presence. Thermal analysis showed an increase in the maximum decomposition temperatures of modified silk and CHX incorporation. The drug release was higher for sutures with 4% of CHX but a rapid burst release effect was obtained. Then, we can conclude that chlorhexidine was effective incorporated in these surgical silk sutures. Other modifications are being made to better control the chlorhexidine release and the antibacterial activity will be studied.

**Keywords:** Silk suture; Chlorhexidine; Drug Release.


# CHITOSAN – LEMONGRASS ESSENTIAL OIL FILM AS ANTIAGING FACIAL MASK

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**Abstract:** Chitosan films are characterized by biodegradability, low toxicity, flexibility, resistence and antimicrobial properties. Several researchs indicated the chitosan film application in cosmetic, food, biomedical and other products. The objective of this work was developed and characterized chitosan active films containing lemongrass essential oil (LO) with potential application as antiaging facial masks. Different concentrations of LO (0, 0.5, 1.0, 1.5%, w/w) were incorporated into chitosan filmogenic matrix (1.0%, w/w) forming the active chitosan films. The antioxidant properties of active chitosan films increased in function of LO concentration ($EC_{50}$ = 0.024 mg/µL for 1.5% of LO), measured by DPPH method. Similar results were observed to water vapor permeability and water solubility that increasing the LO concentrations, it was observed higher water vapour barrier and less water solubility. Chitosan active films containing 1.0 and 1.5% of LO presented a reduced celular viability (30%). Chitosan active films containing 0.5% of LO presented cellular viability over than 70%. In this way, the active chitosan films containing lemongrass essential oil (0.5%) has potential to apply as antiaging mask with antioxidant capacity, seletive permeability, integrity and safety (citoxicity).

**Keywords:** Chitosan; Films; Lemongrass Oil; Facial Mask.


# ANTIMICROBIAL WOUND DRESSING BASED ON BACTERIAL CELLULOSE MEMBRANES CONTAINING SILVER NANOPARTICLES FOR WOUND TREATMENT

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**Abstract:** Bacterial cellulose (BC) is a natural polymer synthesized by gram–negative bacteria such as *Komagataeibacter rhaeticus,* without impurities as lignin, pectin and hemicellulose usually present in vegetable cellulose. In addition to amazing physico–chemical properties, BC can be applied in biomedical field as drug excipient, nerve recovery, wound dressings, among others. Seven® industry has fabricated dressing based on BC that exhibits benefits such as healing and regenerating properties to treat chronic wounds. Specifically, porous BC wound dressings can promote healing been applied for moderate and high wound exudation. Despites good healing properties induced by BC, these kind of dressings do not show antimicrobial properties, which makes difficult to cure effectively wounds with high contamination degree by pathogens. To overcome this drawback, this work proposed to ally wound healing properties of BC with silver nanoparticles (AgNP) labeled BC@AgNP, since these biocomposites have proven efficacy against microorganisms. Methodology: Porous BC Nexfill® dressings were impregnated with distinct concentrations of AgNP as 500, 250, 125 and 62.5 ppm corresponding to the BC@AgNP1, BC@AgNP2, BC@AgNP3 and BC@AgNP4, respectively. AgNP distribution onto BC, cell viability and bactericidal and bacteriostatic effects were evaluated. Results and discussion: MEV results showed a homogeneous distribution of AgNPs onto BC nanofibers network. Cell viability assays displayed non–toxicity of BC@AgNP in healthy cells, with values above 70 % of viability for all biocomposites. Beyound that, commercial wound dressing was evaluated showing high toxicity (30 % of cell viability) regarding to our BC@AgNP samples. Antimicrobial assays using contact method exhibited no microbial growth by using BC@AgNP for ***E. coli*** and ***S. aureus*** bacteria. Death Curve assays demonstrated that the fabricated BC@AgNP can be used for more than 24 h being efficient in maintaining its bacteriostatic abilities. Conclusion: All results from BC@AgNP suggest these biocomposites as potential and effective dressings to avoid wound infections.

**Keywords:** Bacterial cellulose; Silver nanoparticles; Antimicrobial wound healing.


# BIODEGRADATION OF PROGRAMMABLE RELEASE FERTILIZER BASED ON PHB–TPS AND NANOCELLULOSE

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**Abstract:** The currently farming system uses a large amount of agrochemicals in the planted area. The loss of these essential components arises by different physical–chemical processes (leaching, volatilization, solubilization in the soil), but also contribute to environmental pollution. In order to reduce this waste and prevent pollution, programmable release fertilizer (PRF) has been a viable alternative. However, these are expensive and are usually formulated with materials that accumulate in the soil due to low natural degradation, causing damage to the environment. So some polymers, natural or synthetic, are alternatives to this problem because of their physical and microbial degradation characteristics. In this work the biodegradation profile of PRF based on cellulose, cellulose nanofibers, starch, thermoplastic starch and poly (hydroxybutyrate), PHB, according to NBR 14283 was obtained. It considers carbon dioxide a product of degradation and aerobic activity of microorganisms, making it possible to quantify biodegradation. Thus, the biodegradation profiles show that PHB, starch or TPS composites formulated with nanocellulose have a high biodegradation rate. And only the fertilizer in the matrix also accelerates this mechanism when compared to the profile of the matrices. Therefore, nanocellulose is an efficient factor for biodegradation, and may improve soil cultivation properties.

**Keywords:** Starch; Programmable release, PHB.


# CHARACTERIZATION OF COMPOSITE MICROSPHERES OF SILK FIBROIN AND SODIUM ALGINATE

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**Abstract:** In the nature is possible to found a diversify varieties of compounds. In these compounds are situated the high structured polymers, that are molecules made by the junction of small structures called "monomers". Furthermore, is also possible to found the biopolymers, which are attracting more attention lead these proprieties. Two examples of biopolymers are the sodium alginate (A) and the silk fibroin (SF), the sodium alginate are originated from the brown algae, while the silk fibroin is a compound present in the composition of cocoon of silkworm (*Bombyx mori*). The SF have high biocompatibility, mechanical properties and low toxicity, and the alginate had been applied in large scale in food industries. For this reason, our objective was to develop and to characterize microspheres of sodium alginate and silk fibroin potentially applicable for food and drug sciences. The hydrogel A+SF was obtained using a solution of 1%SF with 2%A (w/w) in 25mL of ultrapure water. Then the hydrogel was submitted to a syringe pump to drip the hydrogel in a solution of 5% calcium chloride for reticulation. After, it was washed with ultrapure water for obtaining the microspheres cleared of reticulation agent. The characterization was made by water permeability, thermogravimetry and differential scanning calorimetric, the results suggests that the composite obtained by silk fibroin and sodium alginate had molecular interactions after the preparation. Thus, the microspheres obtained were promissory for the next studies.

**Keywords:** Sodium Alginate; Silk Fibroin; Microspheres.


# OBTENTION OF CARBOXYMETHYLCELLULOSE MICROSPHERES: THE RETICULATION EFFECT WITH $CA^{2+}$ AND $AL^{3+}$ IONS

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Area: ( x ) Food and Agriculture ( ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** The carboxymethylcellulose (CMC) is a water–soluble derivative from cellulose, and cellulose is a natural polymer found abundantly in nature. The CMC has the capacity of deriving itself to gels, which possibility the obtainment of microspheres. The microspheres are solid particles or droplets obtained through a process of reticulation. Moreover, the reticulating agent causes a link by crossing bond between the polymers chain, allowing getting a network, resulting in differences in the materials properties. The reticulation process can be chemical or physical. The last one is reached by using multivalent ions solutions. Thus, the objectives of this work were to obtain CMC microspheres and to study the reticulation effect of $Ca^{2+}$ and $Al^{3+}$ ions. The microspheres were obtained by dripping a solution of 2% (w/v) CMC into the reticulating agent using a syringe pump, washed with ultrapure water and dried by lyophilization. After, they were characterized by visual analysis, swelling studies in water and thermogravimetric analysis (TG/DTG/DSC). The results showed that with the use of the $Al^{3+}$ ions was possible to obtain the microspheres, while with $Ca^{2+}$ the material was not spherical and showed a coalescence effect. Furthermore, the TG/DTG/DSC data showed variations in the thermal properties of the materials by the reticulation process. The swelling results showed that the $Al^{3+}$ reticulated microspheres showed twice the volume of absorbed water than the reticulated with $Ca^{2+}$. In conclusion, the CMC microspheres with the desired characteristics were obtained using $Al^{3+}$ ions as reticulating agent. These microspheres will be used for the encapsulation of entomopathogenic fungi in the next step of this work.

**Keywords:** Carboxymethylcellulose; Microspheres; Reticulation.

**Acknowledgments:** To The São Paulo Research Foundation (FAPESP), process No. 2017/50334–3.


# BIOINSECTICIDAL ACTIVE PACKAGING MATERIAL BASED ON CHITOSAN–LEMONGRASS OIL COATING CARDPAPER

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**Abstract:** Active packaging system is an excellent solution for a wide range of applications in the food industry. Flour and grain–based products are an important kind of food susceptible to microbiological and entomological infestation. The insects' infestation causes the reduced grain quality, which results in economic losses. The objective of this work was developed an active and sustainable grain–products packaging material with insecticidal action against *Sitophilus zeamais* insects. The active material consisted in chitosan coating cardpaper containing lemongrass essential oil (Cymbopogon citratus), as a botanical insecticide. A Factorial Design $2^3$ was developed to evaluate the effect of chitosan and lemongrass essential oil concentrations, and the number of coating layers, on the bio–based coating's properties. SEM images showed that active chitosan–oil coating filled the void spaces between the cellulosic network, improved the barrier, reducing water vapor permeability (WVP), increasing the grease resistance and maintaining a microbial impermeability. Increasing $C_{oil}$ from −1 (20%) to +1 (40%), reduced the WVP in the order to 0.24 $g.mm.h^{-1}.m^{-2}.KPa^{-1}$, indicating that the lipid presence increased the hydrophobicity of the chitosan coating matrix. The grease resistance was improved increasing the total solids ($g.m^{-2}$) on the cardpaper surface. The chitosan–lemongrass oil coating on cardpaper is an active and environmental friendly alternative to grain–products packaging material to reduce the insects infestations.

**Keywords:** Chitosan Coating; Botanical Insecticide; Active Packaging.


# DEVELOPMENT OF A NEW METHOD OF EXTRACTION OF HYALURONIC ACID (HA) FROM EGGSHELLS BY SONICATION FOR USE IN DENTISTRY

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**Abstract:** Differences in the rate of crosslinking of hyaluronic acid (HA) gels can alter their properties and compromise their biological performance and biodegradation. The objective of this study was to evaluate the efficiency of a new method of hyaluronic acid extraction from eggshells by son–ication. A controlled, parallel group, double blinding laboratory study was conducted to evaluate the efficiency of the sonication method to extract hyaluronic acid from eggshells. For sonication extraction 15 mg of crushed egg shells were mixed with 15 ml of sodium chloride.The ultrasound was applied for 10 and 20 minutes with amplitude of 20 hertz. The control group was defined as 15g of eggshell powder and 15mL of acetic acid (4M) were mixed and the solution was stirred (200 rpm) for 24 hours at 9 ° C and constant pH (~ 3.5). Subsequently, an equal volume of isopropanol was added and the solution centrifuged (18,000 x g, 20 min and 4 ° C). The hyaluronic acids extracted was precipitated gel was suspended in 1 L of 3% sodium acetate, 2% silica gel and activated charcoal and centrifuged (20,000 x g, 20 min and 4 ° C) to remove impurities. The purified hyaluronic acids was filtered (0.45 and 0.20mm) and lyophilized. The hyaluronic acid extracted by each method was characterized by UV–Vis chromatography (Carbozole reaction), infra red spectroscopy (FTIR), pH evaluation and rheometry. The results obtained were compared with scientific standard (sigma aldrich) and commercial Rennova Lift (Croma GmbH). The extraction process had a yield of 0.5%. The AH extracted from the eggshell is similar to the stan–dards and can be classified as medical grade and showing potential to be a more ecological alternative to obtain this biopolymer

**Keywords:** Biopolimer; Hyaluronic Acid; Crosslinking.


# ETHYLENE–ADSORBER/CHITOSAN–COATED KRAFT PAPER FOR ACTIVE PACKAGING APPLICATION

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**Abstract:** Ethylene ($C_2H_4$) is a plant hormone that strongly affects climacteric fruits and vegetables post harvest quality as it accelerates ripening. Therefore, maintaining ethylene on the lower level is essential for prolonging shelf–life of climacteric fruits. The present work aims to develop an active packaging system capable of adsorbing ethylene gas inside climacteric fruits packaging. The systems consist of Kraft paper coated with chitosan–ethylene adsorber. An experimental design $2^2$ + central points was formulated to understand the best coating condition. Dependent variables were chitosan and ethylene adsorber concentrations (w/w). Ethylene adsorber was dispersed in chitosan solution and applied as a coating on kraft paper with a film spreader equipment. Grammage (weight per area), thickness, and Taber stiffness (bending resistance) were measured. Despite the presence of a chitosan–adsorber coating, it was not observed higher grammage and thickness for any of the coated systems when compared to non–coated Kraft paper, possibly, due to low concentrations of both chitosan and adsorber chosen in this experiment. Taber stiffness of coated paper systems presented higher values when compared to non–coated paper. It indicates that even though the solids (chitosan+adsorber) on the coating were low enough not to affect grammage and thickness, they were sufficient to improve the mechanical property of bending resistance, especially in the fibers alignment direction. For further studies, it is required to evaluate other properties of the material. For example, barrier properties and especially its effectiveness on ethylene adsorbing and prolonging climacteric fruits shelf life. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. This work is also supported by São Paulo Research Foundation (FAPESP; grant#2016/25120–7).

**Keywords:** Ethylene; Packaging; Kraft paper.


# EVALUATION OF CHITOSAN FILMS CONTAINING ALOE VERA EXTRACT AND/OR COPAIBA OIL IN HEALING TESTS WITH WISTAR RATS

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**Abstract:** Polymeric films have been used as wound dressings for burns. Healing, anti–inflammatory, and antibiotic properties can be potentiated with the incorporation of bioactives. Traditionally healing phytotherapics such as ***Aloe vera*** extract and copaiba oil were incorporated into chitosan films. In previous studies, the physical and biological properties of the films were evaluated and then some films were selected for the ***in vivo*** tests with Wistar rats. The animals were anesthetized and partially epilated on the back. The deep wound was obtained by total excision of the dorsum skin and removal of the fleshy pannicle with the aid of scalpel and surgical scissors, removing a fragment of skin and adding a dressing (film) in place. The animals were randomly distributed in seven groups of five animals: Group I – commercial dressing Membracel; Group II – chitosan film 2%; Group III – 2% chitosan film + silver sulfadiazine; Group IV – chitosan film 2% + Copaiba 0.5%; Group V – Chitosan film 2% + Aloe Vera 0.5%; Group VI– chitosan film 2% + Copaiba 0.5% + Aloe Vera 0.5%; Group VII – control group (dry gauze). After the surgery, visual follow–up was performed, the area of the wound was measured and the healing progress was documented. The monitoring was daily until the day after the wound closure. The results were compared at three different levels: wound closure rate; the macroscopic appearance of the healed wound and histological evaluation. It was concluded that the film containing 0.5% copaiba oil (Group IV) presented the best result in terms of skin regeneration rate, with complete wound closure in 11 days, that is, three days earlier than the control. The other films showed full wound closure at times equal to the control, although these presented a higher regeneration rate in the initial stages (Grant 2010/17721–4, São Paulo Research Foundation – FAPESP).

**Keywords:** Chitosan films; *In vivo* tests; phytotherapics.


# SWELLING AND RELEASE OF HERBICIDE IN SALINE MEDIUM FROM NANOCOMPOSITE HYDROGELS BASED ON CHITOSAN ANDZEOLITE

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**Abstract:** The conventional application of highly water–soluble agrochemicals, such the diquat herbicide, can provoke the contamination of water bodies destined to the supply of rural and urban populations. The controlled released of these compounds from biodegradable polymeric matrices can minimized the damage caused in the human health and the environment caused by these chemical products. The potential of nanocomposite hydrogels composed by chitosan and zeolite supported in poly (methacrylic acid)–co–polyacrylamide in agriculture was investigated by swelling and desorption analyses on different salt solutions. The swelling results showed that the water absorption by the hydrogel and their nanocomposite reduces with the increase in the concentration and valence of the ions presents in the swelling medium. In contrast, the desorption ratio and the diquat mass released by the nanocomposite significantly increased in the presence of bivalent and trivalent ions such the $Ca^{+2}$ and $Al^{+3}$. However, the nanocomposite released a smaller amount of diquat in all desorption test made in this studied, in comparison to the amount released by the pure hydrogel. Indicating that the zeolite insertion in these materials can produce a more controlled released of herbicides, and the desorption analysis also showed that diquat released was controlled by the ionic diffusion, proving that the nanocomposite can decrease the damages in the roots of plants caused by ions $Al^{+3}$, reducing costs of other processes such as liming.

**Keywords:** Nanocomposite Hydrogel; Sweeling; Diquat; Zeolite; Desorption Properties.

**Acknowledgments:** UNESP, FAPESP and CNPq. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – "Finance Code 001"


# EVALUATION OF THE SWELLING RATE OF HYALURONIC ACID BASED FILLERS WITH DIFFERENT CROSSLINKS

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**Abstract**: Advances within Orofacial Harmonization in Dentistry have sought filling materials with better physico–chemical properties in orofacial procedures. Crosslinking of Hyaluronic Acid (HA), for example, which aims to crosslink the molecules of the gel, is intended to increase the product's durability and to modify it's elasticity and viscosity, providing a perfect clinical indication. Therefore, the crosslinking agent, the concentration, and the crosslinking method can modify the rate of fluid absorption in implanted tissue directly interfering with the treatment of patients. Thus, the purpose of this study was to evaluate the swelling rate and the crosslinking degree of HA with 2 differents crosslinking agents: I–polyethylene glycol diglycidyl ether (PEGDE) (150 uL and 300 uL) and II–butanediol diglycidyl ether (500 uL and 800uL). Fourier transform infrared (FTIR) and Swelling Test were performed to compare the products. Both tests were effective in qualifying the products for their crosslinking rate and fluid absorption. The results showed that the higher the crosslinking rate the lower the fluid uptake (BDDE 300uL> PEGDGE 800uL> BDDE 150uL > PEGDGE 500uL). The data obtained from the FTIR corroborated with those obtained by the Swelling test. It can be concluded that the concentration, the method and the different crosslinkers directly interfere with the absorption of fluids.

**Keywords:** Hydrogels; Hyaluronic Acid; Swelling Kinetics.


# DEVELOPMENT OF THE HPMC FILMS REINFORCED BY BIOCELLULOSE NANOFIBRILLATED

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**Abstract:** The disposal of the plastic materials has been caused an environmental problem. For this reason, research has been concentrating on development of the new materials "ecological friendly". The present work consists in the development of films based on a natural polymer to be used as packaging material. Films were produced by solvent casting method. Colloidal dispersion BCNC film–forming was obtained through the addiction of 78.5 g of the distilled water, 1.6 g of Hydroxypropyl methylcellulose (HPMC) and 0.08 g of Bacterial Cellulose Nanofribrillated (BCN) provided by BioPolMat–UNIARA. Films were analyzed by Tensile test through a Universal Mechanical Testing machine following ASTM 882/02 Method. HPMC film (control) exhibited tensile strength (MPa) of 60.81 ± 6.5 After BCN addition the values increased to 72.69 ± 6.2. The percent elongation at the break of the films change from 14.62± 2.3 to 12.78 ± 1.8 after BCN were added. The nanostructures addition contributed for increase of the film resistence due the interaction resultant between BCN and polymer matrice. This results is very important in this area and showed satisfactory properties of these films suggesting potential use as packaging material.

**Keywords:** Biopolymers; Biodegradable Pack; Renewable Resources.


# RIFAMPICIN SUSTAINED RELEASE USING BACTERIAL CELLULOSE SPHERES

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Area: ( ) Food and Agriculture ( x ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Bacterial cellulose (BC) is a biopolymer of great interest for application in various industrial and medical areas due to its peculiar characteristics such as biocompatibility, hypoallergenicity, tensile strength, high water retention capacity, purity and crystallinity. In addition to nutrient sources, other factors such as methods of cultivation (static or agitated), oxygen availability, temperature, pH and the bacterial growth phase may influence BC production. The aims of this work were to obtain BC spheres, by *Komagataeibacter hansenii* ATCC 23769, in agitated culture, using media containing different carbon sources, to produce supports for sustained release of rifampicin (RFM). From a pre–inoculum in log phase of growth, BC spheres were produced in media containing different sources of carbon such as fructose (FRU), glucose and sucrose (MS1), sucrose (Y) and glucose (Z and HS) kept under stirring at 130 rpm for 24 hours. The spheres produced were processed in 0.5M NaOH solution at 65 ° C to remove the bacteria and residues from the culture media, washed in distilled water with periodic exchange, until the pH reached neutral, and after lyophilized. The dry mass yield and the swelling percentage were analyzed in addition to the characterization by scanning electron microscopy (SEM) and infrared spectroscopy (IR). The spheres produced were RFM impregnated and tested for sustained release ability of this drug. The BC spheres with the highest RFM release capacity were produced in the FRU and Z media and presented specific characteristics of composition and purity of BC (by IV) and high density and fiber interweaving (by SEM), when compared to those produced in the other means. These results demonstrated the great potential of these BC spheres to be used as a support for the sustained release of antibacterial agents, such as RFM.

**Keywords:** Bacterial Cellulose Spheres; Rifampicin Release; Different Culture Media; Agitated Cultivation.


# DEVELOPMENT OF STRATEGIES FOR ADMINISTRATION OF AN ANTIVIRULENCE COMPOUND TO AVOID CONTAMINATION IN FOOD BY *SALMONELLA* BACTERIA

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**Abstract:** In 2017, Brazil was the second–largest producer of poultry meat and the fourth–largest pork producer in the world market, according to the ABPA (Brazilian Animal Protein Association) and 33.1% of the production and 18.5% of pork were intended for export. The genus *Salmonella* consists of some species and numerous serovars that can be zoonotic, infecting humans and animals. Moreover, *Salmonella* can be carried through food resulting in a target of trade barriers. These bacteria are also involved in enteric disturbances in pigs and chickens influencing meat production due to low yield animal. Antibiotics are used in the animals' feed as the prevention of bacterial infections as well as AGPs (Antibiotic–Growth–Promoters). The intensive and indiscriminate use of antibiotics contribute to the emergence of multidrug–resistant bacteria increasingly frequent. The main objective is to enable the use of the molecule LED209 by developing an innovative controlled release system initially as a preventive agent to livestock in infections caused by pathogenic bacteria, especially *Salmonella.* One approach that has been highlighted is the use of compounds antivirulence disarms bacterial pathogens interrupting the disease progression, as the LED209, which has potential capability antivirulence, being capable of inhibiting the signaling cascade QseC. The virulence factors are regulated at many levels, one of the two–component systems such as QseBC responsible for regulating many virulence genes in more than 25 animal and plant pathogens. QseC functions as epinephrine/norepinephrine sensor and produced by the host autoinducer–3 chemical signaling between bacteria (quorum sensing), contributing to the transduction of signals from host stress and intraspecific and interspecific communication. Then as a vehicle to the LED will realized of a nanoemulsion followed by microencapsulation with polymers. LED209 blocks QseC, impairing its histidine kinase function by not transferring the phosphate to QseB that results in inhibition of the cascade regulation of various virulence genes.

**Keywords:** *Salmonella;* LED209; Antivirulence.

**Development Agency:** FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and BioSmart Nanotechnology Ltda.


# EVALUATION OF BIODEGRADATION OF EDIBLE ONION FILMS (*ALLIUM CEPA* L.)

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Area: ( x ) Food and Agriculture ( ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** There is an increase in demand for bioplastics from renewable sources, mainly in the areas of medicines, cosmetics, food films, among others. In this context, new biodegradable materials emerge, such as the film derived from the onion pulp. The biodegradation of the samples of Onion films, which were developed by UNESP/UNIARA and Vegetable Cellulose (reference), was performed in the soil column of the Carolina® brand, being removed every day until the 5th day and after the 10th day. The samples were cleaned and placed in a greenhouse for drying for 1 hour. They were characterized by the analysis of infrared spectroscopy and thermogravimetric analysis, in addition to visual analysis. The Onion films began degradation on the 1st day and degraded total on the 10th day. The vegetable pulp continued in the same size and thickness, however, as it is used as a reference pattern of degradation, it was already expected. All infrared spectra showed the chemical bands characteristic of the materials studied. The thermograms of the vegetable cellulose showed that they remained stable during the degradation time. The original Onion films had 3 stages of decomposition, being in 65 °c probably of the sample moisture and the remaining steps at 200 ° C and at 341 °c referring to the decomposition of the sample, but on the 4th day the Samples lost 1 step, leaving only the moisture and at 354 ° C its decomposition. The decomposition evaluation will be analyzed in 10 days for a better understanding of the thermal event.

**Keywords:** Biodegradation; Biopolymers; Soil.


# EVALUATION OF THE DEGRADATION OF THERMOPLASTIC STARCH COMPOSITES WITH PULP RESIDUES AND POST–CONSUMPTION PAPER IN THE INTEMPERISM

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**Abstract:** The intemperism is considered a destructive factor that can cause the deterioration of several materials by abiotic factors, being responsible for the physical and chemical modifications that alter the microscopic structure of the materials. In relation to the life cycle of renewable composites, it is not yet accurate to affirm the time of its durability. The aim of this study was to evaluate the abiotic intemperalism of thermoplastic starch composites with pulp and post–consumer paper residues. Thus, two formulations of thermoplastic starch composites developed by the casting technique were evaluated, containing 30% of cellulose residues and the other with 30% paper. Films with 100% of thermoplastic starch were also produced in order to compare them with the composites. As for the exposition in the natural environments of Feevale University (Brazil) and HAMK–Häme University of Applied Sciences (Finland) (ISO 877–1:2009 and ISO 9370:2009), the materials were subjected to intemperism for 42 days and, after that, were characterized SEM and IR. The results indicated that the intemperism in Feevale caused a more pronounced abiotic degradation compared to HAMK, but there were no chemical modifications in the materials. Possibly by the radical difference of solar radiation, climatic conditions and relative humidity of the air, which were incidents on the samples. In general, the samples that suffered the most degradation were those of TPS, since the pulp and paper composites presented similar results. These composites can be used as support films for plants or as disposable quick life cycle packaging.

**Keywords:** Thermoplastic starch; Weathering; Biodegradable polymers.


# EFFECTS OF SOLVENT ACIDITY ON RHEOLOGICAL PROPERTIES OF GELATIN SOLUTIONS

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**Abstract:** Gelatin is an animal protein produced by denaturation of collagen. These natural polymers are biocompatible, biodegradable, present healing activity and low antigenicity and toxicity. The low cost and the greater availability of gelatin stimulate its use in a wide range of applications, including the biomaterials development. Several methods can be used for production of scaffolds, such as freeze drying, casting or fiber production techniques such as electro–spinning or solution blow spinning. In these methods it is first necessary to produce a solution that will be further processed. Some processing methods are applicable only to solutions with certain rheological properties, properties affected by the concentration and acidity of the solvents used. Gelatin scaffolds are reported to be produced by aqueous or weak acid solutions, such as acetic acid. This study therefore aims to evaluate the effect of acidity on gelatin solutions on their rheological properties, in order to provide guidance for the preparation of feasible solutions for different processing methods. Gelatin solutions (5% w/w) were prepared by stirring in aqueous solutions of acetic acid at concentrations of 2.5, 5.0, 10, 30, 60 and 90% (w/w). The solutions were stored overnight (4°C) and analyzed in a stress–controlled Rheometer (AR–1000N). The increase in acidity resulted in changes on the rheological properties of the gelatin solutions, such as the conversion of gels to fluid solutions, indicated by the change of materials with G'>G'' to fluids with G''>G' and the suppression of the protein transition temperature above 10% of acetic acid. The samples with 30– 90% of acetic acid produced solutions suitable for fiber production. For applications that require gel samples, such as porous scaffolds production, concentration of acid in the range 2.5–5.0% is recommended in this polymer concentration. Solutions with acid concentration of 10% are indicated for production of films by casting.

**Keywords:** Gelatin; Rheological Properties; Biomaterials.


# CINETIC AND STRUCTURAL ANALYSIS OF PRODUCT BASED ON HYALURONIC ACID RETICULATED BY BDDE

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Area: ( ) Food and Agriculture (x) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Hyaluronic acid (HA) is an important compound for increasing the volume of the skin, because it is biocompatible, non–immunogenic, and can be stabilized by chemical crosslinking, making this product with water–insoluble viscoelastic characteristics less susceptible to enzymatic degradation. The objective of this work was to obtain gels crosslinked from hyaluronic acid (HA) by 1,4 butanediol dyglycidyl ether (BDDE) and to evaluate the kinetic characteristics present in the process of gels and the degree of crosslinking. As samples were prepared from differentcategories: degree of swelling (% Q), diffusional exponent (n), diffusion coefficient (D), mean mass between reticles (MMc), density of reticulation (q) number of chains actually elastic per unit volume (Ve).After the swelling kinetics measurements, the different gels samples with and without buffer solution (pH 7.0; NaCl), one can observe the increase of mass of the gels, which is of 5 to 9 times, in relation to its initial mass caused by the absorption of the solvent and / or the buffer solution. Thus, the presence of a mixed diffusion process with $0.45 < n < 1$ (Fikian + relaxation of the polymer chains) for gels with low degree of crosslinking, and predominantly Fikian behavior for gels with a high degree of crosslinking were evidenced.It is concluded that, due to swelling curves, the behavior of the gels presents specific parameters according to the% BDDE incorporated in the crosslinking sys–tem. It is concluded that, due to swelling curves, the behavior of the gels presents specific parameters according to the% BDDE incorporated in the crosslinking system. Thus, the structural characteristics related to the degree of crosslinking promoted in the gel express an important indication in increasing the resistance to enzymatic degradation.

**Keywords:** Hyaluronic acid; BDDE; Crosslinking.


# BACTERIAL CELLULOSE MEMBRANES INCORPORATEDWITH HERBAL EXTRACTS

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Area: ( ) Food and Agriculture ( X ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Bacterial cellulose (BC) is a natural biopolymer known for its biocompatibility, non– toxicity and biodegradability. Such material is very attractive for medical and pharmaceutical aplications because it is free of contaminants and has superior mechanical properties due to its nano–fibrillar structure. Current studies have been conducted on the incorporation of substances, such as herbal extracts, that add specific properties to the BC. Marigold, propolis and witch hazel are examples of medicinal plants, whose extracts are promising in the treatment of wounds due to their antimicrobial and anti–inflammatory properties. The present work aimed to synthesize BC using *Komagataeibacter hansenii* and incorporate those herbal extracts on the membranes surface. For this study it was used aqueous (AEH) and glycolic extract (GEH) of witch hazel, hydroglicolic extract (HEM) and concentrated glycolic extract (GEM) of marigold and propolis extract (EP) in different concentrations. Dried samples were characterized by thermogravimetry (TGA), fourier transform infrared spectroscopy (FTIR) and antimicrobial activity. The incorporation of the extracts on the membranes was confirmed by the presence of chemical bonds from aromatic compounds, which were not found in the pure membrane structure. TGA analysis demonstrated a new degradation step, which was related to the degradation of the extracts. Besides, the incorporation reduced the thermal stability of the biocomposite in 21 °C for membranes with the witch hazel extracts, 45 °C for the marigold extracts and 56 °C for the propolis extracts. Anti–biogram revealed that the membranes incorporated with witch hazel extracts were not efficient against *S. aureus*, *E. coli* and *C. albicans*. Membranes with hydroglicolic extract (HEM) of marigold showed a 64% reduction in the colonization of *S. aureus*, while the glycolic extract (GEM) did not have an expressive antimicrobial activity. For the membranes incorporated with propolis extracts (EP), all the concentrations used were efficient against *E. coli* and *S. aureus*.

**Keywords:** Biocellulose; Herbal Extracts; Incorporation.


# DEVELOPMENT ALGINATE FILMS WITH COTTONSEED PROTEIN HYDROLYSATES FOR APPLICATION AS AN ACTIVE FOOD PACKAGING

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**Abstract:** The use of protein hydrolysates (PHs) obtained by the enzymatic hydrolysis of underutilized proteins as active ingredients may be a promising strategy in the development of bioactive packaging. The effects of the incorporation of hydrolysed cottonseed proteins into alginate films were investigated in terms of their physical, chemical, barrier, optical, antioxidant and antimicrobial properties and the release of peptides in two different alginate film food simulants. PH incorporation did not affect the moisture content, biodegradability, solubility or oil barrier properties of the films but did increase the thickness and water vapor permeability. The increase in the PH concentration increased the barrier properties to visible light, and the film colour became darker, reddish and yellowish. The total phenolic content and the antioxidant activity (as tested by the DPPH, FRAP and ABTS methods) also increased. The addition of PH modified the structural arrangement of the surface of the alginate films, which was modified from a continuous smooth surface with a more homogeneous structure (control film) a heterogeneous, rough and crystalline structure as the PH concentration increased in the films. The PH films showed an inhibitory effect against *Staphylococcus aureus, Colletotrichum gloeosporioides* and *Rhizopus oligosporus* but not against *Escherichia coli*. In migration tests in aqueous media, the active films released more than 60% of their peptides in 30 min. Meanwhile, there was a controlled and gradual diffusion of the compounds embedded in the film when fatty foods were simulated. The results showed that alginate films with PHs show promise as active packaging for the preservation of fatty foods.

**Keywords:** Lipid barrier property; Antioxidant activity; Visible light barrier.


# COLLAGEN MANGOSTEEN BIOMATERIALS. OBTAINMENT AND CHARACTERIZATION

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Area: ( ) Food and Agriculture (X ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Collagen is an excellent biomaterial found in tissues of living organisms and widely used in the tissue engineering and medicine fields due to its properties such as biocompatibility, biodegradability and tissue regeneration ability. Due to ethnical, regulatory and contamination reasons, collagen sources such as fish skin are being currently used and studied. Among them tilapia (*Oreochromis niloticus)* is a cheap and abundant potential source. Polyvinyl alcohol (PVA) is known by its multiple applications in tissue engineering. Mangosteen extract (*Garcinia mangostana L)* has antioxidant, anti–inflammatory, antibacterial properties offering a great array of applications in medicinal areas. The present study aims to obtain gels and scaffolds of collagen, PVA, ethanol and mangosteen extract for the tissue engineering area. Type I anionic collagen was extract from tilapia skin, and was characterized by FT–IR and SDS–PAGE, showing characteristic collagen bands and a $\alpha 1 \alpha 2$. To prepare the gels collagen (3%) was solubilized in lactic acid (0.5%) and the other components were added in proportion to the collagen dry mass, PVA (0 and 5%), extract (0 and 10%) ethanol (0 and 10%) and characterized by rheology. Scaffolds were made from the same gels by lyophilization and characterized by SEM. Rheology analysis show a pseudoplastic behavior and a lower viscosity at higher shear rates when ethanol or mangosteen were added. Oscillatory analysis determined the viscoelastic region and show a lower tan $\delta$ when extract was added, reinforcing the elastic behavior. Scaffolds photomicrographs show a reduced pore size with ethanol and mangosteen addition. DSC analysis show two denaturation temperatures for the treated tilapia skin, the first corresponding to triple–helix denaturation and the second one to the peptide chains break, and rheology temperature analysis showed a small increase in denaturation temperature when mangosteen was added. PVA addition did not interfere in morphological characteristics or rheological properties.
CAPES

**Keywords:** Collagen; Mangosteen; Biomaterial.


# PREPARATION AND CHARACTERIZATION OF POLYURETHANE/TIO$_2$ NANOCOMPOSITES

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Area: ( ) Food and Agriculture ( x ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** The research of new materials has always occupied a prominent position in the scientific environment. A noteworthy area of research is the application of materials for medical and biological purposes. Such materials are called biomaterials. There are studies on biomaterials composites that aim to combine properties of several materials into one. Following this concept, in this work, we synthesized a composite with the combination of polyurethane of vegetal origin (PU) and nanoparticles of titanium oxide ($TiO_2$) for use in bone implants. The synthesis of PU and $TiO_2$ specimens was performed by adding 0%, 25% and 50% $TiO_2$ (by weight) to a certain amount of vegetable polyol. The mixture was allowed to stir until it reached its complete homogeneity. To this mixture, we added diisocyanate in the ratio 1:1 to the polyol and the stirring system. The mixture was then placed in molds which were placed for 48hrs in a vacuum. The materials were characterized using Thermo–Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and Mechanical Analysis (MA). TGA analyzes showed that the thermal resistance increased relative to the amount of added $TiO_2$. SEM images showed that the doped samples formed some foci of $TiO_2$ agglomeration. These sites can be considered as fragility points of the mate–rial that were proved by the results of stress–strain tests. Materials doped with $TiO_2$ presented lower tension values for the same deformation when compared to the pure material by increasing the load/load interaction and not the PU/load interaction, thus making the material very fragile. Based on the results, we concluded that the biomaterials analyzed presented superior thermal properties and inferior mechanical properties compared to the pure polymer.

**Keywords:** Biomaterials; Polyurethane; Titanium Oxide.


# POMEGRANATE PEEL EXTRACT CROSSLINKING EFFECT ON COLLAGEN SCAFFOLDS

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**Abstract:** Collagen is the most abundant protein of the extracellular matrix and is used as a component of scaffolds. For the preparation of collagen the use of pepsin removes the telopeptides, leading to a slower fibrillogenesis and generating collagen micro and nanofibrils. Furthermore, mechanical and thermal stability can be enhanced with the addition of crosslinking agents. Pomegranate peel extract contains vegetal polyphenols capable of interacting with amino acids and also has some antioxidant and antibacterial activities. The aim of this study was to study the crosslinking effect of pomegranate peel extract on collagen. Bovine tendon was used to prepare pepsinized collagen (7.5% in pepsin mass). Gels were prepared in acetic acid pH 4.2 (0.5% collagen). Extract was added to one gel (1.3 mg extract/g gel). From the gels, scaffolds and thin films were prepared. The materials were named C0E and C13E, for collagen without and with extract respectively. Scanning electron microscopy (SEM), differential scanning calorimetry (DSC), ***in vitro*** biological stability tests using collagenase and FTIR were used to study the effect of extract addition. The addition of extract generated matrices with higher denaturation temperature (Td), being 47.3°C (C0E) and 50.1°C (C13E). Biological stability increased with extract addition since C0E had a degradation of 70.5%, while C13E was only 37.1%. The increase in both thermal and biological stability is an indicative of the interaction between the hydroxyl groups of the pomegranate peel extract and the amino acid chains of the collagen. Besides that, the SEM showed that the matrices had porous surface with irregular pore sizes. Extract addition increased pore size and irregularity. FTIR spectra showed an enlargement in the O–H stretch bands, which represents the extract incorporation. Thus, it was possible to conclude that the extract acted as a crosslinking agent, improving the collagen stability. FAPESP

**Keywords:** Collagen; Pepsin; Pomegranate Peel Extract.


# CONFECTION AND PHYSICAL–CHEMICAL CHARACTERIAZATION OF HYDROGELS BASED ON BACTERIAL CELLULOSE CONTAINING EPP–AF®

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Area: (x ) Food and Agriculture ( ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Exposure of ultravioleta (UV) rays to the skin can cause severe damage to the skin's structure, such as burns and even lead to the development of cancer. It is known that bacterial cellulose (BC), a natural polymer extremely pure and biocompatible, acts as a barrier against UV rays from the sun. For its advantages, it is already marketed in the form of wound dressing and as cosmetics masks. Propolis, in turn, is a resin produced by bees from pollen and plant residues, which has excellent biological activities, such as antimicrobial, antioxidant, anti–inflammatory, besides presenting an important role in protection against UV rays. For these reasons, it was thought to aggregate the properties of BC and propolis throught the production of a hydrogel with the function of sunscreen. Therefore, the BC membranes were grinded resulting in a pulp containing cellulose particles and, under mechanical shaking, emulsifiers and surfactants were added to it and also the standardized propolis extract obtained from the company Apis Flora (EPP–AF, from Portuguese "Extrato Padronizado de Própolis da Apis Flora"), creating the hydrogels. They were characterized by Fourier–Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). Through the bands generated by FTIR, it was possible to observe characteristic vibrations of both BC and propolis, thus confirming the formation of the composite BC/propolis. Thermal Analysis shows that the BC/propolis hydrogel remained thermally stable, with thermal behavior similar to the pure BC. These informations prove the viability in the production of hydrogels of BC containing propolis, which present potential photoprotective action, but that still need to undergo tests to validate this activity.


# BIOPOLYMERIC SYSTEMS BASED ON ALGINATE AND CELLULOSE NANOFIBER AS POTENTIAL PLATFORM FOR DRUG DELIVERY

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**Abstract:** Recently, polymers from natural origin had been applied in several innovative technologies from alimentary to pharmaceutical uses due to its biodegradability, biocompatibility, renewability and sustainable nature. Cellulose is the most abundant biopolymer on earth, therefore a great example of this class of material. This polymer is present in various natural sources like wood, cotton and vegetable biomass and for mechanical disintegration of its fiber is possible to obtained cellulose nanofiber (CNF). Besides the biological properties mentioned, CNF exhibit low density, excellent mechanical and barrier properties. When associate with other polymers in drug delivery systems (DDS), CNF had shown improvement in encapsulation efficiency and promising sustained release profile of the drugs. Regarding this application, alginate (ALG) based biocomposites are widely applied in DDS due to its non–toxicity, biodegradability, biocompatibility and unique gel–forming characteristics. Nevertheless, macroporous structure, poor mechanical and weak water resistance properties results in low retention efficiency and sudden release of drugs. Thus, the present study aims to investigate the effect of CNF in alginate matrix for future drug delivery applications. For this purpose, alginate 2% (w/v) was processed as beads by dropping its dispersion in $CaCl_2$ 5% (w/v) crosslinking solution. To obtain ALG–CNF systems, different amounts of CNF were incorporated in alginate dispersion, resulting in final materials with CNF ratios of 0.25, 0.50, 0.75 and 1.0%. The beads were freeze dried for 24 h and then their surface groups and thermal behavior were investigated by FT–IR and TG–DSC techniques. In the FT–IR spectra, some differences were observed after CNF addition into alginate matrix. An increase of intensity related to –O–H bond were noticed, especially in ALG–CNF 0,25%, suggesting hydrogen bonding interactions between ALG and CNF. Regarding to TG–DSC analysis, ALG–NFC systems exhibited a much more complex degradation process than pristine polymers, which can also suggest interactions between ALG and NFC.

**Keywords:** Alginate; Nanofiber Cellulose; Biomedical Applications.


## OKARA AND OKARA FLOUR AS SOURCE OF FIBER IN FOOD PROCESSING

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**Abstract:** Introduction: The processing of the soybean water extract produces, as a by–product, okara, an inert mass of high nutritional value. In order to evaluate the potential of the by–product as a source of fiber in food formulations, okara and its flour were characterized in relation to dietary fiber content. Food fiber consists of carbohydrate polymers that are not hydrolyzed by the endogenous enzymes in the small intestine.
Methodology: Drying of forced air circulation, followed by grinding and sieving to obtain okara flour and enzymatic–gravimetric method for the determination of total, soluble and insoluble dietary fiber samples. Results: Okara in natura presented values of 3.66% of insoluble dietary fiber and 0.04% of soluble fiber and okara flour had values of 16.58% and 0.17% for soluble and soluble dietary fibers, respectively. Discussion: Large quantities of Okara produced in Brazil are destined to feed animals and could be used to fight hunger. Researches were carried out using okara as a substitute ingredient in several foods as an alternative to reuse this product, in morning cereal, chicken burger, biscuits and breads. The fiber content of the soybean helps in the reduction of cholesterol and triglycerides, besides having mechanical activity in the formation of the fecal cake, property related to the insoluble fraction. Conclusion: The results suggest that the use of okara and flour are adequate as a source of fiber in the preparation of food and that the use of flour has advantages in relation to the use of okara in natura because it presents concentrated fiber contents. In addition, dehydration increases shelf life of the product, promotes volume reduction, facilitates storage and transportation, and enables storage at room temperature.

**Keywords**: Okara; Okara Flour; Dietary Fiber.


## EFFECT OF YEAST EXTRACT ON THE PROPERTIES OF SCHIZOPHYLLAN

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**Abstract**: Schizophyllan (SPG) is an extracellular polysaccharide produced by the basidiomycete ***Schizophyllum commune***. SPG is able to form a gel when aqueous solutions are cooled below 6 °C and SPG solutions show two highly cooperative conformational transitions on heating, one at 6 °C and the other at 135 °C. SPG shows antitumor and immunobiological activities. The aim of this study was to analyze the effect of the yeast extract on the composition of the final polysaccharide because it may contain water–soluble polysaccharides that interfere with the final analysis. Mycelia suspension was prepared by suspending mycelia discs from culture plates. Discs from culture plates was used to inoculate 200 mL sterile culture (0.2% de peptone, 2% de glucose, 0.05% de $MgSO_4.7H_2O$, 0.05% de $KH_2PO_4$ and 0.1% de $K_2HPO_4$) that was incubated at 30 °C and 100 rpm for 21 days in a shaker incubator. After 21 days, to increase the contact surface of the mycelium with medium, the broth was homogenized and a total of 10 mL of the mycelia suspension was added to 100 mL medium (18 g/L yeast extract, 10 g/L malt extract, 38 g/L glucose, 1 g/L $KH_2PO_4$, 1 g/L $K_2HPO_4$, 0.6 g/L $MgSO_4.7H_2O$ and 2 g/L $(NH_4)_2SO_4$). The culture was incubated at 30 °C, pH 6.5 in an incubator shaker at 150 rpm for 7 days. The culture broth was then filtered and reduced. After that, alcohol was added, and the culture broth was centrifuged. The SPG produced was characterized using Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR/ATR), thermogravimetric (TGA) and Differential Scanning Calorimetry (DSC) analysis. Both spectra of SPG showed peaks that confirmed that the polysaccharide is schizophyllan. TGA analysis showed that the decomposition profile is similar. However, there are various stages of degradation that may suggest impurities in the medium.

**Keywords:** *Schizophyllum commune*; Schizophyllan; Biopolymer.


# CHARACTERIZATION AND CYTOTOXIC ACTIVITY OF THE CASHEW, ANGICO, LEMON AND SERIGUELA GUM

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**Abstract:** Polysaccharides are biopolymers of complex structures, which may be formed by one or more carbohydrate units. Structural characteristics and physicochemical properties that polysaccharides have, including water solubility, molecular weight, monomer composition, types of glycosidic bonds of the main chain and branching, may contribute to good biological activity. With this, the objective of this work is to analyze the influence of properties of cashew (CG), angico (AG), lemon (LG) and seriguela gum (SG) in front of its cytotoxic profile. The gum was isolated and characterized, where its composition of carbon, hydrogen and nitrogen was determined through elemental analysis besides determination of the surface charge of the gums, molar mass and composition of monosaccharide. The cytotoxic profile of gums by the method of 3–(4,5–dimethyl–2–thiazole)–2,5– diphenyl–2–H–tetrazolium bromide (MTT) salt against normal and cancerous lineage of cells. The polymers analyzed had relatively negative zeta profiles, a certain difference in the percentage of carbon, hydrogen and nitrogen, where the SG and LG gums had a higher percentage, concomitant to the molar mass. About the cytotoxic effect against cancer cell lines, CG and LG have been shown to be more effective for leukemic, melanoma and colorectal lines. When related to cytotoxicity in normal cells, CG presented lower values of inhibition. Demonstrating great for biomedical applications.

**Keywords:** Natural polymers; Antitumor activity; Biocompatibility.


## *IN VITRO* ANTITUMOR POTENTIAL OF QUATERNIZED ANGULARGUM

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**Abstract:** The angico gum (AG) obtained from the red angico exudate (Anadenanthera colubrina var. Cebil (Griseb) Altschul) shows heteropoly–saccharides composed of arabinose, galactose, rhamnose and glucuronic acid, exhibiting great potential for biotechnological applications. In this approach, the objective of the present work was to investigate the cytotoxic profile of the modified red angico gum with the CHPTAC etherifying agent. For this, the gum was isolated, quaternized (QAG) and characterized, where its composition of carbon, hydrogen and nitrogen was deter–mined through elemental analysis besides determination of the surface charge of the gums. The cytotoxic profile of AG and QAG by the method of 3–(4,5–dimethyl–2–thiazole)–2,5–diphenyl–2–H–tetrazolium bromide (MTT) salt against normal and cancerous lineage of cells. From the charac–terization data, it was possible to observe that the polymer that passed through the quaternization process presented an increase in the percentage of carbon, hydrogen and nitrogen, concomitant to the increase of the surface charge due to the inserted group. Both gums presented cytotoxicity to normal cells at concentrations higher than those that showed activity against tumor cell. Therefore, the results demonstrate that the QAG presented in this study is a very promising biomaterial for biotechnological applications.

**Keywords:** Angico red; cytotoxicity; biocompatible.


# SHELF LIFE, REOLOGY AND MORPHOLOGICAL CARACTERIZATION OF BACTERIAL CELLULOSE/ALGINATE HYDROGELS

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**Abstract:** The number of people affected by hard–to–heal wounds (chronic wounds) has been increasing in recent years, and the most affected group are the diabetics and hypertensives. One of the natural biomaterials used in the treatment of these wounds is the bacterial cellulose (BC), a product excreted by gram–negative bacteria, due to its properties such as biocompatibility, non–toxicity and the nanometric size of its fibers, which mimic natural collagen fibers, favoring the healing process. Alginate obtained from the extraction of brown algae is also a non–toxic and biocompat–ible material. When alginate is in contact with a wound, it causes an ion exchange between the sodium present in wound and the calcium from the alginate, forming a gel with exudative activities, aiding the electrolytic debridement. Due to the these biomaterials properties, bacterial–cellulose/al–ginate hydrogels were prepared. Their morphological structures were analyzed by Scanning Electron Microscopy (SEM). In addition, rheology assays has been performed in order to evaluate the viscosity as well as the scatterability of the hydrogels. Aiming the final product, the shelf life experiments are being carried out in order to evaluate the rheological behavior of the hydrogels.

**Keywords:** Bacterial Cellulose Hydrogel; Wound Healing; Shelf Life.


# PHYSICOCHEMICAL PROPERTIES OF PECTIN AND KONJAC GLUCOMANNAN FILMS ADDED WITH SUGARCANE VINASSE

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Area: ( X ) Food and Agriculture ( ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** The use of plastics in agriculture allowed significant improvements on productivity and quality. However, environmental concerns about the excessive use and disposal of synthetic polymers have boosted the interest for biodegradable polymers to produce alternative materials. Biodegradable materials can be integrated directly into the soil where are converted by microorganisms into carbon dioxide or methane, water, mineral and biomass, with no negative environmental impact or ecotoxicity. Vinasse, the main wastewater from ethanol industry, is rich in nutrients and usually applied in sugarcane crops through fertigation. However, its use is limited to the areas near the processing units and when indiscriminately applied can lead to soil salinization and groundwater contamination. The present study proposes the use of vinasse as the solvent for pectin (PEC) and konjac glucomannan (KGM) composite films. Vinasse had its pH lowered and then biopolymers were added in different proportions of PEC:KGM (100:0, 75:25, 50:50, 25:75 and 0:100). The solutions were casted into molds and dried at 40°C/18h. After drying, films were crosslinked by immersion in ethanolic 2% calcium chloride solution for 30 min. Films were characterized by their thickness, moisture content, water vapor permeability (WVP) and mechanical properties. All films were homogeneous and easy to handle. Non crosslinked films were completely water soluble and shower higher WVP and elongation compared to calcium crosslinked films. Moisture content, WVP and elongation decreased by increasing pectin concentration. Pectin/KGM films added with vinasse exhibit suitable functional attributes with good perspectives for agriculture applications.

**Keywords:** Pectin; Konjac Glucomannan; Vinasse.


## CHARACTERIZATION OF CHITOSAN PARTICLES ADDED WITH VINASSE INTENDED FOR SOIL FERTILIZATION

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**Abstract:** Technologies aiming to control or hinder the release of nutrients from fertilizers have been studied in order to improve agricultural systems efficiency with minimum environmental impact. In this context, biopolymers, such as chitosan, can be used to develop slow/controlled release systems with a focus on agriculture. Sugarcane vinasse is the wastewater from ethanol industry (about 12 L per liter ethanol) being mainly applied as fertigation in sugarcane crops. However, this disposal practice has been questioned due to potential effects on the soil and on groundwaters caused by nutrient lixiviation such as potassium. So, the use of vinasse as a solvent to produce chitosan particles was proposed. This study evaluated the properties of chitosan and vinasse particles (Chi–V) intended for soil fertilization applications. Particles were obtained by dripping the biopolymeric solution (3% in vinasse) into a crosslinking solution (sodium tripolyphosphate 5%) followed by drying. Chi–V particles were characterized according to average diameter, bulk density, pH, moisture content, water solubility, swelling degree, chemical composition and morphology. Results indicate that vinasse nutrients were properly incorporated into the chitosan matrix. Particles showed spherical shape with an average diameter of 2 mm, bulk density of 846 kg $m^{-3}$, pH 5.8, 13% moisture content, 46% water solubility and equilibrium swelling degree of 8 g $H_2O$/g. According to the Brazilian legislation Chi–V particles could be classified as organomineral fertilizer class A, added with Ca, S, Cu, Fe, Mn and Zn. Particles showed potential to be applied as fertilizer in agriculture, allowing the recycling of nutrients from vinasse to the soil and, above all, represent a novel alternative for the use of this expressive wastewater from the sugar and alcohol industry.

**Keywords:** Chitosan; Vinasse; Slow Release Fertilizer.


# SURFACE CHARGE OF COLLOIDAL SOLUTIONS OF GELATIN AND/OR CHITOSAN AT DIFFERENT PH

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**Abstract:** In recent years there has been an increase in research using natural polymers due to their peculiar properties that make them suitable for the most diverse applications, extending from the food to the pharmaceutical industry. In this study, characterization and rheological properties of pure components and blend of two biopolymers at different pH were investigated. The solutions were prepared separately, first pig gelatin type A (4%) was hydrated for 30 minutes and then was stirred for 30 minutes at 55 ° C in a thermostatic bath. Chitosan (2%) was diluted with acetic acid (2%) and kept under stirring for 12 hours at 40 ° C using a magnetic stirrer. Then, the solutions with 50:50 ratios were mixed for 2 hours at 50 ° C with constant stirred. The pH was adjusted (3,5 until 6,0) with 0.05M NaOH or 0.05M of acetic acid for further rheological analysis in steady state and dynamic tests (strain, frequency and temperature using a rheometer (AR2000 Advanced Rheometer; TA Instruments, New Castle, DE, EUA)). The determination of zeta potential NanoBrook Zeta Plus Zeta Potential Analyzer, BTI, USA) was done with pure solutions and blends separately, pH was adjusted aft er diluting solutions in the ratio of 1:10. The results indicated that for 4% of pure gelatin solution, zeta potential showed a decreasing of positive charges, the isoelectric point was 9.0; for pure chitosan (2%) two peaks of positive charges were obtained due to its cationic nature, showing some instability, and for the blend negative charges were close to 50. Due to the work being in its initial phase there are still many results that will come later.

**Keywords:** Biopolymers; Isoelectric Point; Zeta Potential.


# DEVELOPMENT AND CHARACTERIZATION OF FILMS BASED ON CELLULOSE NANOFIBERS AND QUATERNIZED ANGICO GUM FOR BIOLOGICAL APPLICATION

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**Abstract:** Several studies have been carried out on materials that can replace the existing dressings in the market, for example, some biopolymers, such as chitosan, gelatine, collagen and bacterial cellulose, which have excellent biological properties for tissue regeneration. The chemical modifications in the structure of the polysaccharides, such as quaternization, involve the introduction of functional groups allowing the production of new biomaterials with new properties and applications. The objective of this work is the development of a new product for the development of films functionalized with bacterial cellulose nanofibers (BC) and quaternized angico gum (QAG) for future biological applications. The films were produced following the casting technique, which has the principle of evaporation of the solvent, using the biopolymers. The films were characterized by: Infrared absorption spectroscopy with Fourier transform (FTIR), thickness, measured with MED25 digital external micrometer and atomic force microscopy (AFM). Of the films developed, two were chosen to be functionalized with BC and QAG, both with polymer matrix composed of agar to 3% and 1% respectively. The thickness of the developed films presented a variation of 0.0225 mm to 1.2 mm, being characterized as ultrafine films. For the FTIR, the spectra of the films presented some of the main characteristic bands of BC and QAG, with some displacements and changes of intensity, evidencing that there was chemical modification in the polymer. In the analysis of the films by AFM, the BC presents a greater roughness compared to QAG and the characteristic nanofibers of BC are very evident. In addition, the QAG film was more homogeneous and with less irregularities. It was concluded that the developed films have potential for the application as new biomaterial, for example, possible substitute for traditional dressing and further studies will be done to test the antibacterial activity of film with QAG.

**Keywords:** Biopolymers; Quaternization; Biomaterials.


# PHYSICOCHEMICAL PROPERTIES OF BILAYER FILMS BASED ON GELATIN WITH NATAMYCIN

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**Abstract:** Traditionally, packaging had a passive role. Nowadays, packaging can have an active role protecting foods and contributing to the reduction of synthetic additives added into foodstuff, with active agents are into packaging material, such as biopolymer–based films, incorporated with natural antimicrobials. The aim of this project was the development of antimicrobial double layer films, loaded with natamycin, and the evaluation of their physicochemical properties. To produce these films, gelatin was hydrated for 30 min at room temperature and solubilized in thermostatic bath (60 °C/15 min). Glycerol was used as plasticizer, at a concentration of 30 g/100g of gelatin, and the natamycin was added at the concentration of 0 and 0.5 g/g of gelatin. The film–forming solution was poured in a plate and dried in a forced–air circulation oven at 30 °C/24 h. The first layer of film was composed only by gelatin and glycerol, while the second one, a little thinner, was composed by gelatin, glycerol and natamycin. Both layers were produced by casting, and after being dried, films were conditioned into desiccators containing saturated solutions of NaBr (RH = 58% at 25 °C) for seven days. Films were characterized for moisture content and solubility, optical and mechanical properties, water vapor permeability (WVP), and water contact angle (WCA). The addition of natamycin did not affect the moisture of the films, remaining around 14%, but increased the film solubility from 38 to 46%. Regarding the mechanical properties, the natamycin increased the tensile strength and Young's modulus, and significantly decreased the elongation. Still, the natamycin also had high influence on the optical parameters, decreasing the values of $\Delta E^*$ and film opacity. Natamycin also increased, although not significantly, the WVP of the films, and decreased the values of WCA. The addition of natamycin can promote an antimicrobial activity to films, without affect their main physical properties.

**Keywords:** Natural Compounds; Moulds; Active Films; Physicochemical Properties.


# TRATMENT AND MANAGEMENT OF PRESSURE LESION WITH BIOCURATIVES OS BIOCELLULOSE CONTAINING PROPOLIS EXTRACT EEP–AF

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**Abstract:** Hospital–acquired Pressure Injuries (HAPI) develops by compressing soft tissue to rigid surface such as bone prominence. These are classified into four stages ranging from intact skin with dermal discoloration to necrotic tissue that may expose subepithelial muscles and structures. Care for the treatment of this lesion includes necrotic tissue debridement and dressing with Silver sulfadiazine 1% or other formulas, such as: 10% sulfanamide acetate and 02% nitrofurazone and 0.1% Gentamicin creams, among others. However, these treatments have adverse reactions, such as: leucopenia, allergic processes due to the oxidation of the components, and discomfort for the patient, who need to have their dressings changed daily, which makes the healing process slow and painful. In order to improve the treatment and management of HAPI, the evaluation will be quantitative in character descriptive, using the bacterial cellulose membrane containing propolis in the treatment and management of these lesions, evaluating the cicatricial process, using as a method of evaluation of effectiveness of membrane use or ImageJ software (version 1.48v). The research site will be the Santa Lydia Hospital located in the city of Ribeirão Preto, São Paulo and the subjects of the research will be the patients of the institution that accept to participate in the research signing the Term of Free and Clarified Compromise. The project is being evaluated by the Research Ethics Committee of the University Center Estácio of Ribeirão Preto. The membrane of biocellulose containing EEP propolis extract will be supplied by the company APIS FLORALTDA. It is intended to obtain a sample of 30 LPP patients in stage one and two, according to the National Pressure Ulcer Advisory Panel (NPUAP),objectifying with a positive evolution of the cicatricial process with reduced healing time compared to the therapeutic measures already implemented in the health services.

**Keywords**: Pressure lesions; Membrane; Propolis; Bacteral cellulose membrane.


# NATURAL LATEX MADE WITH SILVER SULPHADIAZINE FOR THE TREATMENT OF BURNS

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**Abstract:** Every year millions of people are treated by burns, with children and low–income people being the most affected. As burns achieve the first protective barrier of the human body, the epidermis, they increase the risk of infection and the number of fatal victims. The use of antibiotics assists in the fight against infection, but not in tissue healing, making the patient's organism susceptible to microorganisms. The objective of this study is to associate the cicatricial and angiogenic characteristics of natural latex with silver sulfadiazine (SFZ), an antibiotic commonly used in the treatment of burns. The adhesives were molded by mixing 4 mL of natural latex with 4 mL of SFZ solution (1 mg / mL) to complete polymerization. The material was characterized by using MEV, FTIR, mechanical resistance and the antibiotic release through the polymeric matrix was monitored by spectrophotometer (UV–Vis) at $\lambda$ = 241nm for 196 hours. Through the FTIR technique, it was possible to observe that the incorporation of SFZ in the latex did not present the appearance of new bands or covalent bonds, which would result in the entrapment of the drug or the appearance of toxic compounds. By using MEV, it was observed that the drug was uniformly distributed in the membrane. The strain–strain tests showed that there was an increase of 22% in the maximum deformation of the material after the incorporation of SFZ and an increase in the elasticity of the material. Optical spectroscopy revealed that 23.7% of the sulfadiazine was released by the latex membrane in 196 hours, where the kinetics followed a biexponential equation, with a faster release in 24 hours, in which 18.8% of the antibiotic was released. The latex membrane incorporated into silver sulfadiazine has shown to be a good release matrix and it can be used in places of great body movement.

**Keywords:** Natural Rubber Latex; Silver sulfadiazine; Burn.


# GELAN GUM MICROPARTICLES REINFORCED WITH VEGETAL CELLULOSE NANOFIBERS AS A DRUG DELIVERY STRATEGY

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**Abstract:** Microparticles are multiparticulate systems of size between 1 and 1000 μm and defined shape that have been used successfully in the design of controlled drug release systems Such systems are advantageous over conventional release systems, since they allow temporal and/or spatial release control, effectively contributing to the increase of the therapeutic effect and reduction of side and toxic effects. The physico–chemical, structural, thermal, mechanical and control properties of microparticle release can also be optimized through reinforcement using cellulose nanofibers, which have unique properties such as high mechanical strength and stiffness. Morin is a flavonoids that has been identified in a large number of medicinal herbs, is well known for its highly potent anti–hyperuricemic, anti–inflammatory and anti–cancer activities, In view of the above, the objective of this work was to obtain and characterize gellan gum microparticles by the ionotropic gelation method and to evaluate the effect of reinforcement with cellulose nanofibers on the fundamental properties and performance of the microparticles in the encapsulation and release of morin. Partial results: Scanning Electron Microscopy, revealed that the microparticles have roughly spherical structure and rough surface. The increased addition of NFC showed a significant reduction of the particle diameter in relation to the control sample, for the introduction of 3 and 5% NFC, respectively. On the other hand, the same behavior was not observed for the highest concentration of 7%, which presented a similar size to the control sample. The swelling results of the microparticles were analyzed at different concentrations of NFC. Sample control,swelling of 25% and 90% after 2 min and 90 min of assay. In the sample containing 3% NFC, swelling of 24% and 55% was observed after 2 min and 60 min. A distinct behavior was observed in the other samples. In those with 5% NFC they suffered a swelling of 85 and 88% after 60and 90 min, contributing to the increase of their speed of absorption. This behavior can be corroborated by the liquid absorption profile of the microparticles containing the highest NFC load (7%), which reached 110% swelling in 90 min.

**Keywords:** Microparticles; Gellan Gum; Cellulose Nanofibers; Controlled Drug Release Systems; Morin.


# FABRICATION, CHARACTERIZATION AND IN VITRO CELL STUDY OF SCAFFOLDS OBTAINED FROM NATURAL POLYMERS FOR SOFT TISSUE ENGINEERING

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Area: ( ) Food and Agriculture ( x ) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Over the past two decades, numerous biomaterials, including polymers, ceramics, and metals have been actively investigated for tissue engineering (TE) applications. TE aims to create medical devices that can repair or regenerate tissues impaired by disease or injury, those structures are typically fabricated by seeding scaffolds with cells. Scaffolds provide the necessary support as artificial extracellular matrices to serving as tem–plates in guiding the development of new tissue. Gelatin (G), a soluble protein obtained by hydrolysis of collagen, it´s suitable as a biomaterial for TE, has low cost, adequate biocompatibility and biodegradability. This protein can be blended with Chitosan (CH) to improve its biological activity since it contains an Arg–Gly–Asp (RGD)–like sequence, which promotes cell adhesion, migration, and forms a polyelectrolyte complex. In this study we have chosen G and CH, incorporated with bioactive components of natural origin like Aloe vera (A) and snail mucus (S). For production of porous scaffolds, first, scaffold forming suspensions (SFS) of (G) 2% w/w and (CH) 1% w/w were prepared separately with constant stirred for 2 h at 50°C in thermostatic bath, then were mixed with 50:50 ratio. Later (A), (S) and both (A+S) were added at 0.15% w/w. SFS were frozen at –80°C for 2 h, prior to freeze–drying at –58°C for 18h. The resulting sponge–like material was crosslinked and freeze–dried again. Fibroblasts and mesenchymal stem cells were used to study the adhesion capacity to scaffolds and morphological changes during 28 days of incubation. Scaffolds showed to be a highly porous sponge displaying interconnected porosity and homogeneous pore diameters, as well as pore wall thickness. Important microstructural changes were observed in the scaffolds as a function of additives. SEM revealed that the cells appeared to attach and spread well in all scaffolds, forming multiple protrusions and cellular aggregates that gradually increased in size.

**Keywords:** Scaffolds; biomedical applications; mesenchymal stem cells.

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# CHARACTERIZATION OF THE BIODEGRADABILITY OF PLASTICS USING *GALLERIA MELLONELLA* LARVAE

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**Abstract:** The intense consumption of plastic materials, characterized by its high durability, summed up to the fast and undue waste, results in the accumulation of debris and consequently a pollution that has reached the most remote areas of the planet. In 2017, however, it was observed that moth larvae of *Galleria mellonella* are able to digest polyethylene (PE), a type of plastic produced by the chemical industry, turning it into ethylene glycol, which can be used in the automotive industry. Given that Brazil is considered the fourth largest producer of plastic waste in the world and one of the countries that recycles the least, the ability of *G. mellonella* larvae to biodegrade materials made from various types of plastics such as PE and polystyrene (PS) proves to be extremely important, justifying the urgency of its study. Thus, the present proposal aims to evaluate the biodegradability of these larvae in bags, trays and bottles, analyzing the loss of mass, wettability and composition of the material, before and after contact with the animals. To perform the experiment, each group of larvae composed by ten subjects was placed in contact with approximately 2g of a type of plastic, which were: high density polyethylene (HDPE), low density polyethylene (LDPE) and PS. After two weeks, loss of mass was observed for LDPE and PS, while larvae did not appear to interact with HDPE. In this way, it can be observed that the density of the material is directly related to its biodegradability.

**Keywords:** *Galleria Mellonella;* Biodegradation; Plastics.


# PRODUCTION OF BIOFILMS FROM KALE PUREE AND SODIUM ALGINATE FOR USE AS WRAPS

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**Abstract:** The objective of this work was to produce edible films based on kale *(Brassica oleracea L. var. Acephala)* and sodium alginate that have satisfactory properties and market potential. The kale leaves were sanitized and blanched. A puree was made from kale and water for incorporation into sodium alginate and glycerol (plasticizer), and the mixture was homogenized in mechanical stirrer for 3 hours at 1000 rpm, degassed for 20 minutes in vacuum pump, cast on Mylar substrates, and left to dry at 25 ° C for 30h forming the non–crosslinked film. After drying, the film was immersed still attached to the Mylar surface and soaked on a 2% calcium chloride solution, used as a crosslinking agent, and the film was dried again at 25 ° C for 2h, forming the crosslinked film. The films have been analyzed for: thickness, swelling in water and contact angle. While the non–crosslinked film completely solubilized after 5 minutes in water, releasing pigments to the water, the crosslinked film got low degree of swelling in water (<50% of the initial mass). The crosslinked films presented a reduction in the water absorption and increase the contact angle in relation to the water, indicating a significant decrease in the hydrophilic nature of the films. The absorption time found for the crosslinked film treated samples was 1 min and the contact angle were 63°. The results showed that the developed films present characteristics similar to fresh kale as characteristic color and thickness, in addition to presenting hydrophobic properties, making them interesting for applications in the food sector.

**Keywords:** Films; Kale puree; *Brassica oleracea L*; var. *acephala.*

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# DEVELOPMENT OF LATEX MEMBRANE WITH *ALOE VERA* GEL EXTRACT AS AN ADDITIONAL PSORIASIS TREATMENT

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**Abstract:** *Aloe vera* brings in its composition active principles that have healing properties and its application for the treatment of dermal wounds is excellent. In view of this, this work aims to develop latex membranes with *Aloe vera* gel extract, for complementary treatment to psoriasis, a disease that affects 2–5% of the world population, causing injuries to the patient's skin and compromising his emotional stability, professional life and social. Latex from *Hevea brasiliensis* was processed and centrifuged. The *Aloe vera* gel extract was withdrawn from the in natura leaves of the plant, centrifuged at 2000 rpm for 15 minutes, the supernatant was discarded and the pellet lyophilized. In Petri dishes, 4 ml of latex and 2 ml of the aqueous solution containing the lyophilized gel (10 mg/mL) were added. The chemical interaction between gel and natural latex was evaluated by a Fourier transform infrared spectrometer (FTIR, Bruker Tensor 27) with an attenuated total reflectance (ATR) accessory. The liberation of the vegetal extract was evaluated for 96 hours in 50 mL of water and quantified from the spectrofluorescence, λem = 528 nm. With the FTIR technique it was possible to observe that there was no molecular interaction, demonstrating that the pharmacological properties of the extract were preserved. In the release kinetics there was release for 72 hours, showing that the membrane can be changed every 3 days. Approximately 85% of the total extract was released, and in only 24 hours it had been released 40%, confirming the effect of burst release, where the extract present on the membrane surfaces are released faster in the first hours and slower release. The total concentration released is satisfactory because it already induces healing activities. It is concluded that the bioproduct may have a great potential for complementary treatment of psoriasis.

**Keywords:** Natural Rubber Latex; Psoriasis; *Aloe vera*.


# INFLUENCE OF POWDERED CAMU–CAMU (*Myrciaria dubia*) BY– PRODUCT IN STARCH–BASED FILMS

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**Abstract:** Processing of camu–camu generates a large volume of by–products, which can be used for active films development. The aim of this work was the development of films with antioxidant activity based on starch and camu–camu by–product (CCBP). Initially, by–product was dried (70°C/5 h), ground and granulometry was standardized (100 mesh). Films were prepared by tape–casting technique with sorbitol (40 g/100g starch) and pre–gelatinized starch (5 g/100 g solution). The CCBP was incorporated into the concentrations of 0 (control film), 1 (CC1) and 2 (CC2) g CCBP/100 g of filmogenic solution). The films were characterized in relation to: water solubility, contact angle, mechanical properties and antioxidant activity (was measured by ORAC). The water solubility was not affected by the addition of CCBP. The contact angle increased from 63 ± 9° (control film) to 84 ± 8° (CC1) and 85 ± 3° (CC2), indicating films with hydrophobic surfaces. Tensile strength increased from 8.4 ± 0.6 MPa (control films) to 12.7 ± 1.2 MPa (CC1) and 15.3 ±1.5 MPa (CC2). Young's modulus increased from 347.1 ± 36.5 MPa (control film) to 639 ± 51 MPa (CC1) and 861.2 ± 67.5 MPa (CC2), while elongation at break decreased. CCBP addition acted as reinforcing agent of the starch film, increasing its resistance. The films with CCBP incorporation presented antioxidant activity, it was observed an increase from 0.47 ± 0.22 (control film) to 50.27 ± 7.02 (CC1) and 68.63 ± 5.40 (CC2) µMol Trolox Equivalent/g of dry matter. Thus, the addition of CCBP improved mechanical properties of the starch–based films, besides promoted antioxidant activity. These films have potential to be used as active films for food packaging.

**Keywords:** Active films; waste; antioxidant activity.


# EFFECT OF DIFFERENT RELATIVE HUMIDITY CONDITIONS ON DRYING PROCESS OF GELATIN FILMS PLASTICIZED WITH TRIBUTYL CITRATE

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**Abstact:** A limiting problem in biodegradable films based on natural macromolecules is their susceptibility to ambient humidity, since both, macromolecules and plasticizers, normally used in these materials production are hydrophilic. The aim of this study was to produce biodegradable gelatin–based film using hydrophobic tributyl citrate plasticizer (TBC) and evaluate the drying conditions effect (T=30°C and RH=45, 60 75%) on its properties. Films were produced by casting technique using 2g gelatin/100g of film–forming solution (FFS), 50g TBC/100g gelatin, 60g lecithin/100g plasticizer and 20g ethanol/100g FFS. Films were dried for 24 hours at 30°C and 45, 60 and 75% RH, using a climatic chamber with temperature and humidity control. Films were conditioned in desiccators with NaBr (58% RH) for 7 days and submitted to the following characterizations: mechanical properties, moisture content and solubility. The increase of drying relative humidity caused a decrease in films moisture content, from 10.9±0.2 to 9.3±0.6 g$H_2O$/100g FFS, being the drying time for the films of 8 hours for 45% of relative humidity (RH), 10 hours for 60% RH and 12 hours for 75% RH. There was no significant difference in films solubility (8.5g/100g FFS). For mechanical properties, the increase of drying relative humidity caused a decrease in tensile strength (from 44.9±4.1 MPa to 40.8±2.8 MPa) and Young's modulus (from 1359±115 MPa to 1208±83MPa), and did not provide significant differences in elongation (7.9±1.3%).Thus, although the use of different relative humidity in drying process of films caused some differences, it is possible to affirm that they have a reduced impact on its properties, forming concise films and with possibility of application as packaging in food industry.

**Keywords:** Hydrophobic Plasticizer; Drying Conditions; Mechanical Properties.


# EFFICACY OF HEALING IN DIABETIC FOOT ULCERS USING NATURAL RUBBER LATEX MEMBRANE

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**Abstract:** Cutaneous ulcers are those that compromise the integrity of the tissue regardless of its extent, reaching the subcutaneous region and underlying tissues. Several factors and diseases can influence the healing process leading to chronic wonds, such as Diabetes Mellitus, leading to diabetic foot ulcers. These chronic injuries require a high financial investment and are difficult to heal. Natural rubber latex is widely used in biological applications because of its good mechanical strength, flexibility and elasticity. It also has angiogenic capacity, which accelerates the healing process. The aim of this study was to evaluate the efficiency of the treatment of diabetic foot ulcers using the natural rubber latex membrane. We selected 10 participants with diabetic foot ulcer to compose the study. The natural rubber latex membrane was made by the casting method with a final thickness of 1millimeter. Of the 10 participants selected, only 8 followed with treatment. The wounds ranged from 45 days to 20 years of existence and were completely reepithelialized in 75% of the participants, with the shortest treatment time being 9 days and the longest treatment time was 139 days. The shorter the duration of the lesion, the faster the healing. The same applies with respect to its extent. The dressing was efficient in the healing process, with angiogenic properties, debridement capacity and consequently, the appearance of the granulation tissue, aiding and effecting the chronic wound healing.

**Keywords:** Natural Rubber Latex; Membrane; Diabetic Foot Ulcers.


# MORPHOLOGICAL CHARACTERIZATION OF HYALURONIC ACIDS AND THEIR CLINICAL INDICATIONS

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**Abstract:** The versatility of Hyaluronic Acid (HA) as a skin filler makes it a product with diverse characteristics depending on the manufacturer, type of crosslinking, and product concentration. These differences are important in clinical practice because they will determine the correct indication of each product, its local stability, and its durability. The objective of this study was to compare the superficial and internal morphology of lyophilized samples from four different commercial HA presentations, associating their characteristics with the perfect clinical indication. Rennova Ultra® 24 mg / mL (1), Rennova® Ultra® 20 mg / mL (2), Rennova Deep® 20 mg / mL (3) and Rennova Ultra® Deep® 20 mg / mL (4) were freeze–dried at –80 ° C for 2 days and subsequently lyophilized for 2 days using L101 equipment (Liotop, São Carlos – Brazil). Samples were fixed in stubs and metalized with carbon for SEM analysis. Photomicrographs with magnification of 40X, 100X and 500X and 1,500X were obtained using the FEG–MEV scanning electron microscope JSM–7500F (Jeol Ltda, Tokyo – Japan) from the Advanced Microscopy Laboratory (LMA) of the Chemistry Institute of Araraquara – UNESP. Gel 1 presented pores or chambers with larger diameter compared to the other gels, thus showing a looser structure and with less capacity to withstand tensions. The second showed a slightly dense structure compared to 1. The more homogeneous structure was presented in gels 3 and 4, which suggests a higher crosslinking rate indicating greater collectivity, as well as dermal volumizing capacity, supporting higher tensions. It is ob–served that, due to their morphological characteristics, gels 2, 3 and 4 are more suitable to correct greater loss of volume while gel 1 is more suitable for surface filling, since it has a structure with more pores and consequently supports less stress your clinical indication may be the lip contour and small loss of collagen.

**Keywords:** Hyaluronic acid; SEM; reticulation


# PRODUCTION OF NANO/MICROFIBERS OF NATURAL RUBBER CONTAINING REDUCED GRAPHENE OXIDE BY ELECTRO–SPINNING

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**ABSTRACT:** THE ELECTROSPINNING SYSTEM IS CAPABLE OF PRODUCING NANO/MICRO POLYMER FIBERS WITH INTERESTING FEATURES THAT CAN BE APPLIED TO SENSORS, CONTROLLED RELEASE AND TISSUE ENGINEERING. SEVERAL POLYMERS CAN BE USED FOR SUCH APPLICATIONS, BUT NATURAL RUBBER OBTAINED FROM LATEX EXTRACTED FROM THE RUBBER TREE *HEVEA BRASILIENSIS* IS OF LARGE INTEREST FOR THE PREPARATION OF BIOMATERIALS DUE TO ITS MECHANICAL PROPERTIES, STIMULUS TO ANGIOGENESIS, AND THE POTENTIAL APPLICABILITY AS A VEHICLE FOR DRUG RELEASE. IN ADDITION TO ITS EXCELLENT PROPERTIES, LATEX ALLOWS FOR THE POSSIBILITY OF ***IN–SITU*** REACTIONS SUCH AS THE REDUCTION OF GRAPHENE OXIDE. REDUCED GRAPHENE OXIDE (RGO) HAS NUMEROUS PROPERTIES THAT CAN BE ADDED TO NATURAL RUBBER AS THE IMPROVEMENT IN ELECTRICAL, THERMAL AND MECHANICAL PROPERTIES, ALLOWING THE USE OF THIS NATURAL POLYMER IN SENSORS. GRAPHENE OXIDE WAS REDUCED ***IN–SITU*** IN THE LATEX WITH THE USE OF CITRIC ACID, WHICH ADDS A CHARACTER OF "GREEN SYN–THESIS" TO THE REACTION. AFTER THE REDUCTION REACTION, THE LATEX WAS DRIED AT 60 ° C FOR 10 HOURS. AFTER DRYING, THE OBTAINED MATERIAL WAS SOLUBILIZED IN CHLOROFORM, FORMIC ACID AND DMF WITH THE POLYMER SOLUTION CONCENTRATION OF 3% FOR NANO/MICROFIBER PRODUCTION. THE MORPHOLOGICAL ANALYSES OF THE ELECTROSPUN FIBERS PRODUCED WERE CARRIED OUT BY SCANNING ELECTRON MICROSCOPY, WHERE HOMOGENEOUS FIBERS WITH NO BEADS AND AVERAGE DIAMETER OF 2,2 µM WERE OBTAINED. ADDITIONALLY, THE PRESENCE OF RGO ON THE SURFACE OF NANOFIBERS COULD ALSO BE OBSERVED. OUR PRELIMINARY RESULTS INDICATE THAT WITH THE USE OF THE ELECTROSPINNING SYSTEM IT IS POSSIBLE TO PRODUCE NANO/MICROFIBRES OF NATURAL RUBBER WITH RRO, WHICH WILL BE FURTHER APPLIED IN SENSORS AND DEVICES.

**KEYWORDS:** NATURAL RUBBER; OXIDE GRAPHENE; ELECTROSPINNING.


# USE OF POLYSACCHARIDE FROM MACROALGA *GRACILARIA* SP. FOR THE DEVELOPMENT OF POLYMER NANOPARTICLES

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**Abstract:** The red macroalgae consist of a class of aquatic organisms in which polysaccharides with recognized pharmacological activities can be extracted besides presenting characteristics such as availability, biocompatibility and versatility. In their structure they present hydroxyl groups and sulphates which favors the chemical modifications, which means the insertion of new chemical groups in its structure, allowing its use in obtaining new materials. The present work aimed to promote the modification of the polysaccharide isolated from Gracilaria sp., With phthalic anhydride, at the polysaccharide: anhydride ratios of 1: 2 and 1: 5, to obtain a hydrophobic material. The derivative obtained was characterized by FTIR and nanoparticles were synthesized by means of dialysis at concentrations of 0.1%, 0.05% and 0.025%, using as solvent dimethylsulfoxide (DMSO). The nanoparticles were then characterized by Uv–vis and DLS spectroscopy. The FTIR results confirm the modification due to the presence of ester car–bonyl bands and aromatic ring bands. Sizes were observed in the range of 518.7 to 232.9 nm for all concentrations, however 0.05% was outstanding, due to the lower value of PDI. The nanoparticles were successfully prepared and have potential for the drug delivery system.

**Keywords:** Polymer; Synthesis; Nanostructured.


# CHEMICAL MODIFICATION OF ANGICO GUM AND INVESTIGATION OF ITS BIOTECHNOLOGICAL POTENTIAL

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**Abstract:** Gums are complex natural carbohydrates that represent an abundant class of macromolecules of great importance for the industrial area as raw material. Among them, we have the angico gum which are resins exuded from native trees of the Northeast of the genus Anadenanthera colubrina var. cebil. The chemical modification acts in the improvement of the application of the materials as to its advantages, improving the natural characteristics for the use of these polymers as compatible biomaterials or, as polymer matrices for the drug delivery, aiming at the operational interaction of the drug interaction sites and the hydrophilic fields optimizing the therapeutic index. The present work aims at the esterification of gum from angico (GA) with propionic anhydride in order to investigate its activities regarding biocompatibility, antimicrobial activity, antioxidant and nano-structured system formulation. The chemical modification was verified by means of the spectra obtained by the Infrared Spectroscopy (FTIR) analysis comparing the spectra of the angico gum (GA) with the angiotonic gum modified with propionic anhydride (GAMAP) by evaluating the insertion of the functional groups after the modification and by means of the X-ray Diffraction(XRD) verifying the crystallinity of the obtained material comparing with the starting material. The GAMAP derivative had no antibacterial effect against S. aureus and E. coli. However, it was satisfactory in hemolytic assays with human erythrocytes. The GAMAP derivative showed antioxidant activity against the capture of the ABTS • + radical. Derived biopolymers are presented as promising biomaterials for use in biotechnological applications.

**Keywords:** Exudate; Chemical Modification; Biotechnology.


# INVESTIGATION OF BACTERIAL CELLULOSE MODIFIED WITH MERCAPTOSILANE IN THE ADHESION OF HUMAN FIBRO–BLASTS

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**Abstract:** Cellulose is the most abundant polymer on earth, and it can be produced by plants, algae, fungi and bacteria. Cellulose synthesized by bacteria presents unique properties as high water holding capacity, biocompatibility, biodegradability and three–dimensional architecture similar to the extracellular matrix. Despite being a promising support for cell cultivation, bacterial cellulose (BC) does not allow proper cell adhesion on its surface. So the purpose of this study is to modify the BC surface in order to improve the adhesion of cells, particularly of human fibroblasts. Therefore, BC membranes synthesized by *Komagataeibacter xylinus* were modified with (3– mercaptopropyl)trimethoxysilane, being evaluated the following parameters: silane concentrations (0.538, 0.135 and 0.034 mmolmL–1), solvent (acetone and a mixture of ethanol and water) and drying method (at room temperature and at 120°C). The modified membranes were analyzed by FTIR, TGA, SEM and XPS. XPS analysis demonstrated that the surface modifications were modest in all the tested conditions, however the applied treatments increased the thermal stability of the platforms. SEM analysis showed that the most significant modification occurred for the surface treated with the highest silane concentration using ethanol and water as sol–vent and dried at 120°C, yet all membranes maintained the BC characteristic three–dimensional nanometric structure. Cell adhesion and proliferation using human fibroblasts as well as resazurin cell viability assays are being performed.

**Keywords:** Bacterial cellulose; Mercaptosilane; Human Fibroblasts.


# MULTILAYERED FILM ARCHITECTURE AS A STRATEGY TOCONTROL DRUG LOAD AND RELEASE PROPERTIES

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**Abstract:** The layer–by–layer technique has been widely used for the self–assembly of multilayered films, with a particular interest in the production of drug carriers for biomedical use. Despite the considerable interest in post–assembly techniques for drug loading, such as drug conjugation with the film moieties, little attention has been driven to the effect of film deposition conditions in the drug release performance. Herein, we use the traditional poly (acrylic acid) (PAA)/poly (allylamine) (PAH) multilayered platform for understating the influence of polyelectrolyte solution pH and drug loading method in the release of calcein (CAL), a chosen model drug molecule. Films were assembled over glass substrates using the dipping layer–by–layer method, using different polyelectrolyte solution pH (4.5 or 8.8), while the drug loading process was carried out during or post film assembly (pH 7.1 in both cases). We also tested the barrier effect of the biopolymer– based multilayered carboxymethylcellulose (CMC)/ chitosan (CHI) films deposited over the payload region as a strategy to avoid the drug burst release. Results show higher CAL loading capacity for films assembled at pH 4.5 and post–assembly drug loading; the more sustained drug release profile is observed for films deposited at pH 8.8 and drug loading during film deposition. These results also indicate that film area, rather than the number of bilayers deposited, controls the drug loading capacity, suggesting that CAL molecules are majorly adsorbed in the outer layers of the film by electrostatic interaction with the non–complexed carboxylate groups of PAA. Finally, CMC/CHI barrier deposited by LbL spraying method drastically reduces burst release effects, extending the drug release profile for up to 10 days. Our findings indicate that simple film deposition parameters may be used to control the performance of multilayered films suitable for drug delivery applications.

**Keywords:** Layer–By–Layer; Drug Delivery; Chitosan.


# INFLUENCE OF BIOPOLYMER BASED THIN FILMS ON MICROPATTERNED PDMS PROPERTIES

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**Abstract:** Surface properties play a key role in how biomaterials interact with the environment. In the context of biological phenomena, several studies report changes in cellular behavior promoted by changes on chemical and physical properties of surfaces involved in the interaction process. Surface topography has also been reported to be a key player for cell adhesion in some research, although its effect is still not well elucidated. To investigate these effects on the cellular behavior, it is necessary to implement a methodology with a strict control over the surface properties. By combining lithographic techniques with multilayer coating strategies, such as layer–by–layer technology, it is possible to functionalize patterned surfaces for the study of biological phenomena, such as cell adhesion. As regards the functionalization materials, natural polymers are particularly interesting as they are usually weak acids and bases that can be fine–tuned by pH and ionic strength. In this study, thin films of hyaluronic acid (HA) and chitosan (CHI) were developed by Layer–by–Layer technique on a micropatterned polydimethylsiloxane (PDMS) to evaluate its performance in the modulation of cellular behavior. Briefly, the PDMS substrates were treated with oxygen plasma and pre– coated with a polyethyleneimine precursor layer before films assembly. The characterization of these coatings was carried out using the Atomic Force Microscopy (AFM), Contact Angle and UV–Vis. AFM and UV–Vis results confirmed the deposition of films with 29 nm of thickness across the substrate extension. This coating was able to considerably reduce the hydrophobicity of the PDMS, reducing contact angle measurements by up to 50%, improving its biocompatibility for cell culture. Although much work is still needed, the findings highlight the functionality gains promoted by nanostructured coatings of biomaterials through the Layer–by–Layer deposition technique and can contribute to the development of new biomaterials with applications in biomedical systems.

**Keywords:** Biopolymers; Layer–by–Layer; Surface Properties.


# MORPHOLOGICAL CHARACTERIZATION OF HYALURONIC ACIDS AND THEIR CLINICAL INDICATIONS

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**Abstract:**The versatility of Hyaluronic Acid (HA) as a skin filler makes it a product with diverse characteristics depending on the manufacturer, type of crosslinking, and product concentration. These differences are important in clinical practice because they will determine the correct indication of each product, its local stability, and its durability. The objective of this study was to compare the superficial and internal morphology of lyophilized samples from four different commercial HA presentations, associating their characteristics with the perfect clinical indication. Rennova Ultra® 24 mg / mL (1), Rennova® Ultra® 20 mg / mL (2), Rennova Deep® 20 mg / mL (3) and Rennova Ultra® Deep® 20 mg / mL (4) were freeze–dried at –80 ° C for 2 days and subsequently lyophilized for 2 days using L101 equipment (Liotop, São Carlos – Brazil). Samples were fixed in stubs and metalized with carbon for SEM analysis. Photomicrographs with magnification of 40X, 100X and 500X and 1,500X were obtained using the FEG–MEV scanning electron microscope JSM–7500F (Jeol Ltda, Tokyo – Japan) from the Advanced Microscopy Laboratory (LMA) of the Chemistry Institute of Araraquara – UNESP. Gel 1 presented pores or chambers with larger diameter compared to the other gels, thus showing a looser structure and with less capacity to withstand tensions. The second showed a slightly dense structure compared to 1. The more homogeneous structure was presented in gels 3 and 4, which suggests a higher crosslinking rate indicating greater collectivity, as well as dermal volumizing capacity, supporting higher tensions. It is ob–served that, due to their morphological characteristics, gels 2, 3 and 4 are more suitable to correct greater loss of volume while gel 1 is more suitable for surface filling, since it has a structure with more pores and consequently supports less stress your clinical indication may be the lip contour and small loss of collagen.

**Keywords:** Hyaluronic acid; SEM; reticulation


# VISCOELASTIC PROPERTIES OF CROSS–LINKED HYALURONIC ACID POLYMERS BY RHEOLOGY

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**Abstract:** Hyaluronic acid is a polymer used in a wide medical application, being a natural polymer that can be stabilized by chemical crosslinking, resulting in a water–insoluble viscoelastic polymer, less susceptible to enzymatic degradation and better elasticity compared to the in natura product. The objective of this work was to obtain crosslinked gels from hyaluronic acid, using the crosslinking agent 1,4–butanediol diglycidyl ether, in relation to different mass / mass ratios of the agent, seeking the preparation of high hydrophilicity gels. In agreement with the objectives of this work it was possible, so far, to confirm the production / preparation of gels with particles showing sphericity observed by optical microscopy, which adds effective gain to the viscoelastic properties of the product, important with regard to subcutaneous application. No aspects of rheological properties; The gels prepared in this work were evaluated within the range of the physiological stress region (0.1 to 2) Hz from the G 'parameter evaluation. Gels produced with 13 25% BDDE have accepted measurements for use as a facial filler and the 37% BDDE gel has been classified as a viscosupplementation. Gels prepared with 37 and 50% BDDE showed high G 'values and were indicated for applications as strong gels. From the evaluation of parameter G ", the gels prepared with 13 and 25% BDDE presented modules with values below 100 Pa; values close to the gels present in the market. Gels prepared with the addition of 37% and 50% BDDE may be final polished with the addition of the addition of solubilized HA to facilitate gel extrusion. Values obtained for tan δ, varying in the range of $0.33 \leq \tan \delta \leq 0.43$ for 13 to 50% BDDE crosslinked gels, which are presented as gels with higher elastic behavior, and for 13 to 50% crosslinked gels. 37% BDDE is indicated for use in regions that require parameters similar to synovial fluid.

**Keywords:** Hyaluronic acid; Crosslinking; Polymers in medicine; Thermal analysis


# THERMAL EVALUATION OF CROSS–LINKED DERMAL FILLERS WITH HYALURONIC ACID AND BDDE

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Area: ( ) Food and Agriculture (x) Medical and Pharmaceutical ( ) Multifunctional Applications

**Abstract:** Dermal fillers are as medical indications in routine clinical practice, the base substance for their formulation is hyaluronic acid (HA) is important substance to maintain the youthful appearance of the skin. This natural polymer can be modified at the carboxylic, hydroxyl, acetamide group and at the reduced terminal by crosslinking, esterification, and etherification reactions, among others. The objective of this work was to obtain gels by crosslinking reaction from hyaluronic acid, using the crosslinking agent 1,4–butanediol diglycidyl eter, against different mass / mass ratios of the agent, seeking the preparation of gels and evaluation by analysis. thermal TG–DTA and DSC confirmed the obtention of different gels. Ther–mogravimetric results obtained in water mass loss in relation to HA concentration in the different formulated polymer systems. In the DSC evaluation, the degree of cross– linking, in the absence of ionic strength adjustment, was obtained from the solvent peak crystallization temperatures ($T_c$) in the polymer lattices according to the order: $T_c$13% ~ Tc25% (– 22, 3–22.5 ° C) > $T_c$36.6% (–23.6 ° C) > $T_c$50% (–27.0 ° C); the minimization of melting temperatures, $T_f$, $\Delta T_f$, for the composition gels 50 and 13%, from $\Delta T_f$ = 9.9 $^{O}$C (pure water) to $\Delta T_f$ = 3.8 $^{O}$C (buffered solution with just ionic strength), reflecting the minimization of solvent–solvent and solvent–polymer interaction energies compared to gels prepared in pure water; ionic strength adjustment being an important aspect of the gel adjustment to the physiological behavior that will be applied. In addition, allotropic transition, change from cubic to hexagonal structure (characteristic structure of frozen water), based on endothermic / exothermic peaks present at T = –18 / –50 oC (heating / cooling), present only for swollen gels in buffer solution with NaCl–adjusted ionic strength; determining the glass transition temperature, Tg, only for gels with a high degree of cross–linking: Tg36,6% and 50% = –53 oC (heating branch); Tg for low cross– linking gels possibly below –80 $^{O}$C.

**Keywords:** Hyaluronic acid; Crosslinking; Polymers in medicine; Thermal analysis.