IJAMB

International Journal of Advances in Medical Biotechnology

International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Bioinorganic applications of gold and platinum coordination compounds: a brief historical overview and recent advances in 2017

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ARTICLE INFO

Keywords:
Gold
Platinum
Bioinorga
Bioinorganic
Arthritis
Anticancer agents
Antiviral compounds

ABSTRACT

Gold-based metallodrugs have been studied for a wide variety of medical–related applications, although the antiarthritic auranofin is the only representative within this class that has reached the clinic. Platinum compounds, on the other hand, are the leading class of metallodrugs used against cancer, with very successful representatives worldwide, such as cisplatin, carboplatin and oxaliplatin. In this mini review, we will briefly present the development of gold- and platinum -based metallodrugs throughout the year of 2017.

1. Introduction

Medicinal applications of gold date to antiquity. The oldest records, dated to around 2500 BC, indicate that the Chinese and Arabians were the first to use gold for therapeutic purposes.[1,2] In the 8th century it was considered as an elixir of youth.[2] In the 19th century, sodium tetrachloridoaurate(I), $Na[AuCl_4]$, was prescribed to treat syphilis and chronic alcoholism. In the end of the 19th century, Robert Koch first described the activities of potassium dicyanidoaurate(I), $K[Au(CN)_2]$ for the treatment of tuberculosis.[2] With the development of modern Medicine and the emergence of new technologies, the empirical use of gold was replaced by a more rational design of gold-based medicine, to circumvent the toxic effects of $K[Au(CN)_2]$. This approach was responsible for the development of aurothioglucose (Solganol), myocrisin and later of auranofin, compounds used in the treatment rheumatoid arthritis. Curiously enough, the first uses of gold(I) compounds in treatment of rheumatoid arthritis were based on the idea that such illness was caused by a bacterial infection, which was shown to be incorrect.[1] Mirroring the historical application of gold and gold-containing compounds for the treatment of such a wide variety of diseases, the 2016-2017 period has introduced gold-based metallodrugs for applications including the molecular understanding of the mechanisms of interaction with protein targets[3–8] and development of new therapeutic agents for HIV, cancer, [9,10] bacterial infections[11] as well as parasitic infections.[12]

Platinum compounds, on the other hand, were intensively studied since the middle 1960's after the serendipitous discovery of the antitumor activity of cisplatin by Rosenberg.[13,14] Cisplatin revolutionized the treatment of testicular cancer, leading to cure rates higher than 95%. The mechanism of action of cisplatin is generalized as dependent of 4 steps: cellular uptake, aquation (replacement of the leaving ligands by water molecules, DNA binding (leading to a bending along the helical axis) and finally cellular processing, including recognition of the damage, which ultimately leads to cell death.[15] A well-stablished structure-activity relationship for cisplatin has been rationalized, and it relies on a square planar Pt(II) center surrounded by monodentate or chelating N-donors (non-leaving groups) and negatively charged monodentate or bidentate ligands (leaving ligands). Oxaliplatin and carboplatin were rationally developed expanding on the general structure of cisplatin. Both compounds feature bidentate ligands that are replaced by water molecules more slowly than the monodentate chlorides found in the structure of cisplatin.[16,17] Platinum drugs have been listed on the 2013's edition of the *Model List of Essential Medicines of the World Health Organization*, which highlights the relevance of this class of compounds and justifies the extensive research in the field. Some hand-picked examples published in the previous year will be discussed here. Within this period, we also highlight the breakthrough discovery regarding the mechanism of action of oxaliplatin, which was demonstrated to be significantly different from that of cisplatin and carboplatin.[18]

ARTICLE HISTORY:
Received 21 November 2017; Received in revised form 22 December 2017;Accepted 15 January2018
Available online 25 February 2018

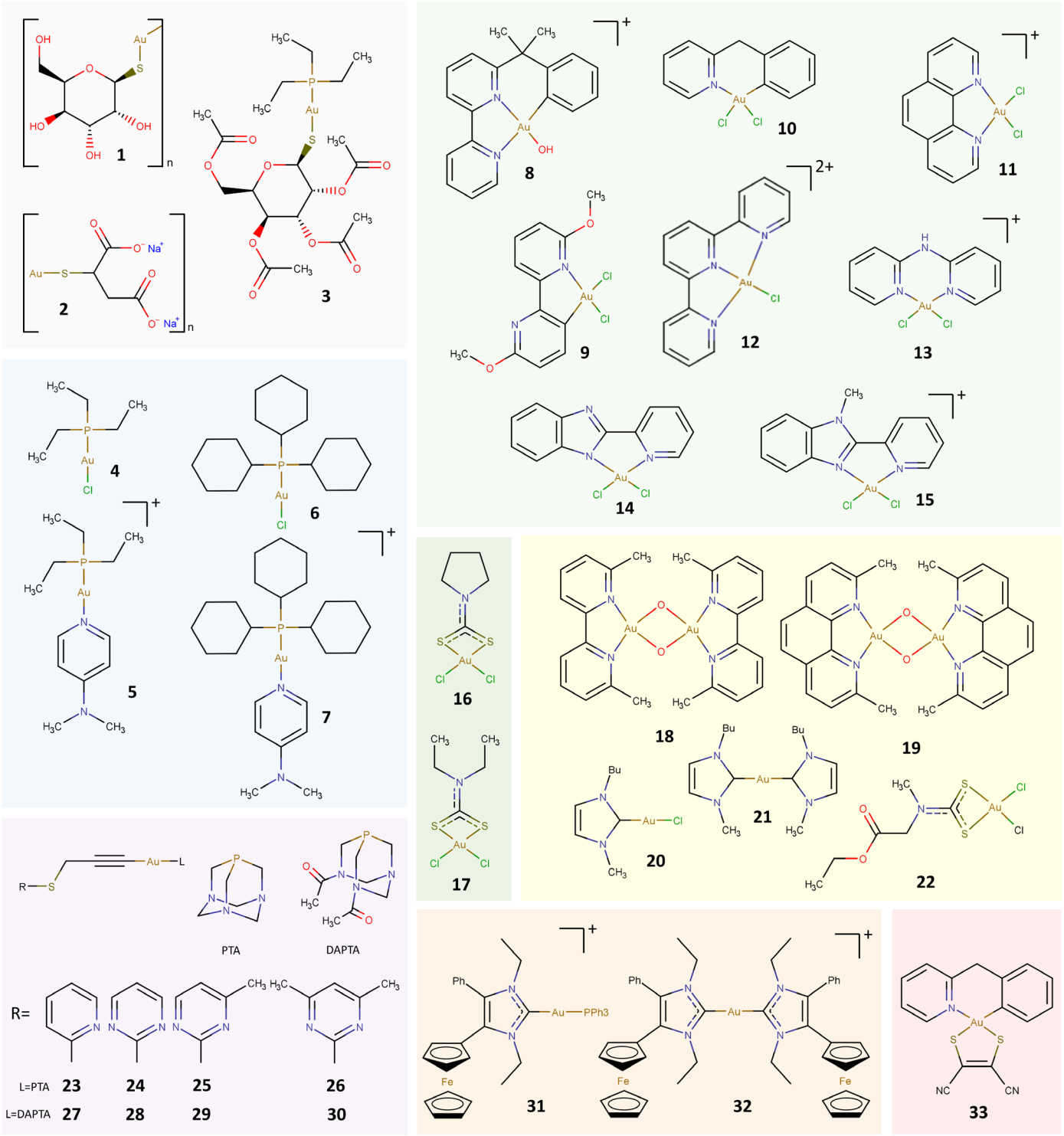

Figure 1 - Grey board: classical Au complexes used in crysotherapy, including aurothioglucose (solganol), aurothiomalate (**2**) and auranofin (**3**). Blue board: Au(I) compounds (**4-7**) designed by de Paiva et al.[3] as probes of the topography of the HIV-1 nucleocapsid zinc finger protein. Light green board: Au(I,III) compounds (**8-15**) studied by Meier et al.[5] and de Almeida et al.[6] for understanding the molecular basis of the interactions with selected biomolecules. Dark green board: gold(III) dithiocarbamate complexes (**16** and **17**) investigated by Wang et al.[7] as inhibitors of amyloid fibril formation. Yellow board: gold(I,III) complexes (**18-22**) evaluated by Massai et al.[8] as Cys-protease inhibitors and as antiparasitic agents. Purple board: Alkyne gold(I) compounds (**23-30**) studied as anticancer agents by García-Moreno and co-authors.[9] Orange board: Au(I) carbene compounds coupled to ferrocene moieties (**31** and **32**) investigated by Muenzner et al.[10] as anticancer agents. Red board: gold(III) organometallic compound (**33**) evaluated against Gram-positive and Gram-negative bacterial strains.[11]

2. Recent advances on Au(I,III) chemistry in the context of Bioinorganic Chemistry

Figure 1 shows gold-based metallodrugs published in the literature from 2016 to 2017.

Protein targeting and inhibition

A series of thiophilic Au(I)-phosphine compounds (**3-7**) was evaluated by Paiva et al.[3] for chemoselective auration of the C-terminal HIV nucleocapsid protein NCp7 zinc finger 2 (F2) and the full-length HIV NCp7 (NC), as probes of nucleocapsid topography. The nature of the phosphine and the co-ligand affect the reactivity with the C-terminal NCp7 F2 and the full-length NC. ^{31}P NMR spectroscopy showed the formation of long-lived {Au(PR_3)}−ZnF species in all cases, but a selective interaction was observed for the dmap-containing compound **5** with NCp7 F2. Auranofin (**3**) led to an unusual Au−His (rather than Au−Cys) coordination to NCp7. Modification of the fully functional NC zinc finger by Cy_3P-containing species (**6** and **7**) inhibited the NC−SL2 DNA interaction, as evaluated by fluorescence polarization.

Traveling Wave Ion Mobility-Mass Spectrometry (TWIM-MS) was used by Du, Paiva and Farrell[4] to investigate the possible coordination isomerism of Au(I) metallopeptide ions obtained by the interaction of compound **4** with the zinc fingers NCp7 F2 and Sp1 F3 (where Sp1 is the human transcription factor). Two conformers of the NCp7 F2 "gold finger" were identified in the gas phase using TWIM-MS, while a single conformer was identified for the Sp1 F3 "gold finger". Collision induced dissociation allowed an unequivocal assignment of the Au(I) binding sites for the major conformers obtained in each reaction. A Cys-Au-Cys coordination was identified for NCp7 F2 "gold finger", while a Cys-Au-His coordination was observed for the Sp1 F3 "gold finger".

Meier et al. studied the interaction of a series of gold(III) compounds (both organometallic and coordination, **8-12**) with biologically relevant nucleophiles by ESI-MS.[5] Compound **8** reacts readily with 9-ethylguanine (EtG). Organometallic compounds **9** and **10** show only very minor MS signals for EtG adducts even after 24 h. Readily detectable EtG adducts were observed for **11**. Compounds **11** and **12** form adducts with cytochrome c (cyt) to a greater extent than **9** and **10** do. Compound **8** did not form any adducts with either ubiquitin (ub) or cyt. Compounds **9** and **10** formed mono- and bis-adducts of the type $[protein+(L)_nAu(III)]^+$ (L is the respective C,N bidentate ligand; n = 1 or 2) with both proteins to a similar extent. Complexes **11** and **12** reacted similarly with both proteins, even leading to the formation of higher order adducts. Compound **8** reacted preferentially with Se-Cys, **9** with Cys, and **10** with His, whereas **11** and **12** undergo redox reactions and oxidize Cys to cystine. The molecular reactivity patterns and binding preferences correlated with the inhibition of TrxR1, i.e., Se-Cys binding leads to potent TrxR1 inhibitors and in some cases to a high antiproliferative activity. The binding preferences imply that the families of coordination and organometallic Au(III) anticancer agents follow different modes of action.

The inhibition of human aquaporin (AQP3) was evaluated by a series of Au(III) complexes (**10**, **11**, **13**, **14** and **15**) was investigated by Almeida et al. [6] The cationic complex **15** was identified as the most potent inhibitor of glycerol permeation. The neutral complex **14**, with a similar ligand system, was scarcely active. DFT studies showed a good correlation between the compound's calculated affinity to cysteine residues and their AQP3 inhibition. Electrochemistry demonstrated that AQP3 inhibition is not related to oxidative damage. Molecular Dynamics studies demonstrated that binding of the compounds to one monomer also affects substrate permeability in an adjacent one. The Au(III) complexes were also shown to be cytotoxic *in vitro* and AQP3 inhibition might contribute to the biological effects observed towards cancer cells.

Wang et al.[7] investigated the inhibition amyloid fibril formation by gold dithiocarbamate complexes. Thioflavin fluorescence assay (added to samples of the peptides PrP106-126 and hIAPP incubated with the gold compounds), supported by TEM and DLS measurements, revealed that complexes **16** and **17** effectively inhibited fibril formation. Auranofin (**3**) had only limited effects. In terms of binding sites, histidine was pointed as a potential target in both PrP106-126 and hIAPP.

A panel of Au(I) and Au(III) coordination and organometallic compounds (**2**, **3**, **8**, **18-22**) was evaluated in terms of inhibition of human and parasitic Cys-proteases (Proteasome (CT-L), Cathepsin B, Cathepsin L, Rhodesain and CPB2.8ΔCTE).[8] Compounds **8**, **19**, **20** and **22** were found to be potent inhibitors of human cathepsins (B and L) and of *L. mexicana* cysteine protease CPB2.8DCTE. The compounds showed sub-micromolar antiproliferative activity against *L. infantum*, *T. cruzi*, *T. brucei*, *T. rhodesiense* and *P. falciparum*, but were also shown to be cytotoxic against the model host cell lines MCR-5 and PMM.

Antiproliferative activities

A series of gold(I)-alkyne derivatives containing the water soluble phosphines PTA (1,3,5-triaza-7-phosphaadamantane) and DAPTA (3,7-diacetyl-1,3,7-triaza-5-phosphabicyclo[3.3.1]nonane) (compounds **23-30**) were tested against the human colon cancer cell line Caco-2 (PD7 and TC7 clones).[9] PTA-containing compounds were more cytotoxic than DAPTA-containing analogs, which correlated well with the higher cellular uptake of the former. The anticancer activity of **23** against colon cancer cell lines happens through the apoptotic pathway and induction of S-phase arrest in the cell cycle. An increase in the mean survival time and life expectancy in athymic nude mice xenografted with human HCT -116-luc2 cancer cells was observed, with moderate inhibition of tumor growth.[9]

Muenzner et al.[10] investigated the antiproliferative and antivascular properties gold(I) carbene complexes

featuring 4-ferrocenyl-substituted imidazol-2-ylidene ligands (31 and 32 were selected as examples). The series had low micromolar to nanomolar IC_{50} (72 h) values against a panel of seven cancer cells. The lipophilic cationic complexes **31** and **32** caused an increase in reactive oxygen species by a ferrocene-dependent mechanism and by inhibition thioredoxin reductase. Both complexes led to a G1 phase cell cycle arrest and a retarded cell migration. Antiangiogenic effect was demonstrated by tube formation assays with endothelial cells. The biscarbene complex **32** lead to up to 80% xenograft tumor volume reduction in mice.

Antimicrobial properties

The novel heteroleptic cyclometalated complex [Au^{III}(py^{b}-H)(mnt)] (**33**) was tested against a panel of ten Gram-positive (belonging to the *Staphylococcus*, *Streptococcus* spp. and *Bacillus clausii*) and Gram-negative (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) bacteria and three yeasts belonging to the *Candida* species. Complex **33** showed a remarkable bacteriostatic antimicrobial activity against *Staphylococci*, with Minimum Inhibitory Concentration (MIC) values of 1.56 and 3.13 µg/mL for *S. haemoliticus* and *S. aureus*, respectively. [11]

Antiparasitic properties

In a Phase **1** clinical trial, auranofin (**1**) was identified as a broad-spectrum antiparasitic drug, being effective *in vitro* and *in vivo* against *Entamoeba histolytica* and both metronidazole-sensitive and -resistant strains of *Giardia intestinalis*.[13] Both parasites are the major causes of water and foodborne diseases. Patients were treated daily with 6 mg of auranofin, which corresponds to the recommended dose for arthritis treatment. Besides the 7 days treatment period, the patients were followed for 126 days. A concentration of 13 µM (in auranofin equivalents) was found in feces at the 7th day of treatment, which corresponds to more than 25 fold the IC_{50} for *E. histolytica* and 4 fold that of *Giardia*.

3. Recent advances on Pt(II,IV) chemistry in the context of Bioinorganic Chemistry

Platinum compounds are still the leading metallodrugs and platinum chemistry with bioinorganic applications has been extensively explored. For that reason, we had to handpick the most noteworthy examples of platinum(II,

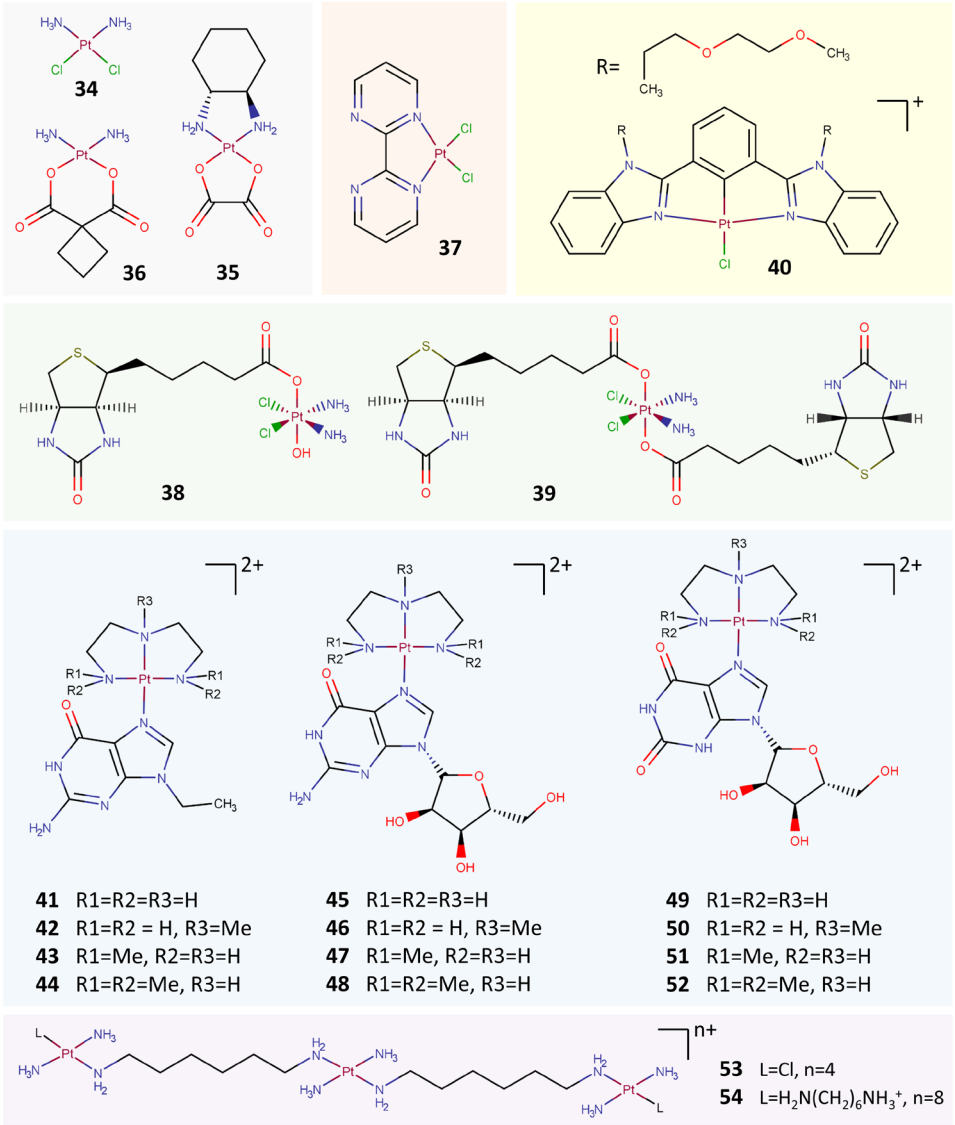

Figure 2 - Grey board: classical platinum(II) compounds cisplatin (**34**), oxaliplatin (**35**) and carboplatin (**36**). Orange board: platinum(II) complex (**37**) used by Rivilla and co-authors[19] for catalyzing 1,3-dipolar reactions of azomethine ylides with maleimides using platinated DNA systems coupled with copper(II). Green board: Platinum(IV) biotinylated systems (**38** and **39**) designed by Muhammad and co-authors as cytotoxic agents.[20] Yellow board: platinum(II) compound (**40**) designed by Zhu and co-authors[21] as probes for lipopolysaccharide endotoxin and discrimination between Gram-negative and Gram-positive bacterial strains. Blue board: platinum(II) compounds (**41-52**) designed by Tsotsoros and co-authors[22] containing the general motif [Pt(chelate)(N-donor)]$^{2+}$ and evaluated as anti-HIV agents. Purple board: Peterson and co-authors[23] demonstrated that the polynuclear platinum compounds triplatin (**53**) and triplatin NC (**54**) can bind to glycans.

has been extensively explored. For that reason, we had to handpick the most noteworthy examples of platinum(II, IV) metallodrugs published in the literature from late 2016 to late 2017 to be discussed in this minireview. Figure 2 shows the structures of the selected compounds.

Expanding on the understanding of cisplatin-like drugs

Bruno, Lippard, Hamman and co-authors demonstrated that oxaliplatin (**35**) kills cells by inducing ribosome biogenesis stress, unlike cisplatin (**34**) and carboplatin (**36**) that have the same effect through a DNA-damage response.[18] This difference in drug mechanism explains for example an observed lack of efficacy for oxaliplatin in the treatment of malignancies conventionally treated by cisplatin and suggests that alterations in the nature of the ligands in platinum complexes have deep implications for primary mechanisms of action. The final consequence is that platinum drugs might not function interchangeably with their derivatives in cancer chemotherapy. The authors suggest that the ability of oxaliplatin to cross-link DNA has questionable relevance in cytotoxicity, but it could still lead to the inhibition of rRNA synthesis, which would ultimately be responsible for ribosome biogenesis stress.

The mechanism of hypersensitivity of testicular germ cell tumors (TGCTs) to cisplatin was investigated by Awuah, Riddell, Lippard and co-authors.[24] The authors demonstrated that the high-mobility group box protein 4 (HMGB4), a transcription repressor preferentially expressed in the testes that binds cisplatin-damaged DNA, blocks excision repair of cisplatin-DNA 1,2-intrastrand cross-links, increasing the sensitivity of TGCTs to cisplatin therapy. CRISPR/Cas9-mediated gene editing was used to knockout the HMGB4 gene in a testicular human embryonic carcinoma. Cell proliferation and apoptosis assays demonstrated that loss of HMGB4 elicits resistance to cisplatin.

Bioconjugation

Rivilla, Cosío and co-authors described a novel catalytic system based on covalently modified DNA that promotes 1,3-dipolar reactions between azomethine ylides and maleimides.[19] The catalytic system makes use of the distortion of the double helix of DNA caused by platination of guanine units, similar in nature to the DNA damage caused by platinum chemotherapeutic drugs. As a proof of concept, compound **37** caused a distortion in salmon sperm DNA and an heterobimetallic system was generated *in situ* using $Cu(OTf)_2$. This system was able to catalyze (3 + 2) cycloadditions between azomethine ylidenes and maleimides.

Muhammad, Guo, Wang and co-atuthos demonstrated that tethering biotin moieties to the Pt(IV) scaffold remarkably increases the cellular uptake of Pt in breast cancer cells, but lowers its accumulation in breast epithelial cells. [20] The mono-biotinylated Pt(IV) complex (**38**) was more active than the di-biotinylated one (**39**) in terms of reactivity and cytotoxicity.

Platinum complexes as probes of biomolecules

A very interesting luminescent probe based on platinum(II) complexes of 2,6-bis(benzimidazol-20 -yl) pyridine with hexaethylene glycol methyl ether groups (compound **40**) was developed by Zhu, Yu and co-authors[21]. The compound can be used for sensing lipopolysaccharide (LPS) endotoxin and, as consequence, it can be applied in a sensor for rapid discrimination between Gram-negative and Gram-positive bacterial pathogens. In the presence of LPS, **40** binds to negatively charged LPS to form LPS–Pt(II) aggregates that enhance the intermolecular Pt/Pt and π- π stacking interactions, leading to a luminescence emission centered at 650 nm. The limit of detection of LPS was of 5.7 nM. As a proof-of-concept, the authors also demonstrated the application of **40** for rapid and washing-free discrimination of Gram-negative *E. coli* and Gram-positive *S. aureus* within 5 min.

Anti-HIV activity

Tsotsoros, Farrell and co-authors presented to the scientific community a systematic strategy to understand the interaction between platinum–nucleobase compounds and the tryptophan-containing HIV NCp7.[22] The inherent π–π stacking properties of the compound $[Pt(chelate)(N\text{-donor})]^{2+}$ were modulated by systematic variation of the tridentate ligand (diethylenetriamine and Me-substituted derivatives) and N-donor (nucleobase or nucleoside), leading to compounds **41-52**. The activity of $[Pt(dien)(9\text{-EtGua})]^{2+}$ (**41**) against HIV-1 strains BaL, NL4-3 and 91-US001 in peripheral mononuclear blood (PBMC) cells showed only modest HIV inhibitory activity for the latter with an IC_{50} = 28.61 mM. Cellular accumulation studies showed no significant correlation with lipophilicity of the compounds, but all compounds had very low cytotoxicity suggesting the potential for antiviral applications.

Novel targets

Peterson, Farrell and co-authors demonstrated that heparan sulfate acts as a ligand receptor for polynuclear platinum anti-cancer agents.[23] Masking of extracellular heparan sulfate (HS) through metalloshielding resulted in very effective inhibition of physiologically critical HS functions including catalytic heparanase (HPSE) and protein growth factor recognition. The interaction of cationic polynuclear platinum complexes with the model HS-like pentasaccharide Fondaparinux resulted in inhibition of its cleavage by the HS-related enzyme heparanase. The end-point of inhibition of HPSE activity and growth factor signaling is the prevention of cell invasion and angiogenesis, demonstrated in HCT-116 cells at sub-cytotoxic concentrations. A competition assay demonstrated that Fondaparinux can sequester the 8+ TriplatinNC from DNA, emphasizing the strength of PPC–HS interactions. Altering the profile of platinum

agents from cytotoxic to anti-metastatic has consequences for future directions in the development of platinum-based chemotherapeutics.

4. Summary and Outlook

Some important contributions were made to the field of gold- and platinum-based metallodrugs throughout the year of 2017. New classes of gold compounds were explored in the field of bioinorganic chemistry, such as gold(I)-alkyne organometallic complexes.[9] A new MS-based technique, Ion Mobility Mass Spectrometry, was also employed to better characterize gold adducts to the nucleocapsid protein of the HIV-1 virus.[4] In the field of platinum chemistry, new insights were published on the mechanism of action of oxaliplatin, which surprisingly differs significantly from cisplatin and carboplatin.[18] Other exciting applications include bioconjugation for both medicinal[20] and catalytic purposes[19]. Finally, glycans were identified as a new class of biomolecular targets for polynuclear platinum complexes.[23]

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Recent advances of synthesis of Boron derivatives and their applications in bioimaging

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ARTICLE INFO

Keywords:
Luminescent
Boron
Synthesis
Bioimaging

ABSTRACT

The increasing interest in the luminescent boron materials is due to their potential application in diverse areas such as solar cells, optoelectronic devices, and biological imaging materials. Continuous search for the compounds with better properties, luminescent organoboron materials have been gaining more importance, especially in the development of new technologies and novel techniques for bioimaging, which is a powerful tool to analyze the cellular organelles with important value into the cell biology and medical research. Synthesis, properties, and applications of luminescent boron compounds and their application in bioimaging are reviewed.

1. Introduction

An interesting alternative for the development of luminescent materials are the coordination compounds derived from main group elements. Recently, there has been great interest in the development of boron compounds, which display an important role in different fields such as supramolecular chemistry,[1] construction of organic light-emitting diodes (OLEDs),[2] photo-responsive materials,[3] sensing,[4] molecular rotors,[5] and in the development of new materials for fluorescent bioimaging (FBI).[6] The FBI is a practical technique to study the localization and movement of molecules in living cells by fluorescence.[7] Recently have been demonstrated that fluorescent compounds derived from boron have several advantages for this application. These benefits include their capability to penetrate into the cells, solubility and stability in biological media, which can be excited and emit non-damaging wavelengths.[8,9] This also reveals the photophysical properties and large Stokes shift for effective discrimination of excitation and emission wavelengths.

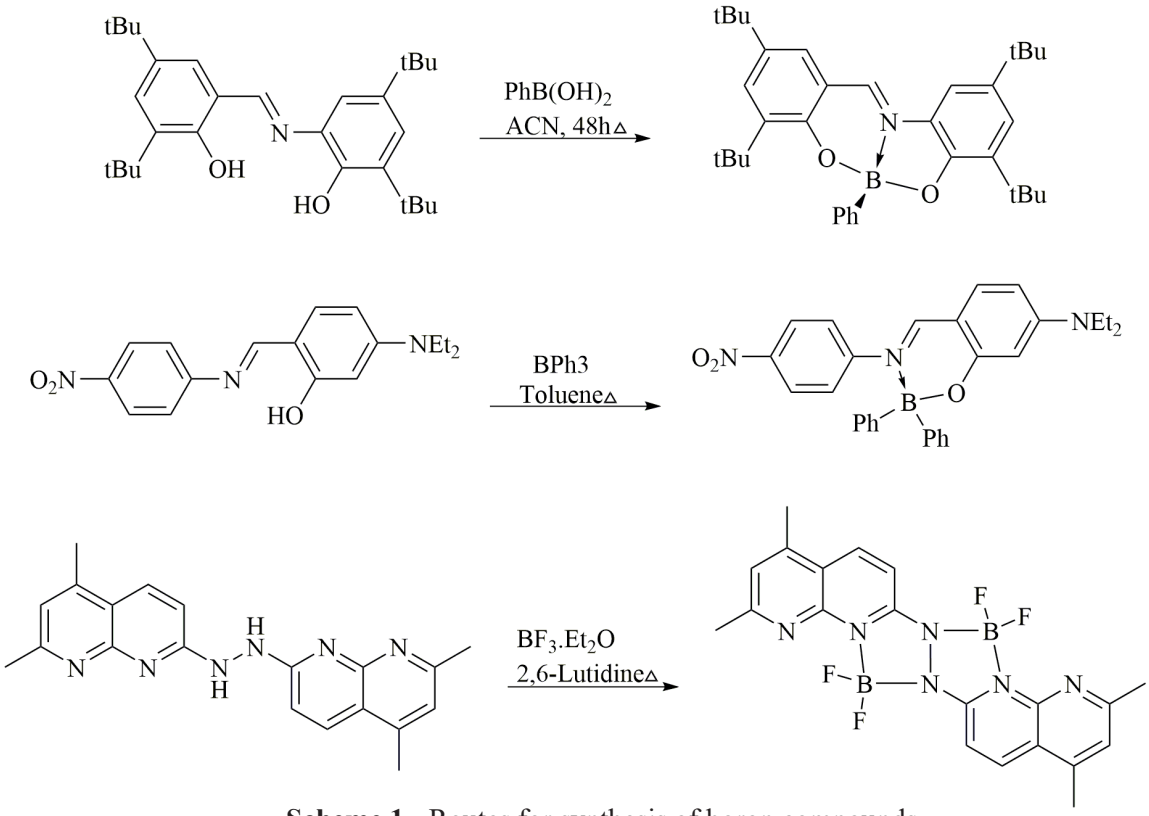
2. Synthesis of luminescent organoboron materials

Fluorescent organoboron compounds are significant and promising materials due to their photonic, biomedical, and optoelectronic applications.[2,10–18] By reviewing the synthetic routes of luminescent organoboron compounds, numerous methods have been carried out by conventional methods, for example simple reflux to open-flask can be isolated tetracoordinated fluorescent Boron compounds derived from Schiff bases and Ph_2BOH or $PhB(OH)_2$.[5,19–21] However, when starting materials of Boron are air-sensitive such as BPh_3,[22–24] $BF_3(OEt_2)$,[25–30] BBr_3,[31,32] and $(Mes)_2BF$ (Mes = 2,4,6-$Me_3C_6H_2$) [33] the synthesis must be carried out in special conditions due to their high toxicity and reactivity (Scheme 1). For example, it has been reported the synthesis of derived from quinolate,[34–38] smaragdyrins,[39] difluoroboron,[40,41] BODIPYs,[42–44] and three-coordinated molecules using Schlenk line,[45–47] with reaction time ranging from 2 to 18 hours, and other multi-step synthesis which takes around 5 days.[48] Chan *et al.* have reported a green synthetic approach of fluorescent organoboron compounds derived from Schiff bases by microwave-assisted synthesis. They described the preparation of four organoboron compounds in acetonitrile and alumina (Al_2O_3) as a support, with reaction time of 5 minutes in both routes and with enough chemical yields (94-97%) with more solvent. The reaction time decreased about of 576 times less than conventional method previously reported.[49]

3. Fluorescent bioimaging by luminescent organoboron materials

Boron-dipyrromethene (BODIPY) derivatives have attracted great interest over the past years due to their high phosphorescence and their photophysical properties, including high stability toward light and chemical agents, large molar extinction coefficients in the higher-

ARTICLE HISTORY:
Received 10 October 2017; Received in revised form 14 October2017;Accepted 02 February 2018
Available online 13 February2018



Scheme 1 - Routes for synthesis of boron compounds.

wavelength visible region, high fluorescence quantum yields, and relatively long fluorescence lifetimes.[50,51] All of these features make this family an excellent option for biological applications in disease therapies and cell imaging.[52,53] For bioimaging purpose, developing water soluble BODIPYs which present a far-red or NIR emission and large Stokes shift, is an important research topic and of particular interest due to the unique advantages of NIR in biological applications. The long-wavelength region would generate minimum photo-toxicity to biological components, deep tissue penetration and minimize auto-fluorescence background by bio-molecules.[8]

The solubility of these dyes in aqueous media can be greatly enhanced by introducing hydrophilic groups, such as quaternary ammonium, sulfonate, phosphonates and the symmetric functionalization of the boron-core, without changing their high fluorescence quantum yields.[58] We can find in the literature NIR BODIPY-based fluorescent compounds capable to penetrate the cell and localize in the cytoplasm[59] or organelles such as mitochondria and endoplasmic reticulum[60,61] also there are reports of fluorescent probes for specific molecules such as enzymes.[62]

Fluorescent molecular rotors of Boron compounds derived from Schiff bases has been reported as well, as favorable molecules to penetrate the cell membrane and stain the cytoplasm. These compounds demonstrate the quantum yield rise when solvent viscosity increases as well, property that is extremely functional in viscosity studies on cellular scale.[5]

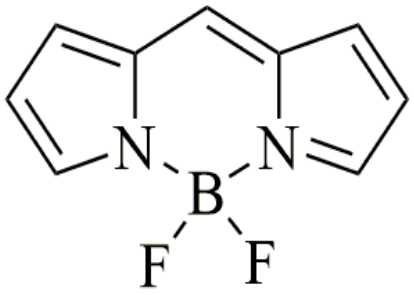


Figure 1 - Structure of a BODIPY fragment.

1) Generate a "*push–pull*" structure and to extend π-conjugation whit the functionalization at α-, β- and mesosites of the BODIPY core.[54,55]

(2) Employment of π-extended pyrrole units instead of the simple pyrrole or fusion of aromatic units to extend the π-conjugation at the [a] bond, [b] bond of the BODIPY.[56]

(3) Substitution of the meso-carbon by an imine type nitrogen atom.[57]

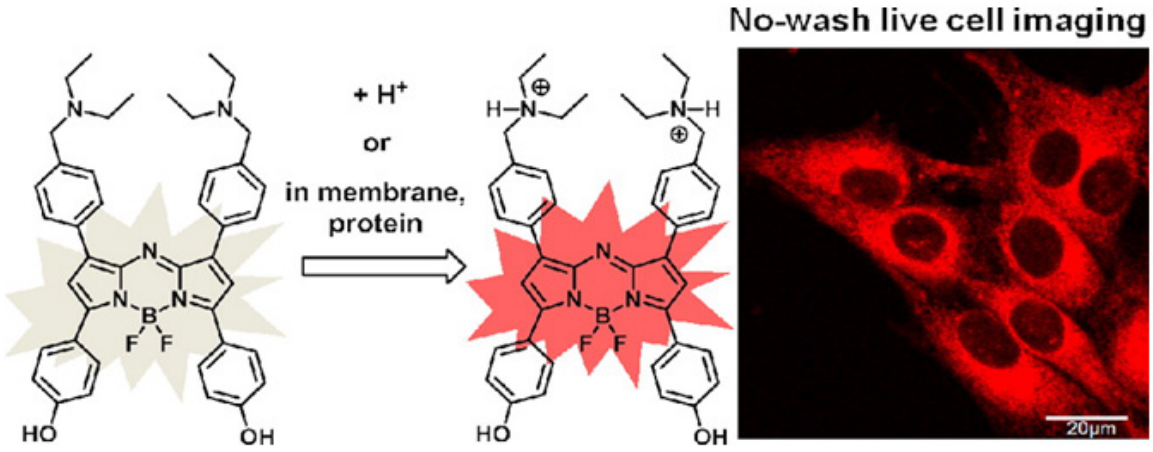


Figure 2 - NIR BODIPY dye displayed off-on at acidic pH and its live-cell image of dye after 3 h. Reprinted with permission from ref.[63] Copyright (2013) American Chemical Society.

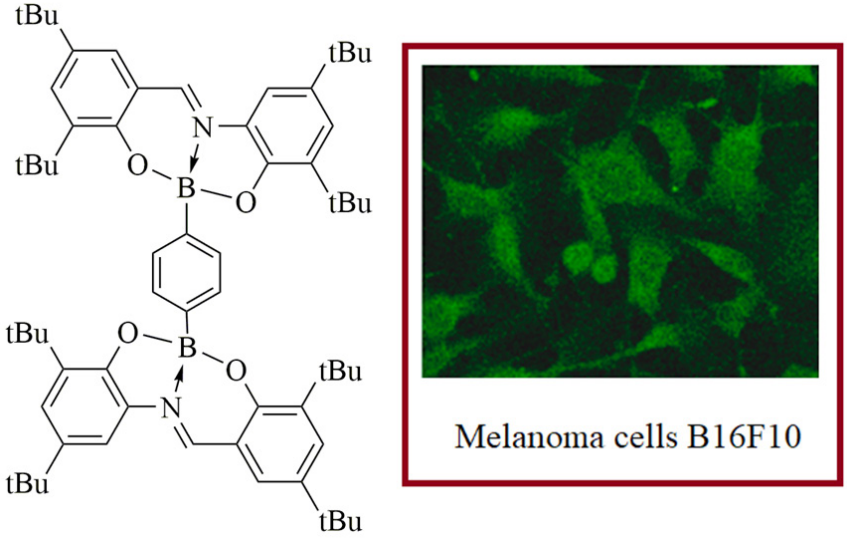


Figure 3 - Organoboron compound and its application as a fluorescent dye of melanoma cells B16F10 treated with 10 μg/mL of the compound for 2 h. Reprinted with permission from ref.[5] Copyright (2017) American Chemical Society.

4. Conclusion and outlook

Luminescent compounds derived from boron have been synthesized by different synthetic techniques, predominantly by conventional methods or using Schlenk techniques. Despite the immense advance in the green synthetic methods, only a few have been reported for the synthesis of these main group compounds, where the compounds of boron, aluminum, and bismuth, have been synthesized by microwaves, mechanosynthesis or via solvothermal respectively. It is therefore expected that future research can focus on greener synthetic methods due to the great advantages that this implies. Fluorescence bioimaging has become a potent and resourceful tool in pharmaceutical, biological, medical and associated sciences in recent years, due to their attractive facilities for studying, both *in vivo* and *in vitro*, living organisms and their live processes with rapid response and being weakly invasive. Nowadays, the design and synthesis of new metallic compounds with potential use as fluorescent imaging agents has become a dynamic research field. There are many reports on boron complexes used for this propose, especially BODIPY compounds, that has been demonstrated their potential use as cellular markers.

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International Journal of Advances in Medical Biotechnology

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RESEARCH PAPER

Intelligent copolymers based on poly (N-isopropylacrylamide) PNIPAm with potential use in biomedical applications. Part i: PNIPAm functionalization with 3-butenoic acid and piperazine

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ARTICLE INFO

Keywords:
Poly (N-Isopropylacrylamide)
3-Butenoic Acid, Piperazine
Thermosensitive copolymers
LCST Temperature

ABSTRACT

The synthesis and characterization of the thermosensitive copolymers based on Poly (N-Isopropylacrylamide) (PNIPAm) and 3-butenoic acid and functionalized with piperazine was carried out. The free radical polymerization of the PNIPA copolymer with 3-butenoic acid was performed under microwave radiation. After obtaining this copolymer, the carboxyl groups present in the copolymer chain were activated with 1-ethyl- (3-3-dimethylaminopropyl) carbodiimide in the presence of N-hydroxysuccinimide, improving its reactivity to incorporate the piperazine through its amino group. The characterization consisted: differential scanning calorimetric and ultraviolet-visible spectrophotometry to determine the LCST phase transition temperature, ranging from (30-35)°C. Structurally it was analyzed by infrared spectroscopy. A morphological analysis was performed using scanning electron microscopy, after simulating an injectable process, with the objective to observe internally the porosity and interconnectivity. The biocompatibility was evaluated through hemocompatibility tests and it was observed that the copolymers obtained were not cytotoxic. In base of the results, the chemical structure of these new copolymers confers a functionality that allows them to serve as nuclei to graft other molecules, such as polysaccharides. Then, the results obtained on the LCST temperature, porosity, interconnected pore network morphology, the ability to be injectable and the biocompatible nature of these copolymers are indicative that these new synthetic biomaterials have the potential to be used in biomedical, pharmacological and for tissue engineering. Also, once their biocompatibility was demonstrate, they may serve to generate interesting compounds having chemical anchor points for the possible addition of polysaccharides using insertion reactions, thereby generating graft copolymers with potential use in biomedical applications.

1. Introduction

Over the years, metals, ceramics and polymers had been the basic materials for the design of biomaterials due their reproducibility and properties[1], but are the polymers (like polyesters and biopolymers) who have presented the broadest and most versatile class of biomaterials that are widely used for biomedical applications, greatly impacting the advancement of modern medicine [2,3]. One of the most important characteristics of a biomaterial that can be used in biomedicine to interact with a biological system is that it be biocompatible [4]. Among the biomaterials, based on polymers, we can find hydrogels, which are hydrophilic polymers that due to their macromolecular chain and at their disposal in the form of a three-dimensional network can absorb water without being soluble under conditions of temperature and physiological pH [5].

Currently, the development of systems called intelligent hydrogels has taken great interest in the area of bioengineering and biomedicine. These novel materials can drastically change their volume upon application of a specific stimulus, such as a change in temperature, pH, electric fields or light, and such changes are reversed once the stimulus is removed [6-8].

In the case of hydrogels sensitive to changes in temperature are characterized by having a lower critical solution temperature (LCST) [9,10]. Among the smart polymers we can mention PNIPAm, which is a non-biodegradable polymer having an LCST of approximately 32-34°C (close to human body temperature 37.4°C) [7,11]. However, being a synthetic polymer, the PNIPAm could present problems

ARTICLE HISTORY:
Received 20 October 2017; Received in revised form 12 November 2017;Accepted 10 December 2017
Available online 04 January 2018

of biocompatibility and biodegradability, so it is of great interest, the incorporation of a phase that can improve this condition, especially if it is intended to be used in some biomedical applications [12]. In addition, it has been reported in the literature that copolymerizing PNIPAm with other monomers provides a way to incorporate a wide range of functionality while retaining thermal response [10]. Considering the above, it is proposed to obtain a copolymer based on PNIPAm modified and functionalized with 3-butenoic acid and piperazine. This research, presented in this first part, may serve to generate interesting compounds having chemical anchor points for the possible addition of polysaccharides using insertion reactions (how it will be done in the continuation of this project and will be presented in a second part), thereby generating graft copolymers with potential use in biomedical applications

2 Materials and Methods

2.1 Materials

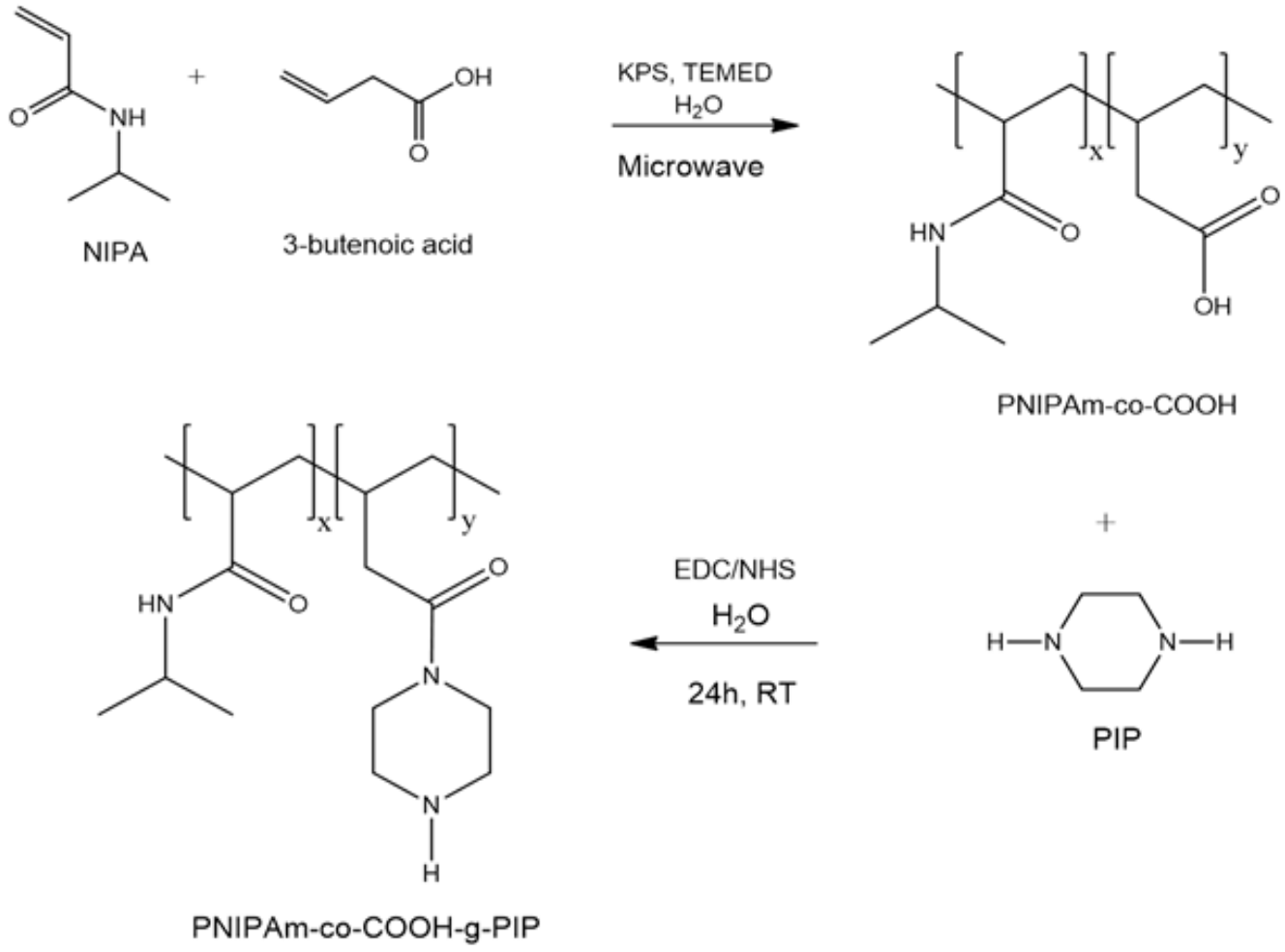
N-Isopropylacrylamide (NIPA), 3-butenoic acid, piperazine (PIP), 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Potassium peroxydisulphate (KPS, Riedel - de Haën). All reagents were used analytical grade.

2.2 Methods

2.2.1 Synthesis of copolymer PNIPAm-co-3-butenoic acid (PNIPAm-co-COOH) via a microwave

PNIPAm-co-COOH was synthesized by free radical polymerization via a microwave. First, the NIPA monomer and 3-butenoic acid as the co-monomer, were placed in a teflon reactor with 25ml of deionized water (19:1 and 18:2 molar ratios, respectively) (Fig. 1). Dried nitrogen ($N_{2(g)}$) was bubbled in to the solution for 5min prior to polymerization to avoid formation of oxidation products. KPS initiator was added and the reactor was closed. The mixture was immediately placed into the microwave. The microwave-assisted polymerization reactions was performed in a microwave mark MAR model CEM and power at 300 W (at 70%) for 5min. The use of microwave radiation allowed to reduce the reaction times to only 5min. By using conventional reaction methods reaction times can be extended up to 24 hours. The system recorded that it reached temperatures between 60-70 ºC and a pressure around 10psi. After the reaction, the purification consisted of the following: the solutions were dialyzed for

Figure 1 - Scheme of synthesis of the copolymer PNIPAm-co-COOH and functionalization with piperazine (PNIPAm-co-COOH-g-PIP).



48h at room temperature, followed by successive washes with deionized water and centrifugation. To complete the purification process, and taking advantage of the phase change undergoing the PNIPAm, the copolymer synthesized were dissolved in 5mL of water, stirred vigorously for a few minutes and heated (40°C) to promote phase separation and forming the gel. The reactants that did not react would remain in the aqueous phase, which was carefully removed. This procedure was performed three times. Finally the samples were frozen and lyophilized for 24h at -50ºC and 500psi.

2.2.2 Functionalization of PNIPAm-co-COOH with piperazine (PNIPAm-co-COOH-g-PIP)

Once the PNIPAm functionalized with 3-butenoic acid has been obtained and characterized, the PNIPAm backbone has -COOH groups available for reaction with an amino group. Therefore, the functionalization of PNIPAm-co-COOH with piperazine will be performed. Carbonyl group of the copolymer PNIPAm-co-COOH was activated with the condensing agents EDC/NHS (2:1 ratio) [13,14]. Improving its reactivity for subsequent incorporation of the amino group present in the piperazine, obtained the copolymer PNIPAm-co-COOH-g-PIP. The synthesis pathway is summarized in Figure 1. The PNIPAm-co-COOH: PIP ratio was 20:1 and 20:1.2. The reaction was carried out for 24h under constant stirring at room temperature. The purification process was performed in a similar manner as when the PNIPAm-co-COOH copolymer was obtained.

2.3 Characterization

2.3.1 FTIR-ATR spectroscopy of the copolymers PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP

The identification of functional groups and changes in bonding environments during reaction were monitored using a Thermo Scientific – Nicolet IS5 Fourier transform infrared (FTIR) spectrometer equipped with a ZnSe Attenuated Total Reflectance (ATR). The experiments run with air as the background. Scans were accumulated 32 sweeps with a resolution of 4cm^{-1} for each spectrum. FTIR spectra were taken in the wavelength region 4000-400cm^{-1} at ambient temperature.

2.3.2 Lower critical solution temperature (LCST) of the copolymers obtained by differential scanning calorimetric (DSC) and UV-spectrophotometry (UV)

Copolymers synthesized were tested in a Perkin-Elmer DSC 7 to determine their lower critical solution temperature (LCST). Samples were prepared as follows: after having been freeze-dried, 5mg of each material was weighed with 5 µL of distilled water in hermetically sealed aluminum pans. After calibrating with indium and obtaining the corresponding baseline under inert $N_{2(g)}$, each sample was submitted to a heating scan: 0°C → 50 °C at 20°C/min.

Additionally, the phase transition of PNIPAm and copolymers solutions was measured using an UV/VIS spectrophotometer HP AGILENT 8452 with diode arrangement for temperature control. The absorbance of visible light at 450nm was measured while the cells were subjected to heating with a water bath to promote the phase change by thermal effect. The temperature range studied was 20-45ºC. LCST correspond to the temperature at which the transmittance is 50% [15,16].

2.3.3 Morphologic study of the copolymers obtained by scanning electron microscopy (SEM)

A JEOL JSM6390 scanning electron microscope was used for the morphological analysis of copolymers synthesized porosity. First, simulating an injection process, given their gel character, these copolymers were dissolved in deionized water and loaded into an injector by passing the thin needle. Then, the solutions obtained were subjected to a bath which reached a temperature equal to or higher than the LCST of these materials (~ 40°C). Once the phase change occurred, the samples were immediately frozen and lyophilized to be observed by SEM. The samples studied were cryogenically fractured in liquid N_2 to ensure the morphological observation of the inside of each sample. Then, they were coated with a thin layer of gold in a Balzers-SCD 030 sputter coater. The voltage of the SEM equipment was set to 20 kV.

2.3.4 Cytotoxicity test by cell hemolysis on blood agar of the copolymers synthesized

Blood compatibility was evaluated with hemolysis assay. The blood was mixed with agar in a totally sterile medium and was gelled for 2h in an oven at 37 °C. Each lyophilized sample was subjected to 1h of irradiation UV, necessary to sterilization. Once the gels were sterile, they were brought into contact with blood/agar system and were transferred under sterile conditions to the surface of the blood/agar gel and then placed at 37 °C and a 5% CO_2 flow in the oven for cell culture. Protocol performed according to ISO-10993-4: 2002. The surface around each gel sample was observed for a total time of 48h and was compared to the controls (negative control = a blood agar gel which had not been in contact with any gel; another or positive control = blood agar gel which had been in contact with a toxic gel using of NIPA monomer).

3 Results and Discussion

3.1 Characterization of PNIPAm and PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP

The signals corresponding to the groups present in the FTIR-ATR spectra of PNIPAm and the copolymers PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP were analyzed by the Fourier Transform Infrared Spectroscopy (Fig 2-A). The PNIPAm, being the majority component, was the starting point and reference for the study of the other infrared spectra. The broad band representative of the amide NH stretching is presented at 3408cm^{-1} and two

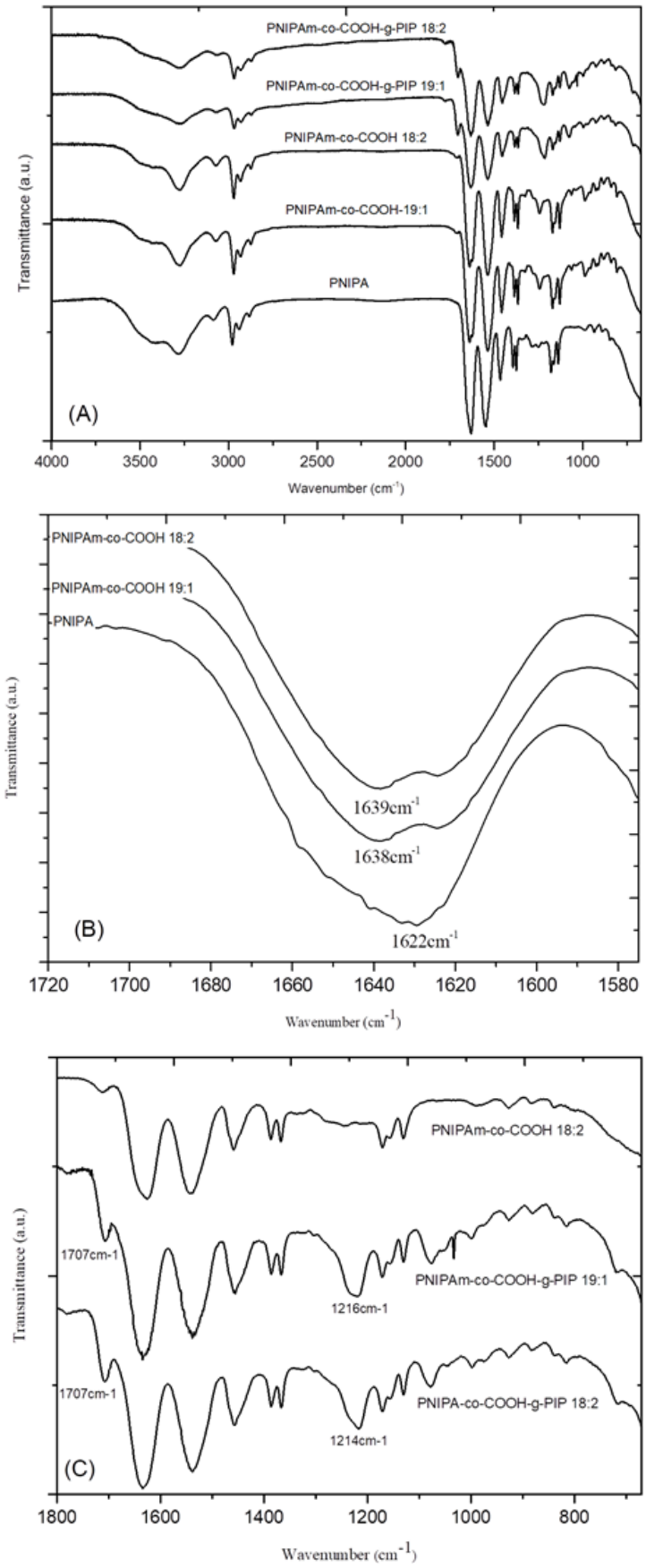


Figure 2 - FTIR spectra of:
(A) PNIPAm, PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP in its 19:1 and 18:2 formulations (region 4000-500cm^{-1}).
(B) PNIPAm, PNIPAm-co-COOH 19: 1 and 18: 2 (region 1700-1500cm^{-1}).
(C) PNIPAm-co-COOH 18: 2 and PNIPAm-co-COOH-g-PIP 19: 1 and 18: 2 (region 1800-700cm^{-1}).

peaks close to 1300cm^{-1} are also observed, representing the flexion vibration of the isopropyl group [17,18]. Once the copolymerization of the PNIPAm with 3-butenoic acid (PNIPAm-co-COOH) is carried out, the carbonyl signal of the synthesized product is displaced at a higher wavelength with respect to the PNIPAm homopolymer and the signal is unfolded, indicating the presence of the 2 types of carbonyl groups present in these news copolymers (See Figure 2-B). However, this shift is not as noticeable because of the high proportion of NIPA relative to the incorporated 3-butenoic acid.

Rejinold (2011) chemically modified the PNIPAm with mercaptopropionic acid by free radical polymerization. In this work the characterization of the copolymers by infrared spectroscopy was made, considering it valid evidence indicating the modification and obtaining of the desired product as a function of the displacement observed for the carbonyl group [13]. Then when the PIP was subsequently incorporated, it was possible to evidence the presence of a new band near the 1700cm^{-1} characteristic of a tertiary amide (-CO-NR2), corroborating the formation of the same between PNIPAm-co-COOH and PIP. Additionally it is possible to identify a band around the 1200cm^{-1} corresponding to the secondary amine (R-NH-R) present in the piperazine (Fig 2-C). When the piperazine is incorporated into the PNIPAm-co-COOH (PNIPAm-co-COOH-g-PIP) by forming an amide bond, another amino group is provided by the piperazine for subsequent modification of the backbone by graft-like reactions.

3.2 Study of the LCST of the synthesized copolymers

In Figures 3A and 3B, it is seen how the phase change occurs in each of the samples analyzed by the selected analytical techniques. When the DSC curves (figure 3B) were used it is observed that all the PNIPAm-co-COOH copolymers decrease the phase transition temperature LCST with respect to the PNIPAm homopolymer. This

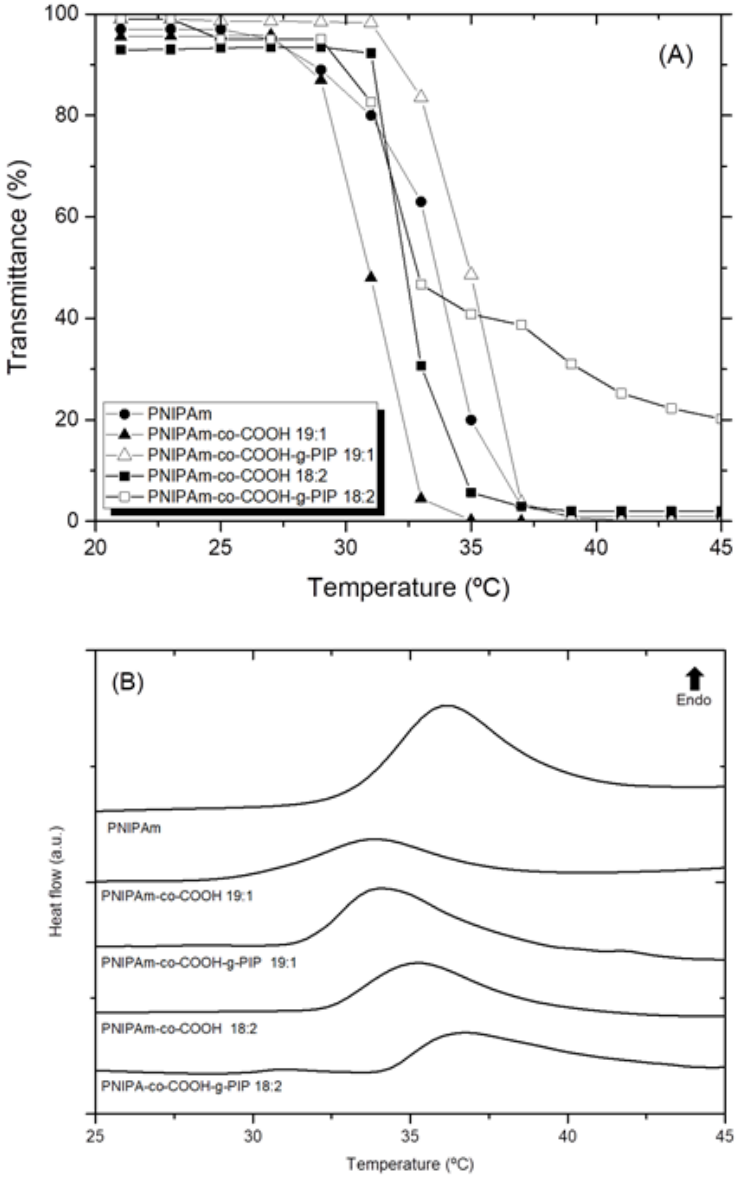


Figure 3 - Determination of LCST transition for PNIPAm, PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP formulations by techniques: (A) UV-vis spectrophotometry, and (B) DSC.

Table 1 - Transition temperature LCST determined by UV and DSC, of the synthesized copolymers.

Sample	UV	DSC	
	T_{LCST} (±1) ºC	$T_{(onset)LCST}$ (±0.1) ºC	ΔH_{LCST} (±0.1) J/g
PNIPAm	34	33.1	17.8
PNIPAm-co-COOH 19:1	31	30.5	14.5
PNIPAm-co-COOH$_{-g-PIP}$ 19:1	32	31.5	22.9
PNIPAm-co-COOH 18:2	32	32.3	6.0
PNIPAm-co-COOH$_{-g-PIP}$ 18:2	34	34.6	7.1

response may be an indication that with the presence of the 3-butenoic acid fraction the hydrophilic character of the copolymer is increased, but in turn the polymer-polymer interactions are favored, which causes it to displace smaller temperatures in relation to the homopolymer. The same effect is observed in the curve obtained by the analysis performed using UV spectroscopy (fig. 3A). In the case of the curve obtained by UV, initially a transparent solution was obtained, for which they had a maximum transmittance. As the temperature in the cells increased, the solutions became opaque giving rise to the phase change (gelation) and decreasing the transmittance due to that turbidity. This change is what allows to determine the average value of the temperature LCST.

These results show how two techniques that are fundamentally different, allow to identify the temperature in which the phase change occurs and therefore the intelligent nature of these materials. However, the advantage of the DSC analysis is the possibility of knowing the value of the energy cost (enthalpy) associated with this transition (see Table 1). It is seen, in Table 1, that as more of the 3-butenoic acid co-monomer is incorporated, the copending capacity of the copolymer is reduced by reducing its enthalpy. But after functionalizing with the piperazine, it appears structurally that the polymer chain is able to order or extend its intramolecular interactions in its gel form, which in turn can translate into a slight increase in its phase transition temperature LCST.

The intelligent character of the copolymers obtained is based on the phase transition LCST that presented around a temperature range T~30-35°C, values close to the body temperature (37.4°C), giving the material potentiality for its use in biomedical applications. In the literature it has been reported that by adding a new component to PNIPAm (covalently bound or by physical mixtures) it is possible to change its LCST transition temperature to higher or lower values [11,12,19]. What has been mentioned is that when some hydrophilic phase is added to the PNIPAm, the LCST is displaced at higher temperatures[5], while in the presence of compounds with greater hydrophobic character, the opposite effect occurs [2,20]. All the formulations of PNIPAm-co-COOH decrease the transition temperature with respect to the PNIPAm homopolymer, despite the hydrophilic character of 3-butenoic acid, which seems to contradict the above; but in this case, it is possible that, if the polymer-polymer interactions prevail, a lower temperature is require to initiate the phase transition.

In order to support this, another effect can be measured, and for that reason an additional measure was to determine the pH of the solution of these copolymers in aqueous medium. By showing that the 18:2 copolymers have a lower pH value than in the case of 19:1, which is to be expected. For polymers such as PNIPAm, it has been reported in the literature that as the pH increases, there is a decrease in the LCST transition. According to this statement, when the pH is high the protonation is weak and the polymer-polymer interactions are favored, which leads to phase separation occurring at a lower temperature [21]. This makes it possible to justify these slight differences in TLCST between formulations 19:1 and 18:2. On the other hand, by incorporating the piperazine as a grafted functional group, an electrostatic repulsion is generated which makes the polymer more soluble and produces an increase in LCST due to an easier protonation of the amino group [21].

3.3 Study of copolymer morphology observed by SEM

The microphotographs of the copolymers allow to appreciate homogeneous porous three-dimensional structures, especially for the 18:2 PNIPAm-co-COOH formulation (Figure 4 (a, b, c, d)); Which has a honeycomb-like morphology formed by interconnected pores. The morphology of the obtained biomaterials was studied after simulating its injectability process, where it was possible to observe scaffold structures with interconnected pores, whose homogeneity varies depending on the formulation. The aim of simulated the injectability process, ie., the passage of the aqueous formulation by an injector, is what would be the morphology once the phase change (gel or solid) inside the organism.

For the case of the PNIPAm-co-COOH-g-PIP samples (Figure 4 (e-h)), a significant variation in morphology is

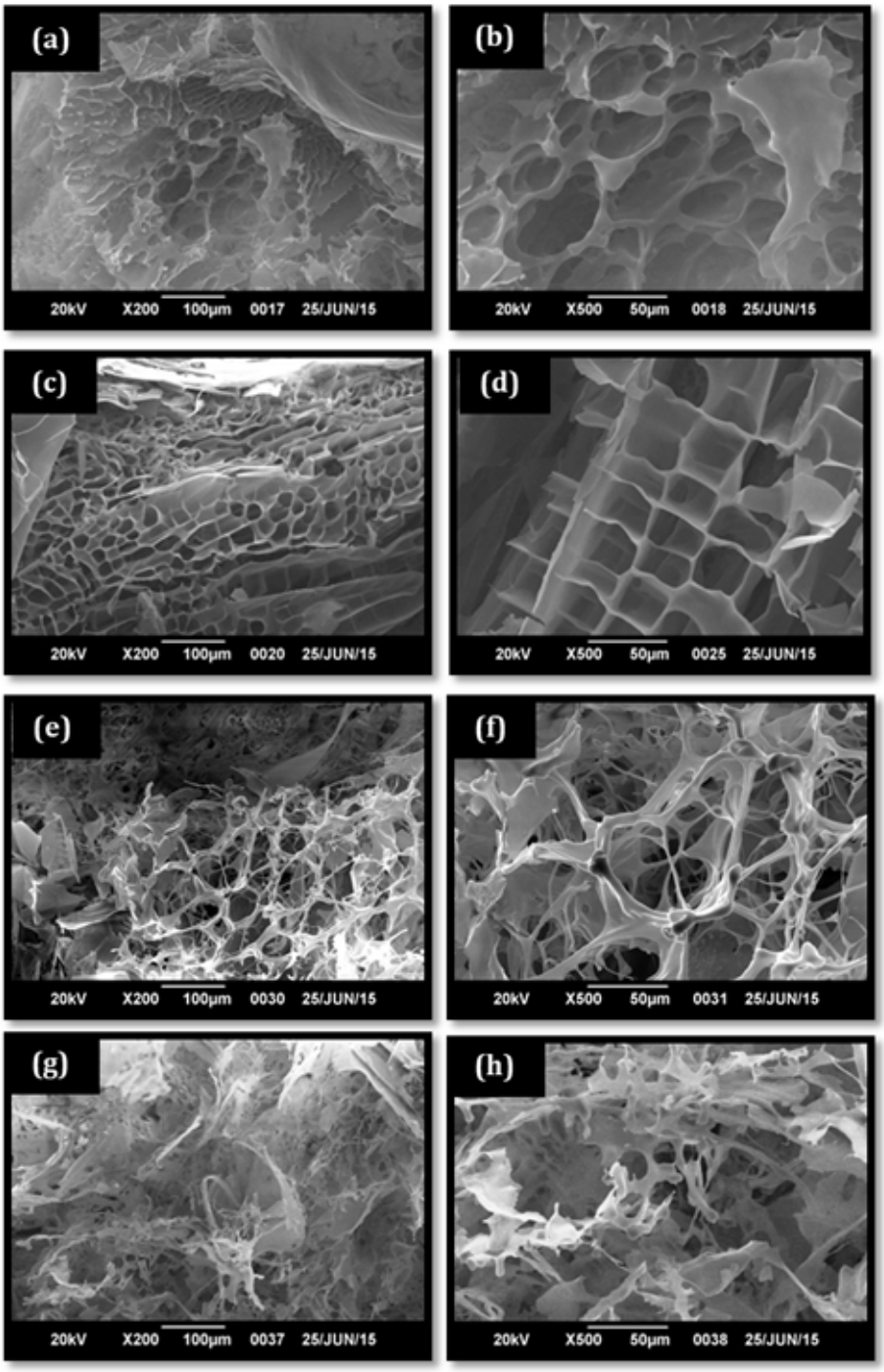


Figure 4 - Micrographs SEM of PNIPAm-co-COOH 19: 1 (a, b), PNIPAm-co-COOH 18: 2 (c, d), PNIPAm-co-COOH-g-PIP (e,f) and PNIPAm-co-COOH-g-PIP (g, h).

observed compared to the starting material. However, it continues to present as a scaffold-like structure, having pore walls much thinner than those obtained in its precursors, ie, porosity of the open and interconnected type.

At present, the study and design of functional biological systems has been demanding the development of interconnected porous and three-dimensional structures (scaffolds) that serve as vehicles for transporting drugs or other biomolecules [22-24], as well as for cell culture and tissue regeneration [12,25]. The purpose of scaffolds in the field of tissue engineering is to support the cells to proliferate and differentiate, given their interconnectivity between the pores necessary to allow vascularization, the passage of fluids that transport cells, nutrients, Oxygen and to remove waste [23,26]. This type of morphology observed for these new copolymers are important requirements to be able to estimate the biomedical or tissue engineering applications that these novel materials can have, where their cytotoxic response plays an extremely primordial role.

3.4 Cytotoxic studies

The hemocompatibility test is very common and

important during the development of biomaterials, as it measures their tolerance when subjected to contact with an active biological system such as blood [27,28]. Hemolysis testing, despite their simplicity, are of great relevance for evaluating medical devices or implants that will be in contact with blood flow [29]. Even more so if think of devices type scaffold [30].

Figure 5 shows the results of the hemocompatibility test of the synthesized samples and their respective controls. As control it is presented to the NIPA monomer (which has been reported to be cytotoxic) [31], and a whitish halo is clearly seen around the sample (hemolysis) deposited in the center of the agar / blood system, due to cell death around the sample. However, for the synthesized copolymers, which were subjected to a thorough purification process, this trend was reversed obtaining copolymers that according to the results and under the conditions evaluated are presented as non-cytotoxic. Based on this, it can be affirmed that the obtained compounds were not toxic, since, none generated evidence of cellular lysis. Therefore,

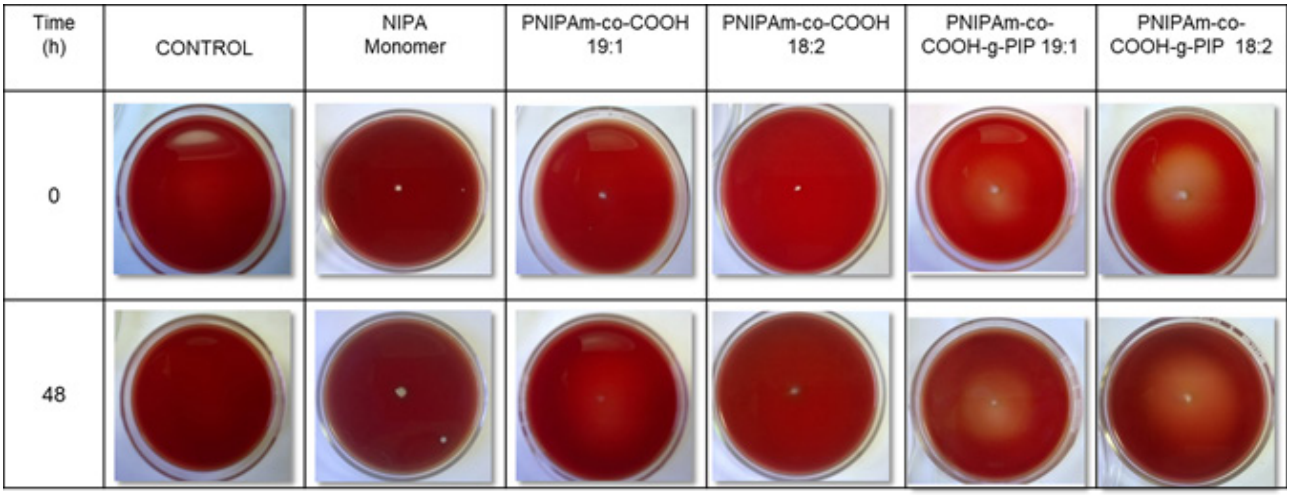


Figure 5 - Non-cytotoxic evidence of PNIPAm-co-COOH and PNIPAm-co-COOH-g-PIP in contact with agar/blood at time 0 and after 48h.

these could be used in more advanced or exhaustive in vitro tests, in order to put these materials in direct contact with primary cell cultures or cell lines; Or in vivo experiments with experimental animals, as these results give indications of their potential biocompatibility [25,32,33]. This obtained biocompatibility is a relevant result, since for this research, these copolymers will serve as an anchor point for the insertion of polysaccharides such as chitosan, hyaluronic acid and alginate, thus leaving evidence that new biocompatible compounds derived from these Which are being studied in this research.

4 Conclusions

A copolymer based on NIPA and 3-butenoic acid (PNIPAm-co-COOH) was synthesized in order to generate a highly reactive block copolymer. From this acid group it was then possible to perform a chemical modification by the incorporation of piperazine (PNIPAm-co-COOH-g-PIP). The synthesized copolymers exhibited characteristics such as: phase change temperature (LCST close to body temperature), porosity, interconnected pore morphology, water solubility, ability to be injectable and biocompatible, indicative that these new biomaterials have the potential to be used in biomedical applications and for tissue engineering. In addition, the structure of the modified PNIPAm has anchor points for future insertion reactions that allow graft-like copolymers to be obtained between this and biopolymers, such as polysaccharides.

Acknowledgment

The authors would like to express their thanks to the following laboratories within the USB: research group B5IDA, Polymer research group GPUSB-1, Laboratory of Surfaces for SEM (Laboratory E), Laboratory of Microbiology of the Department of Cell Biology, Laboratory of instrumental analysis of Chemistry Department. Likewise, the deans of professional studies and dean of research and development (DID).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

RESEARCH PAPER

Morphology study of alginate micro/nano particles for the encapsulation of divalents Mg2+ and Zn2+ ions

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ARTICLE INFO

Keywords:
Alginic acid
Divalent ions zn^{2+} and mg^{2+}
Internal gelation
Micro-emulsification

ABSTRACT

This research work aimed to promote the formation of alginic acid particles and the encapsulation of divalent ions, such as Zn^{2+} and Mg^{2+}; but using a combination of internal alginate gelation and micro-emulsification method. Both ions are essential elements of the human body, i.e., they are present in tissues and body fluids and participates in many bodily functions. The influence of different parameters was evaluated relate to the formation of the particles in micro/nano-scale, and their morphology was observed. The concentration of both ions used in the formulation was varied considering [0.075, 0.15 and 0.25] mol/L. It was found in general that the formation of particles in nanoscale, with a spherical shape and smooth surfaces (also by Atomic force microscopy AFM) after characterizing by electron microscopy (Scanning SEM and Transmission TEM) with energy-dispersed analysis of X-rays (SEM/EDX). The only evidence of formation of particles at higher concentrations of the ion ([0.25] mol/L) was found when the magnesium ion was used ($MgSO_4$) while the smallest particles (≤100nm) were formed when $ZnSO_4$ ([0.25] mol/L) was used. The results suggest that these particles can be used as a coat or carrier for essential nutrients for food fortification, for instance, for others applications in biomedicine or charge drugs in delivery systems.

Introduction

Zinc (Zn^{2+}) and Magnesium (Mg^{2+}) are between the essential elements for the human body since they are present in tissues and body fluids and they participate in many bodily functions linked to the metabolism of proteins, lipids, and carbohydrates, as well as to insulin synthesis, RNA, and DNA. Zinc deficiency affects cell growth, sexual maturation, regeneration, and repair of tissues, affects the functioning of the immune system[1,2] which is why intake and absorption are critical to a human being. Also, the intake of magnesium ion (Mg^{2+}) is very important because it is associated with the operation of several enzymes related to metabolism, protein synthesis, RNA, and DNA, as well as with the maintenance of the electrical potential of nervous tissues and cell membranes and calcium metabolism[3,4]. Magnesium deficiency produces malnutrition, vomiting, muscle weakness, inhibition of natural tissue regeneration, and a prolonged deficiency may conduct to great weight loss[5], and also has been suggested that maintaining low levels of magnesium may influence the onset of heart disease and hypertension[6]. For these reasons, the presence of these ions in the food is very important for being healthy, which is why is so important to study different the ways to incorporate them into our alimentation. Alginate is a biopolymer that has been used for a very long time in the food industry, and also it is a copolymer composed of polysaccharides (β -(1→4)-linked *D*-mannuronic acid (M) and α -(1→4)-linked *L*-guluronic acid (G). Guluronate groups (G) and Mannuronate groups (M) can rapidly cross-link in the presence of divalent cations[7]. Cations such as calcium have been widely used to induce gelation of the alginate[8-11]. They have also been employed for other divalent cations such as Zn^{+2}, Mn^{+2}, Co^{+2}, Sr^{+2}, Ba^{+2}, Cu^{+2} [12-14]. However, the Mg^{+2} had been considered not to induce the formation of alginate gelation for a long time[15,16]. This last idea is about to change because some researchers continue working in that, for example, a recent report from Topuz *et al.* (2012)[17] indicates through some

ARTICLE HISTORY:
Received 05 September 2017; Received in revised form 02 October 2017;Accepted 20 December 2017
Available online 10 January 2018

rheological evidence that the gelation of alginate may occur in the presence of magnesium. Nanotechnology reaches an important role to incorporate these essential elements and their mixture with biopolymers. Then, in the studies related to determining the size of the polymers particles, these characteristics greatly depend on several parameters that can be modified during the preparation of these particles and which can reach sizes of micro or nanoscale. The type of ion used to influence the particle size, surfactant concentration, homogenizer speed, ion concentration, etc.[18-21] Because the ion binding is key to produce homogeneous particles, it could then ensure the nano size. Therefore, the effect of the encapsulation and concentration of cations used in the formulation of particles is one aspect that this research work considers, because understanding the encapsulation mechanism could lead to the delivery of these divalent cations.

Thus the purpose of this research is to provide positive results that were achieved by encapsulating Zn^{2+} and Mg^{2+} ions using sodium alginate through an ionic gelation process combined with the microemulsion methodology. It is expected that the final application of these nanoparticles would help to improve the nutritional value for several food industry applications mentioned above, and also opens the door for this methodology to be used in the preparation of nanoparticles containing these ions as well as to serve as a vehicle for encapsulating drugs or other biomolecules[22].

Materials and methods

Materials. A Sodium Alginate, with structural relation = 0.95 Mannuronic/Guluronic acid (M/G Groups) determined by NMR analysis, was used from Sigma Aldrich. Zinc Sulfate ($ZnSO_4$), Magnesium Sulfate ($MgSO_4$) as a precursor of Mg^{+2} and Zn^{+2} ions. As surfactant Polyvinylpyrrolidone (PVP) with a molecular weight Mw 10.000 g/mol and Tween 80 as a non-ionic surfactant. All these reactive were purchased from Sigma-Aldrich. Also, deionized water was employed.

Alginate Purification

There was used the same method described previously[23].

Preparation of Alginic Acid Solution. It was prepared a solution of alginic acid [1% w/v] and blended with Tween 80 [0,05% w/v] in deionized water.

Preparation of Zinc and Magnesium Sulfate Solutions

Solutions of $ZnSO_4$ and $MgSO_4$ ([0.075], [0.15] and [0.25] mol/L) and PVP [2% w/v] were prepared in deionized water and added to isopropyl alcohol in proportion 80/20 v/v. The emulsion was prepared by using a high-speed homogenizer (IKA T-10 Ultra-Turrax) at 10.000 r.p.m during 15 min.

Preparation of Nanoparticles

Alginate particles were produced by dropwise the alginate aqueous solution into the $ZnSO_4$-PVP or $MgSO_4$-PVP solutions under continuous stirring during 30 min and stop. After this time, the particle suspension was kept at room temperature for 24 hours.

Nanoparticles Recuperation

After 24 hours, the resulting nanoparticles were purified via washing with deionized water by four centrifugation cycles (15 minutes each). The samples were frozen at -4°C for 24 h and then lyophilized during 24 hours, in a Labconco freeze dryer. The PVP used as surfactant has a cryoprotectant action[24] during lyophilization process at –45°C and 0.075 Torr.

Morphological Analysis

Scanning Electron Microscopy with energy-dispersed analysis of X-rays (SEM/EDX) has been used to characterize the size, shape, surface texture and elemental composition of nanoparticles. All samples were gold coated using a Sputter-coater Balzers-SCD-030 unit and then analyzed under a JEOL JSM 6460 microscope at 15 kV. Elemental compositions (semi-quantitative) are reported as weight percents for all tested compounds. A JEOL equipment was used for Transmission Electron Microscopy (TEM), JEM 2100, 200 kV accelerating voltage and filament lanthanum hexaboride (LaB_6). In this case, only the sample with 0,15 M of Zn^{+2} was observed and prepared by suspending wet and deposited on a copper grid of 200 mesh and coated with carbon.

Also, the topography of the alginate obtained particles were analyzed using an Agilent 5500 AFM equipment, Atomic Force Microscope (AFM). Small sections of the particles were introduced and gelled into the resin and then cut using an ultramicrotome. The samples were digitized in an acoustic mode with a resonance frequency of 157.070 kHz. The observation in the AFM was carried out through scanning areas of dimensions (2μm × 2μm).

Particle Size Distribution

This parameter was measured in a Dynamic Light Scattering equipment Zetatrac (from Microtrac, Inc) with zeta potential measurement. For that 20 mg of nanoparticles were suspended in 10 mL of 1:1 ethyl alcohol: deionized water solution. The samples were first sonicated for thirty seconds in a bath-type sonicator Hielscher UP400S, 400 W and 24 kHz (70% frequency) at room temperature to reduce agglomerates between particles and obtaining better results. Each formulation was performed and recorded three times to get the average zeta potential.

Determination of the amount of encapsulated ion

Atomic absorption spectroscopy (Perkin Elmer equipment, model 3300) was used to determine the quantity of the ion encapsulated in the nanoparticles. (10 ± 1) mg of sample was placed with (10 ± 1) mL of concentrated hydrochloric acid in a test tube. The mixture was stirred for 15 min and then left to stand for 24 hours. After that

time, the solution was placed in a 50 mL graduated balloon and leveled. The amount of ion present in the sample was determined from this solution. Knowing the quantity of the encapsulated ion/mass of particles and the total amount of ion that was added in each formulation, it was possible to calculate the efficiency of the encapsulation (% EE) that was reached with the method employed, through the following equation:

$$\%E = \frac{m_{(total-ion)} - m_{(non-encapsulated-ion)}}{m_{(total-ion)}} \times 100$$

Tests for the release of the encapsulated ion. The release of the encapsulated ion (according to USP XXXI 2008 protocol) was evaluated by simulating in vitro gastric pH conditions and pH conditions of the human intestine, both tests carried out at 37 ° C, without considering the presence of enzymes. All samples were analyzed in triplicate, using atomic absorption spectroscopy (Perkin Elmer equipment, model 3300). Protocol was follow: *Release assay in a simulated gastric medium (Part 1)*. 10 mg of particles were placed in graduated and sterile plastic tubes to which 25 mL of HCl solution at pH 1.75 and stirring. Subsequently, a volume of 1.2 mL of the supernatant of the particles was taken every 30 min for a total of 120 min. Each of these aliquots was placed in a 50 mL volumetric flask and filled with deionized water for later analysis by atomic absorption spectroscopy, to determine the amount of ion released as a function of time to that conditions. *Release assay in a simulated intestinal medium (Part 2).* Once the release in the gastric medium was studied, the test was carried out in the intestinal medium. In this case, the test residue in the gastric medium was centrifuged and used for the next test to simulate the passage of the particles through the gastrointestinal tract. The same procedure was used above, but using a phosphate buffer solution at a pH of 6.56. The results obtained under these two gastric and intestinal conditions were then gathered in a single figure to know the in vitro simulated release process.

Results and discussion

The method of preparation is very important to determine the properties, stability and final application of nanoparticles[18-21] and one of the important parameters to evaluate its the effect of ion concentration on internal gelation of alginate. The only process of crosslinking by the ionic gelation process of the alginate is not a guarantee that structures are formed at the micro and nanometric level, because the simple use of ionic gelation allows the formation of a continuous gel. But when doing the formation of this gel in an emulsified system, it is where particles are guaranteed to form on this scale and to be maintained stable once they have precipitated.

The microemulsion method and ZnCl2 were evaluated in a previous research work 23. In this case, $ZnSO_4$ and $MgSO_4$ were used for crosslinking the alginate, and the formation of drops allows obtaining the particles into a micro emulsification system. Fig. 1 and Fig. 2 shows SEM micrographs and EDX analysis of samples prepared with Zn^{2+} and Mg^{2+} respectively.

As shown in Fig 1, all assays tested with $Zn^{2+,}$ produced particles at the nanoscale with narrow particle distribution (as evidenced in Fig 3); the TEM results will verify these dimensions. Fig. 1 shows the spherical morphology of the particles, including some agglomeration, and also verifies the presence of the zinc element using EDX analysis, where results showed that zinc values are proportional with the [$ZnSO_4$] concentration. As has been reported in the scientific literature, researchers have concluded that the M/G ratio of alginate has a major influence on the degree of shrinkage as it affects the gelation mechanism (i.e. 'egg-box' formation). A higher concentration of group G residues in the molecular chain of alginate guarantees the formation of more stable structures[25], which is the case of the alginate that this research utilizes. The formation of the particles is not only the result of the ionic gelation process but also the micro-emulsion system used here for the formation of the particles.

In cases where the magnesium ion was used one can observe at low ion concentrations [0.075] mol/L structures like fibers (see Fig. 2(a)). At [0.15] mol/L a mixture of fiber and particles seems to appear (Fig. 2(b)); but only particles were observed when the maximum concentration of Mg2+ [0.25] mol/L was employed, as shown in Fig. 2(c).

The first step that occurs in the gelation process of alginate is the metal ion complexation with the carboxylate group present in the polysaccharide structure. The affinity of the alginate for the multivalent cations depends exclusively on a guluronic acid fraction (G) present in the polysaccharide because mannuronic acid (M) presents almost no ion selectivity 16. Had been reported that the affinity of the guluronic alginate fraction cross-linked with calcium ions (II) compared to other metals ions, and it is increasing in the following order: Ca+2<Zn+2<Sr+2<Ba+2 [12,15,16]. That is, as the atomic radius of the element in question increases the affinity increases 12-14. As seen in these previous investigations, no magnesium ion comparisons were established, likely because this ion did not produce immediate gelation when used with this polysaccharide. But new interest in this aspect has been presented, and further efforts are being conducted in this particular. There is recent evidence propose for Topuz *et al.* 17, that indeed the crosslinking occurs in the presence of Mg^{+2}, their results support our results shown in Fig. 2. In this work, Topuz *et al.* 17, evaluate the gelation using dynamic rheological studies in the oscillatory mode of alginate with magnesium (at a concentration range of 10-40 mM). Their results were shown in a Sol-Gel graphic and SEM morphology, and this further justifies the fact that a high concentration of magnesium is necessary for the gelation can be facilitated between guluronic acid and this Mg^{+2} ion. These results open a window to use different technologies for sample

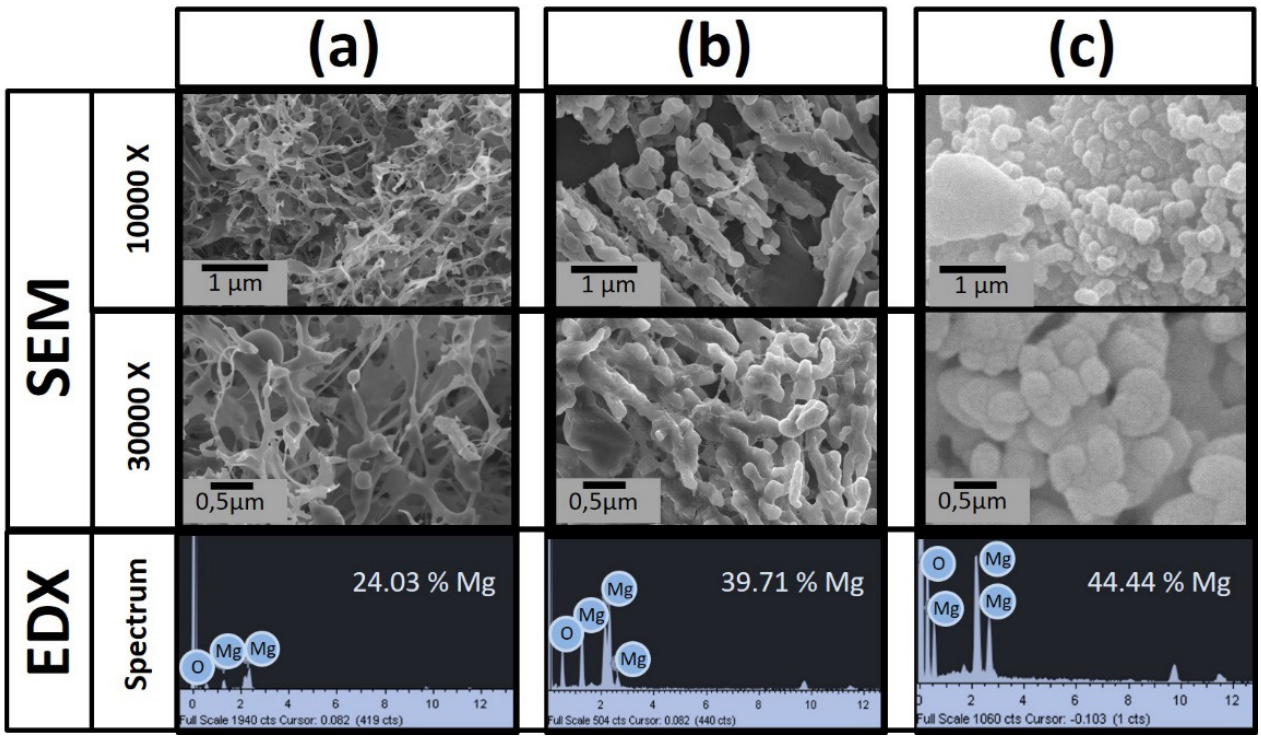

Figure 1 - Scanning electron micrographs, EDX spectrum and elemental composition of nanoparticles prepared with three Zn^{2+} concentrations: (a) 0.075 mol/L, (b) 0.15 mol/L and (c) 0.25 mol/L.

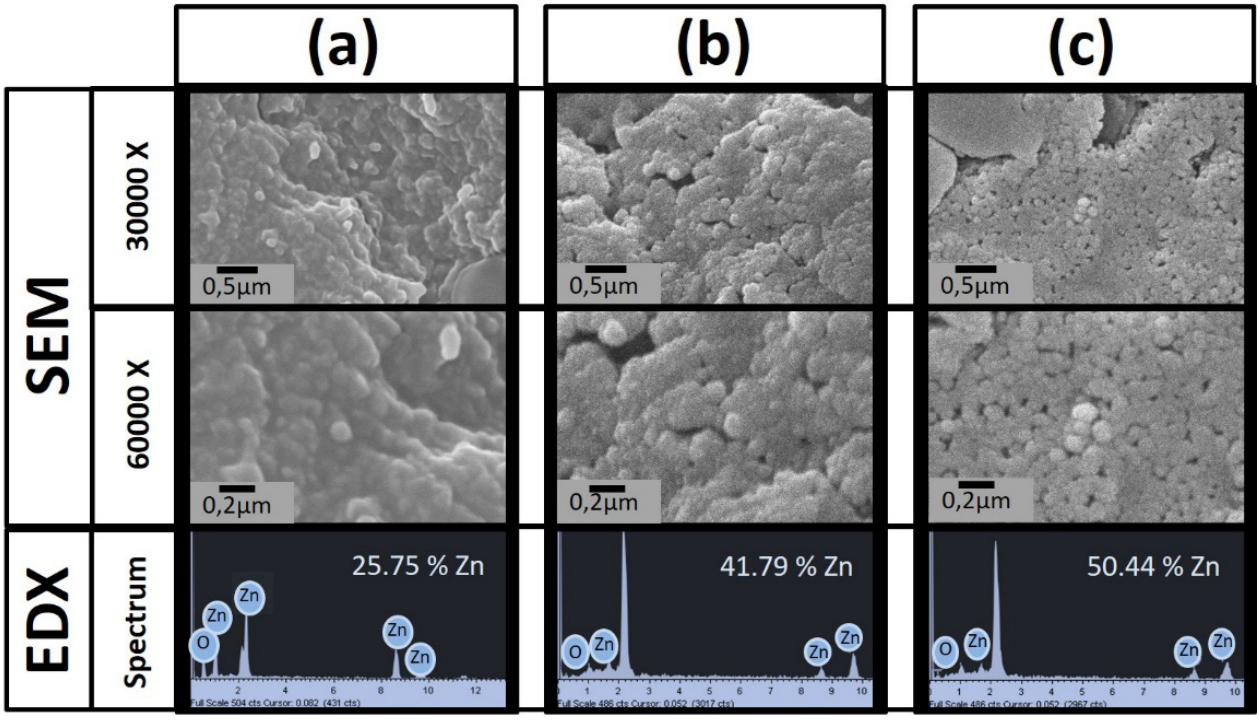

Figure 2 - Scanning electron micrographs, EDX spectrum and elemental composition of nanoparticles prepared with three Mg^{2+} concentrations: (a) 0.075 mol/L, (b) 0.15 mol/L and (c) 0.25 mol/L

preparation, mixing injection, in situ cross-linking, which makes these gels promising candidate for bioengineering and biomedical applications.

Relate to the gelation process, the most widely accepted model in which the divalent cation is bound to two G groups (Guluronics groups) into contiguous alginate chains is known as "egg box" [11,26]. This process would explain the observed in Fig 1(a) wherein the formulation of lower Zn+2 concentration the number of particles formed is low, and these appear to be more agglomerated (which can also be associated to the result reported in Fig 3 (a)). Graphs of figure 3, represent the behavior of Intensity and accumulated frequency vs. particle size.

The difficulty of formation of particles could be further related to the different components of the formulation during the emulsification process. For example, (i) the quantity of surfactant used in the formulation (about the amount of salt added) and (ii) part of the alginate molecule does not form part of the nanoparticles, and it can be formed as a continuous alginate film.

Another hypothesis is that at higher ion concentrations, the ions can saturate all guluronic groups (G), and then, starting to interact with mannuronic groups (M), so the stereochemistry of the molecule appears not to be favorable 26,27. Thus, it could form the more substantial amount of nuclei which will produce particles with better dispersion in size (as shown in Fig 2(b) and 2(c)). Also, a slight change in shape and the surface topography of the particles could appear. The aforementioned is shown in Fig 1(c), where the particles are more uniform in size and its surface seems smoother when compared with Fig 1(a) and Fig 1(b). Additionally, EDX results show that the concentration of Zinc and Magnesium increases quantitatively in the particles as expected.

Although it has been mentioned, others parameters should determine the properties and morphology of the particles and guarantee its size into nanoscale. Between these parameters are: the viscosity generate for the gelation ion-alginate and also for alginate molecular weight, surface area, density and encapsulating capacity of substances (absorption rate and release profile) [18,20,29]. Figure 3 shows a Gaussian distribution for each of the formulations (a) [0.075], (b) [0.15] and (c) [0.25] mol/L, being resulted that increasing the ion concentration will reduce the dispersion and size. Thus the Fig. 3(a) shows sizes range from 100-600 nm, having a quasi-bimodal distribution. In Fig. 3(b) the particle size was among 50-1200 nm, concentrating the largest number of particles with sizes around 480-500 nm and a monomodal distribution. And finally, for the highest concentration of salt, it can reduce the particle size and size dispersion resulting in a monomodal curve with a range of 150-500 nm, with a media around 345 nm.

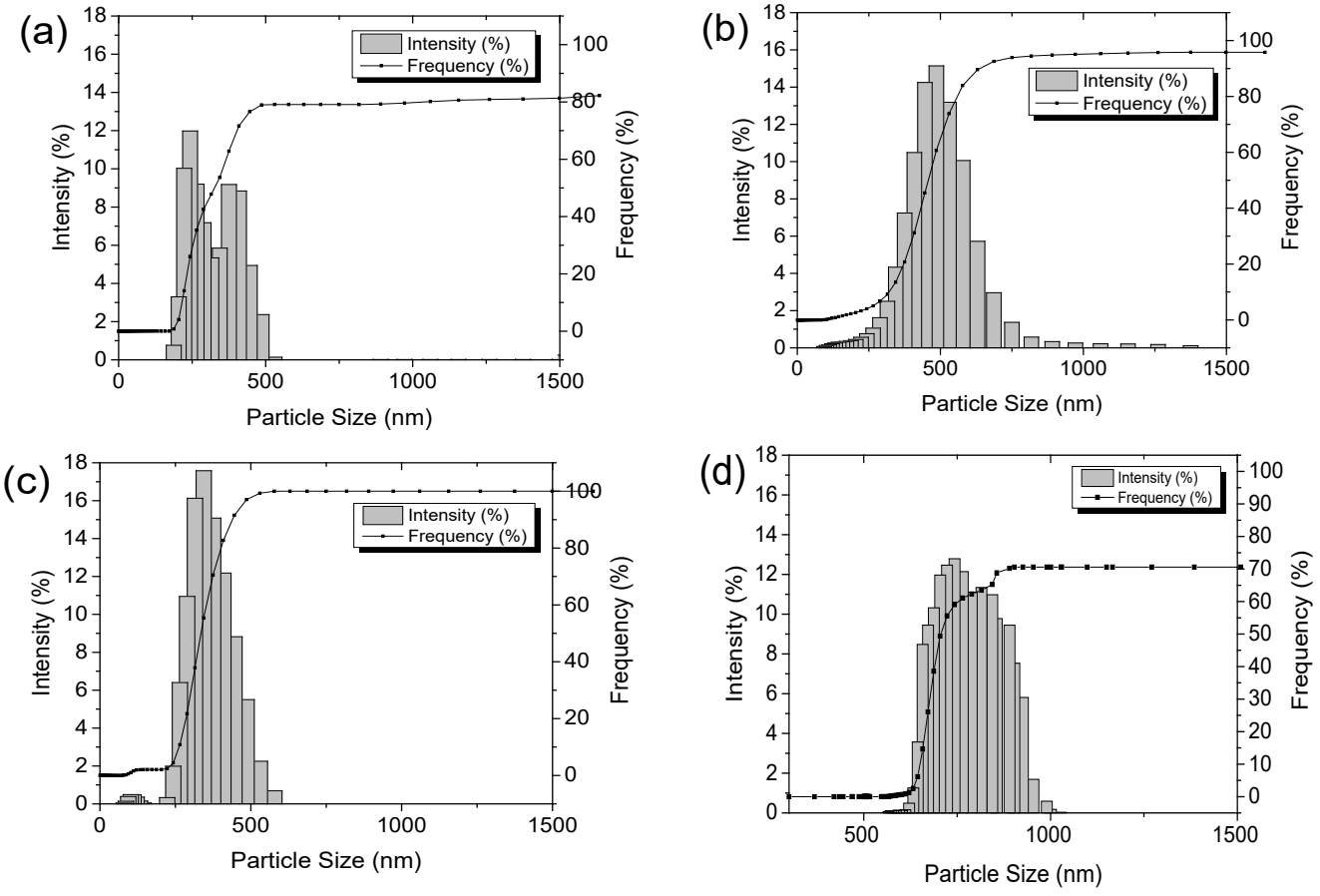

Figure 3 - Particle size distributions of Zn+2 concentrations (a) 0.0075 (b) 0.15 and (c) 0.25 mol/L and Mg+2 concentration (d) 0.25 mol/L.

The particles obtained with Mg^{+2} concentration [0.25] mol/L are shown in Fig. 3(d), because it was the only concentration that showed the formation of particles, as has been mentioned. The particle size distribution, in this case, is such where the unimodal particle size remains in the nanoscale (600 to 1000 nm), these being larger than those obtained with Zn^{+2} at the same concentration. It is possible to think that in the case of Mg^{+2} ion, these larger sizes of particles are obtained due to its ionic radius. Because these Mg^{+2} ions serve as nuclei for gelation, and it can be assumed that they occupy the most space to interact with the biopolymer chains during the formation of particles. It is for this fact that can be assumed that better packing is induced into the structure called box egg and thus generate larger and more compact particles [28,29].

The morphology analysis follows using TEM, is shown in Fig. 4. It is possible to verify the nanoscale reach of the nanoparticles forming using Zn^{+2} ions, and also that they are spherical and appear to be compact (are not nanocapsule) confirming what was appreciated in Fig. 1 and Fig. 3(b). All nanoparticles prepared uniform exhibit size, and it must be pointed out that the largest % of particles present diameters below submicrometer range (< 1 μm).

A detail of the smaller nanoparticles (≤ 100 nm) can be seen at higher magnifications in Fig. 4, and it is clear that there are compact particles. However, it seems that around their surface a thicker wall is formed, which could be defining surface characteristics of these nanoparticles. Also, TEM micrographs demonstrate that the density of crosslinking into the particles is different from their surface radially towards their interior.

In Fig. 5, the entire surface of the sample under study can be seen in a scan of 2μm x 2μm, where a high presence of semi-spherical particles and agglomeration of the same are observed. In Fig. 5b we have a 3D micrograph of the entire surface studied and detailed the valleys formed by the differences in height between the particles. Fig. 5c corresponds to an extracted profile measured horizontally on a group of particles. Through these AFM micrographs show that the particles do not exceed 20 nm in height and a width of between 100-250 nm so that we can confirm the sizes observed by both SEM and TEM.

Encapsulated and release. Two formulations were chosen to perform release assays. Since it was desired to study the release of the ion under controlled conditions, the formulations chosen to study were those prepared with the highest concentration of the ion (0.25 mol / L), both for Zinc and for Magnesium. The sample identified as Zn1 corresponds to the SEM micrographs shown in Fig. 1(c), while the sample identified as Mg1, corresponds to the SEM micrographs shown in Fig. 2(c). These results are summarized in Table 1.

The tests were made with the same formulations Zn1 and Mg1 to which the percentage of encapsulation was determined. Such as shown in fig. 6, particles prepared with Zn^{+2} show a release below 30% in gastric medium, while that of the Mg^{+2} exhibit a release around 80% of the ion in this medium at 120 min. The fact above may be because the interaction of the alginate with the Mg^{+2} ion is weaker as already discussed previously[16,17,30,31] and therefore the bonds could be broken, allowing the release of Mg^{+2} more rapidly than in the case of Zn^{+2}. The results of these tests indicate that indeed, the greater amount of the encapsulated Zn^{+2} ions are released in intestinal conditions.

Our results support other research about alginate because it is resistant to acid hydrolysis and soluble in alkaline solutions[15]; which is very important for the encapsulation of nutrients in alginate is a viable procedure. That means the alginate protects the nutrients during its passage through the upper digestive tract and allows its release in the intestine, which increases the level of usage of a compound encapsulated in alginate particles[32].

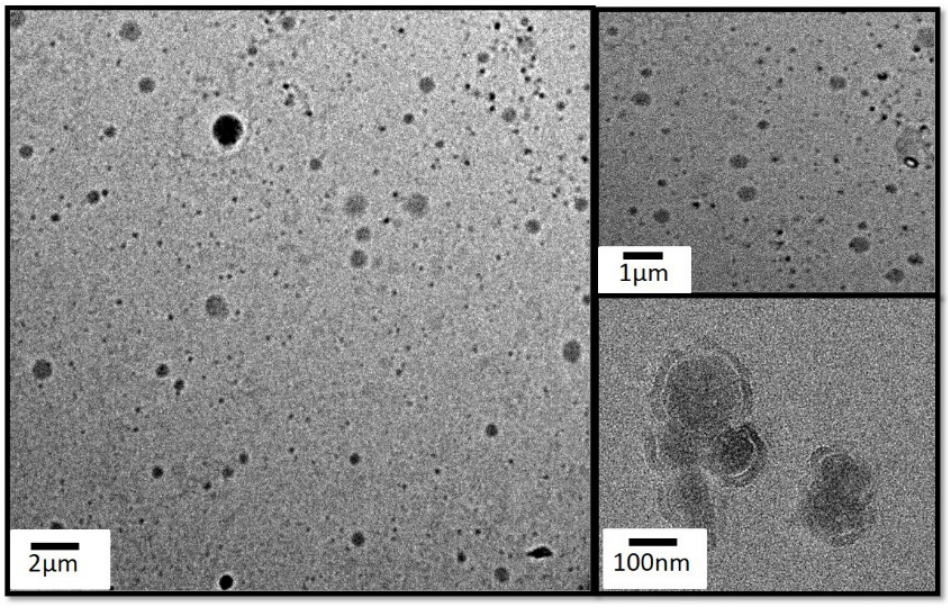

Figure 4 - TEM micrographs of nanoparticles prepared with Zn^{2+} ([0.15] mol/L).

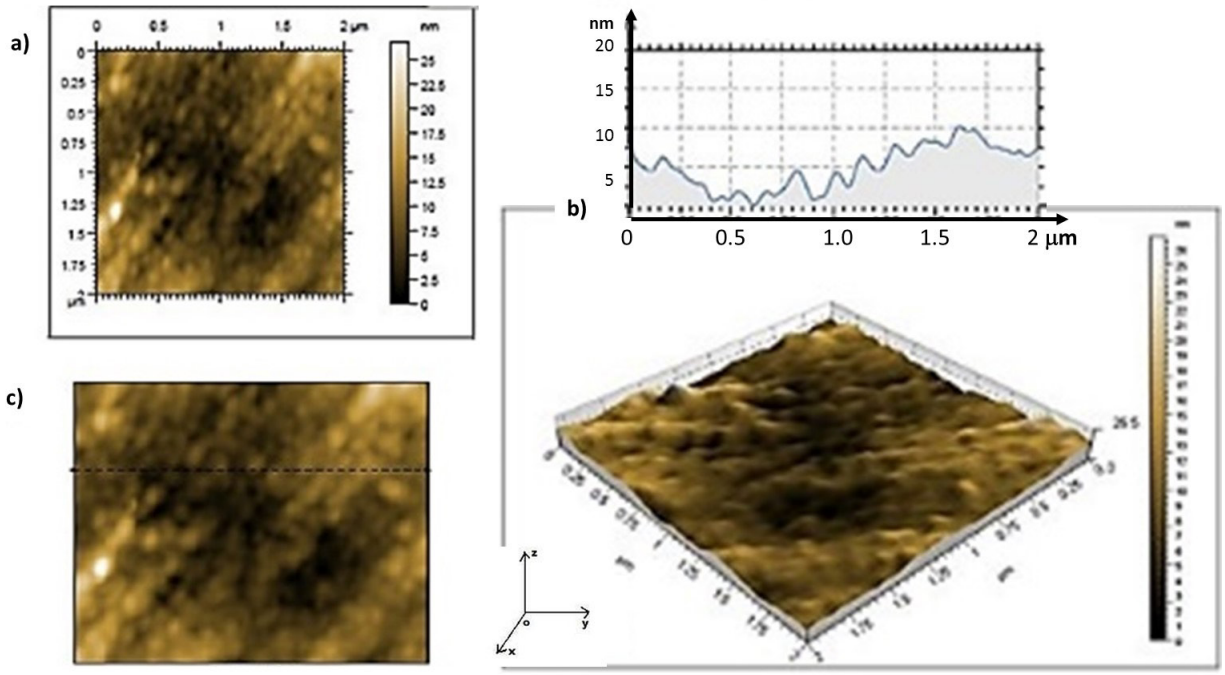

Figure 5 - Atomic force micrographs of alginate particles prepared with 0.25 mol/L of Zn^{+2} (a) Micrograph of an area corresponding to 2µmx2µm; (b) 3D image of the observed surface; c) Profile extracted in horizontal line

Table 1 - Encapsulation efficiency of the ions into each particles.

Sample	%EE
Zn1	71,99
Mg1	61,35

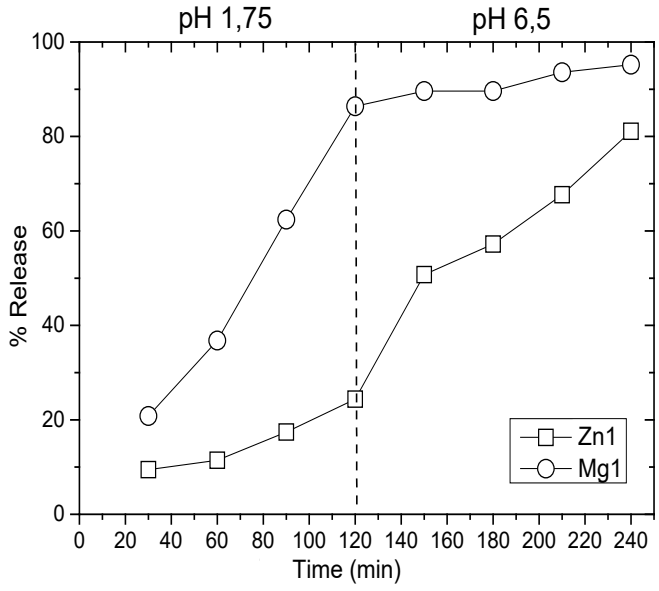

Figure 6 - *In vitro* release of Mg^{+2} and Zn^{+2} contained in the particles in digestive gastric and intestinal medium without enzymes

Conclusions

The ion concentration and its chemical characteristic are parameters with the greatest effects on morphology and size of the nanoparticles, which were prepared with higher Zn^{2+} concentrations show smoother surface morphologies and unimodal distribution with low polydispersity index as compared with lowest Zn^{2+} concentration. Higher Zn^{2+} concentration could saturate the guluronic (G) nucleus, and it is proposed that the ions could be binding both to G and M units of the alginate molecule for forming particles at the nanoscale and increasing their amount, as was shown by SEM and TEM images. Typically, and until now, has been reported that Mg^{+2} ion could not cause gelation of the alginic acid. However, this research demonstrates that gelation occurs. In the case of Mg^{+2} ion, an increase of ion concentration is necessary to obtain particles and additionally considering that ionic crosslinking relies on a micro-emulsion process to guarantee the stabilization of the particles. This result was evident after TEM and AFM microscopy, which also allowed verifying that these particles have sizes in the nanometer scale. The latter suggests that these micro/nanoparticles can be used as a carrier for essential nutrients for food fortification, as well as for others applications in biomedicine or charge drugs in delivery systems. Also, the results of this publication related to the use of Mg^{+2} as an ionizing and nucleating agent for the formation of alginate particles is a significant contribution and expansion of the possible use of this biopolymer, and they also weaken a belief that has been considered for several years that magnesium could not gel alginate.

The experiments carried out in this research, where gastrointestinal media without enzymes was simulated, represent a key tool for the evaluation of future applications of the nanoparticles obtained considering that the utilization of the nutrients occurs to a greater extent in the intestinal tract. Also, the particles obtained can be used successfully to fortify foods, or even to release other types of biomolecules that can be ingested orally.

Acknowledgments

The authors would like to thank National Institute of Technologic (INT), Rio de Janeiro, Brazil for technical support to characterize nanoparticles size (Polymer laboratory LAMAP and Lab Sol-Gel of Materials Department.) The authors also want to acknowledge Electron Microscopy Lab-USB for support with MEB analysis. Also, thank FONACIT (Fondo Nacional de Ciencia y Tecnología) for the financial support to PEII Projects Nº 418 and 1859. The authors also acknowledge Dr. Belsay Borges for proofreading this manuscript.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

REVIEW ARTICLE

Immobilization of biomolecules on natural clay minerals for medical applications

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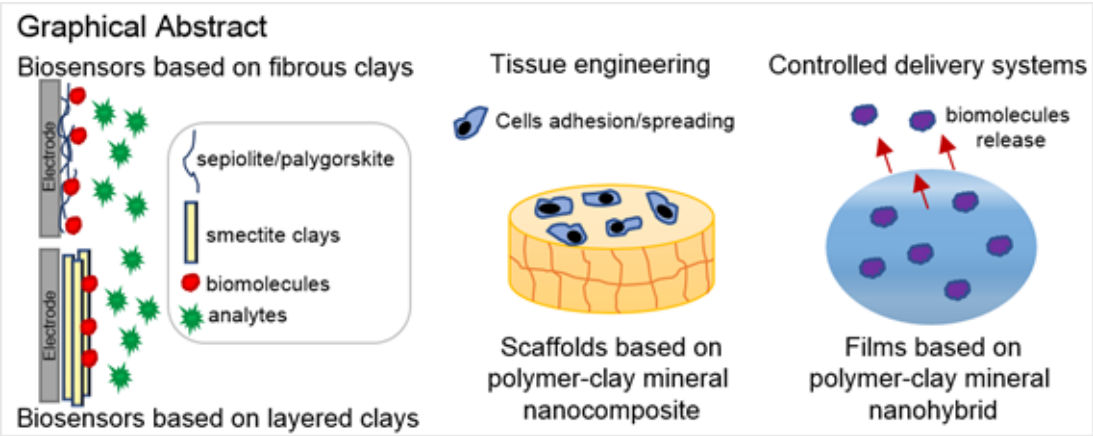
ARTICLE INFO

Keywords:
Bionanocomposites
Biosensors
Tissue engineering
Delivery systems
Biomaterials

ABSTRACT

Biomolecules are a group of organic entities that are important in many areas of research on nanomaterials and for biomedical and pharmaceutical applications. Advanced systems have been developed to attempt to protect the activity of biomolecules from rapid degradation and instability. Among these techniques, the incorporation or immobilization of biomolecules has become popular in the development of biocomposites. As such, clay minerals appear to be promising materials; combining a nanometer-scale size with their adsorptive capacity, lack of toxicity, and biocompatibility would result in enhanced biomaterial properties. This mini-review discusses the recent advances concerning biological molecules immobilized on clay minerals and their biomedical applications as biosensors, in regenerative medicine, and even as controlled delivery systems.



1. Introduction

Biomolecules are a group of organic entities of biological origin, such as polysaccharides, lipids, vitamins, enzymes, amino acids, peptides, proteins, and nucleic acids. A variety of materials have been proposed with properties derived from the functions of these molecules.[1,2] However, systems containing biomolecules often show restricted recovery and reuse because of their lack of stability at elevated temperatures, in organic solvents, and in a gastrointestinal environment.[3]

Biocomposites are materials based on an inorganic solids, such as clay minerals, in association with organic compounds.[4] This approach can protect biomolecules from degradation in arrays derived from natural resources.[5] In addition, molecules immobilized on nanosystems show well-preserved catalytic activity and enhanced properties.[6,7] Systems containing biomolecules have a wide variety of applications in clinical or industrial use; thus,

ARTICLE HISTORY:
Received 17 October 2017; Received in revised form 09 November 2017;Accepted 10 December 2017
Available online 25 January 2018

inorganic solids and their assemblies play an important role as immobilizing matrices.[8]

Clay minerals are solids of the phyllosilicate family with the potential for biomolecule immobilization, resulting from their specific physicochemical characteristics, such as high surface reactivity.[4] In addition, these materials exhibit antimicrobial properties with good biocompatibility.[9,10] The advantages of these materials include their abundance, low cost, and potential as regional products.[4]

According to Jayrajsinh and coworkers[11], the interaction between nanoclays and organic compounds has been studied in different areas of research, such as engineered nanomaterials for biomedical and pharmaceutical applications. The proposed applications for nanocomposites include the use of biomaterials as scaffolds, drug carriers for delivery systems, patches for wound healing, and as modifiers of biological substrates in electronic or implantable devices.

The present review focuses on studies related to different types of biological molecules immobilized on natural clay minerals published in the last ten years. We review the main experimental research in the biomedical application of clay nanocomposites, including their use as biosensors and controlled delivery systems and in regenerative medicine.

2. Clay minerals – major characteristics

Aluminosilicates, such as montmorillonite, kaolinite, illite, sepiolite, and palygorskite (or attapulgite) belong to two types of abundant inorganic solids in nature.[12] There are certain differences between layered and fibrous clay minerals, i.e., expanding and non-expanding types. As an example of layered clay, montmorillonite shows expansibility, 1-nanodimensional particle, high charge density and cation exchange capacity, low-density silanol groups at the edges, and particles in layered stacks. By contrast, fibrous clays, such as sepiolite and palygorskite, exhibit a non-swelling process, 2-nanodimensional particles, low charge density and cation exchange capacity, high-density silanol groups (≡Si-OH) at the broad external surface area, and particles in bundles.[13]

Both classes are micro and nano-sized[14], and present strong adsorption strength and ion exchange abilities, and a high internal surface area. The layers or sheets are constituted by basic arrangements of clay mineral tetrahedral silicates and octahedral hydroxide sheets, giving rise to various classes of clay minerals of type 2:1 or 1:1.[10] The permanent net negative charge of the layers or sheets, resulting from the substitution of Al^{3+} by Fe^{3+} or Mg^{2+}, the presence of hydroxyls at the limits, and compensatory cationic ions located in interlayer/sheets spaces are responsible for cation exchange abilities. Broken edges of clay show pH-dependence, staying positive at low pH and negative at high pH, originating from the surface reactivity of the clay mineral.[14, 10]

Thus, biomolecules, either negatively or positively charged, can be immobilized on the surface, edges, or interlayer/microchanels of clay particles.[10,15] Adsorption of organic molecules on clay minerals reported in the literature[16] includes hydrophobic interactions, hydrogen bonding, protonation, ligand exchange, cation exchange, cation bridging, and water bridging.

Natural clay minerals to be used for medical purposes must be purified to eliminate impurities, such amorphous or organic materials.[6] Preparation of the purified clay sample is beneficial, because the final product is of very high purity. Despite the costs incurred during the purification of natural clay minerals, which are used for medical applications, they remain an attractive choice.

3. Diversity of biomolecules associated with clay minerals

Many biological agents incorporated to clay minerals can be released in organic systems for a range of biomedical applications. The diversity of biomolecules used to form new materials in association with natural clay minerals, as well as the forms of incorporation and the state of the molecules, are summarized in table 1.

As seen in table 1, clay minerals can adsorb various biomolecules, including proteins, nucleic acids, and carbohydrates. The majority of the reports on the use of biomolecules refer to polymer and enzyme immobilization. In the field of release systems, the number of reports on the use of clay minerals has increased in the last four years. Controlled drug delivery systems allow temporal and/or spatial control of release rates, thus, allowing acceleration, delay, or prolongation of release, as well as site-specific targeting.[4]

Among different types of clay minerals, montmorillonite has been studied more frequently in biological applications, possibly because of its high ion exchange capability and because it is widely distributed in nature. Chen and coworkers[50] reported that effective intercalation of proteins within the galleries of montmorillonites can be achieved via space enlargement and exchange processes, while retaining the native conformation of the guest proteins and the multilayered structure of the bioinert host plates. Despite this, according to Ruiz-Hitzky and coworkers,[4] in certain cases, fibrous clays are more interesting than layered clays and can display higher enrichment of the mechanical properties of biomaterials.

Furthermore, bionanocomposites can be processed as films or as porous cellular materials, using solvent casting and freeze-drying processes. The following sections discuss the biomedical applications of clay minerals.

Table 1 - Diversity of immobilized biomolecules on natural clay minerals reported in the literature in the last ten years.

Biomolecule	Clay/clay mineral	Form of Incorporation	Condition of the molecule in the clay	Ref.
Carrageenan	montmorillonite	Suspension in solution	Intercalated	17
Chitosan		Electrospinning from mixing in solution	Intercalated by ion exchange	18
		Mixing in solution	Intercalation with a planar conformation	19
		Dispersion in acetic acid aqueous solution	Intercalated by ion exchange	20
Chitosan-PVA		Electrospinning for mixing in solution	Intercalated by ion exchange	21
Alginate/Ghatti Gum		Dispersion in aqueous solution	Intercalated complexes	22
Vitamin B12		Adsorption in phosphate-buffered saline (PBS) buffer solution	Intercalated with diffusion of vitamin B12 to the interlayer spaces	23
Cellulose		Solution mixing process	Intercalation	24
Lipid		Aqueous solution of clays added in an emulsion containing the lipid to form microparticles	Not determined; however, clay exfoliation was proposed to form the microparticles, as observed by scanning electron microscopy (SEM).	25
Pectin/chitosan	montmorillonite with silica gel and sepiolite	Solubilization of chitosan, followed by addition of the clay under ultrasound, and formation of the hydrogel with the addition of pectin under vigorous shaking	Amorphous phases were identified.	26
Carboxymethyl-starch (CMS)		Clay was dispersed in water and stirred with CMS, glycerol, and citric acid. The mixture was poured into a polytetrafluoroethylene (PTFE) mold to form a film	Intercalated	27
Cysteine, aspartic, glutamic acids		Suspension in solution	Intercalated	28
Dendrimeric peptide		Suspension in solution	Intercalated	29
Cysteine, thiourea, and thiocyanate		Suspension in seawater	Adsorption with a smaller expansion of the layer	30
DNA/RNA		Adsorption in double-deionized water followed by precipitation in a microcentrifuge	Intercalated	31
Polylactide		Melt extruded	Dispersed in polymer	32
Proteins		Adsorption on buffer solution	Not indicated in the text	33
Hemoglobin or methyl viologen		Clay films were prepared by dropping a known volume of clay colloids – soaked in methyl viologen, or by dropping of Hb/clay aqueous mixture onto the electrode	Not indicated in the text	34

►►

Table 1 - Diversity of immobilized biomolecules on natural clay minerals reported in the literature in the last ten years(cont.)

Hemoglobin (Hb) or methyl viologen	saponite	Clay films were prepared by dropping a known volume of clay colloids – soaked in methyl viologen, or by dropping of Hb/clay aqueous mixture onto the electrode	Not indicated in the text	34
L-DOPA (precursor of dopamine)		Adsorption in aqueous solution at pH 7.5	Intercalated – theoretical and experimental study	35
Chitosan		Films were prepared from the solubilization of the chitosan and saponite dispersion in the presence of glycerol	Coplanar alignment of saponite nanoplatelets with two monolayers of chitosan macromolecules in the gap	36
Cysteine, thiourea, thiocyanate	bentonite*	Suspension in seawater	Adsorption with a smaller expansion of the layer	30
Amino acids		Suspension in seawater	Intercalated	37
Homalomena aromatica rhizome oil		Clay was modified with oil, followed by modification with epoxy resin, and cured	Intercalated	38
Amino acids	kaolinite	Suspension in seawater	Intercalated	37
Gellan gum	halloysite	Hydrogel was prepared with dispersion of gellan gum, followed by clay addition under sonication	Not indicated in the text	39
Pectin, cellulose, chitosan		Adsorption	Not indicated in the text	40
Cellulose		Halloysite nanotubes were incorporated into a cellulose NaOH/urea solution to prepare composite hydrogels by epichlorhydrine crosslinking	Not indicated in the text	41
Carrageenan		Film was prepared from a mixture of aqueous solution of Carrageenan and of clay, followed by the addition of glycerol	Carrageenan interacted with the hydroxyl surface groups of clay	42
Starch, alginate	sepiolite	Polymers were dissolved and added to a dispersion of clay, and then mixed using a processor	Strong interactions were indicated by the considerable perturbation of the stretching vibration band of −OH in the silanol groups	43
Chitosan		Chitosan was dissolved in acetic acid, clay was dispersed in water, and the solutions were mixed to form a film	The materials interact on the surface of the clay without penetration inside the tunnels	44
Starch and alginate or chitosan		Dispersion of clay in the solution containing the polymer	Not indicated in the text	15
Arabinoxylan		Polymer and clay were solubilized and suspended in water separately, and mixtures were degassed by ultrasonication and then added onto plates to form a film	The SEM images showed a good dispersion of clay in the films	45
Xanthan gum		Polymer and clay were solubilized and suspended in water separately, and mixtures was stirred to obtain a gel and lyophilized for solvent removal	Polymers interact on the surface of clay via hydrogen-bonding interactions	46

►►

Table 1 - Diversity of immobilized biomolecules on natural clay minerals reported in the literature in the last ten years(cont.)

Poly(lactic-co-glycolic acid) - PLGA	palygorskite (attapulgite)	PLGA was dissolved in a mixed solvent of tetrahydrofuran– dimethylformamide, clay was added to form a homogeneous solution for electrospinning	Not indicated in the text	47
Protein zein	palygorskite with sepiolite	Adsorption in ethanol/water media	External surface	48
Sodium alginate	palygorskite (attapulgite)	Alginate was dissolved in water; for crosslink formation, ammonium persulfate (initiator) was added. Acrylic acid was neutralized with NaOH, and completely mixed with crosslinker, N,N'-methylene-bis-acrylamide, for incorporation of different amounts of clay into the hydrogel formed, and the samples were dried to obtain the nanocomposites	Not indicated in the text	49
Chitosan and sodium alginate		Hydrogel was prepared with different concentrations of palygorskite by graft-copolymerization in association with acrylic acid. After alginate addition, the mixture was dripped in calcium chloride solution to obtain crosslinked microparticles in Ca^{2+}	Not indicated in the text	50

*The term "bentonite" refers to a mixture of minerals from the smectites group with a predominance of montmorillonite clay mineral; however, we prefer to use the term as reported in the articles cited in the table.

4. Applications of biomolecules immobilized on clay minerals

4. 1. Biosensors

Biosensors are chemical sensors in which the recognition system uses a biological mechanism to measure the interaction between the analyte and the sensor device, transforming quantitative or qualitative information into a measurable electrical signal.[51]

Clay minerals have been used as supports or modifiers of substrates in the field of electronic devices for electroanalytical purposes. These inorganic solids have received increasing attention since they were first used in these systems by Ghosh and Bard in 1983[52], who reported the first electrode modified with a clay film. Following the use of natural zeolite on modified carbon paste electrodes for analytical purposes, the clay mineral sepiolite has also been applied for the same purpose[53].

Some studies have described the use of clays as electrode modifiers or as clay-containing matrices.[54-58] However, only a few have studied the biomedical applications of clay biosensors or the diversity of biomolecules associated with clay minerals.

These materials can be used in electroanalysis because of their electrocatalytic properties and capacity to immobilize biocatalytic entities to improve the sensitivity and/or selectivity of the detection process.[3,53,59] Biomolecules immobilized on clay minerals supports to develop biosensors that have been reported in literature are summarized in table 2.

Nanostructured materials can be formed by the intercalation of organic molecules, such antibodies, peptides, proteins, genes, bacteria, cells, polymers, or enzymes within the interlayer, edges, or surface space of the clay minerals.[56] Notably, reports show that enzymes are the most frequently immobilized entities on clay matrices, including their use as amperometric biosensors, because of the sensitivity and specificity of their chemical reactions in these systems. For example, enzymatic biosensors for the detection of catechol, glucose, hydrogen peroxide, and phenol have been developed.

Immobilization should ensure that the biological activity of the immobilized biomolecule is maintained and its stability is preserved or enhanced while providing accessibility to the analytes. In this regard, clay minerals have proved to be suitable materials.[10]

Palygorskite represents an excellent inorganic material for the development of biosensors because of its electrocatalytic activity, which may be attributed to its high adsorption capability and the presence of OH groups on its surface. These features allow electron transfer between the electrode and the detected analytes.[75] Futhermore, its large surface area, high biocompatibility, and stability make it a promising material for enzyme immobilization.[68]

Recently, halloysite nanotubes have been developed by evaporative assembly. They are promising natural materials because of their rough surfaces, which provide higher cancer cell capture efficiency compared with blank

Table 2 - Biosensors based on clay modifier electrodes and immobilized biomolecules.

Biomolecules	Clay modifiers	Electrode	Biosensor	Ref
Pyranose oxidase	montmorillonite/polyglycolide (PGA)	glassy carbon	glucose	60
Cisteyne	bentonite-AuNanoparticles	glassy carbon	ascorbic, uric and folic acid	61
Enzymes	halloysite	glass capillary	cancer cells	62
Horseradish peroxidase	sepiolite/carbon nanotubes (CNT)/PVA	-	peroxidase	63
Glucose oxidase	montmorillonite/ Gly, Lys, Glu	glassy carbon	-	64
Bovine serum albumin (BSA), glutaraldehyde (GA) and pyranose oxidase	montmorillonite/ calixaren-NH2	glassy carbon	-	65
Laccase	montmorillonite/ histidine	glassy carbon	phenol	66
Glucose oxidase	palygorskite-poly(o-phenylenediamine)/glutaraldehyde	-	glucose	67
Horseradish peroxidase	palygorskite	glassy carbon	hydrogen peroxide	68
Lactobacillus bulgaricus, Streptococcus thermophilus	palygorskite	oxygen	lactate	69
Horseradish peroxidase	palygorskite	glassy carbon	hydrogen peroxide (cellular reactive oxygen species)	70
Tyrosinase	palygorskite	glassy carbon	phenol	71
Hemoglobin	nontronite, montmorillonite and saponite/Fe_2O_3	-	hydrogen peroxide	33
Glucose oxidase	palygorskite	glassy carbon	glucose (blood and urine samples)	72
Hemoglobin	palygorskite	glassy carbon	hydrogen peroxide	73
Cytochrome c	palygorskite	glassy carbon	hydrogen peroxide	74

capillary glass surfaces.[63] Their tubular structure make them suitable candidates for biomolecule capture and development of enzymatic biosensors.

The challenges regarding the development of biosensors based on nanocomposites include the ability of detecting lower concentrations of the analyte of interest, often at the trace level, to ensure the selectivity, sensitivity, and reproducibility of the system.[59]

4.2. Regenerative medicine

Different strategies are required to develop a biomaterial, such as a suitable scaffold, which satisfies the requirements of cells in a three-dimensional support system or as a delivery vehicle incorporating bioactive compounds.[77]

Hydrogels, containing including natural polymers, such as chitosan, gelatin, starch, and recently gellan gum,[78] act as integrated networks of scaffolds because of the structural similarity of these components and have the potential to regulate cellular responses. However, their use has some limitations, such as relatively poor mechanical properties, high water sensitivity, or limited ability to support cell adhesion.[4,7] These difficulties can be overcome by modification of their structure or the incorporation of bioactive molecules, such as proteins, peptides, or clay minerals.[7]

Polymer-clay mineral nanocomposites can contribute to this field because of their high porosity and compressive strength, which remains an ongoing challenge in scaffold design, particularly in bone repair.[14] Another challenge is retaining the growth factors in the matrix in the gel network.[7] In this regard, clay concentrations under 5% (w/w) have shown improvements in the modulus and strength of 3-D materials.[14]

Reviews concerning experimental clay research in regenerative medicine were carried out by Dawson and Oreffo[14] Ruiz-Hitzky[13], Chrzanowski[7], and Bramhill and coworkers.[77] In the last four years, about 140 studies have been published concerning the use of clays in these systems, demonstrating the growing interest in this area. Most of the reports concerned montmorillonite and halloysite; however, other used kaolinite, palygorskite, and sepiolite.

Researchers have also examined the cellular response to biomaterials. Among them, Barua and coworkers[38] developed a polymeric matrix based on *Homalomena aromatica* rhizome oil-modified bentonite, which possessed antimicrobial activity. Biocompatibility assays were performed after subcutaneous implantation in Wistar rats. The biomaterial stimulated the adhesion and proliferation of dermatocytes, without any signs of toxicity.

Another study by Mohd and coworkers,[78] described the use of sodium montmorillonite (Na-MMT) modified with trimethyl ammonium bromide (CTAB-MMT) incorporated into a gellan gum (GG) hydrogel to improve its thermal stability. Cell studies showed that the Na-MMT composite was non-cytotoxic to skin fibroblast cells (CRL2522). In contrast, hydrogels with CTAB-MMT caused death and growth depletion of cells after 72 h.

Another advantage of fibrous clays compared with layered silicates is their very high density of silanol groups, which allows hydrogen bonding in addition to Van der Waals forces at the polymer-silicate interface.[13] The incorporation of palygorskite nanorods into poly (lactic-co-glycolic acid) matrices contributed to the osteogenic differentiation of cells, without changing the uniform morphology and hemocompatibility of the scaffolds.[48]

Another study by He and coworkers,[79] showed the use of natural nanopalygorskite to enhance vero cell productivity, without inducing cytotoxicity. This result suggested a useful strategy to reduce the cost of producing mammalian cell cultures for large-scale tissue engineering.

According to Bramhill and coworkers,[77] classical research has focused on bone regeneration; however, recent advances have also enabled the use of clay minerals at the soft tissue sites in the body. For these purposes, greater control of the physico-chemical properties of the biomaterials and their interactions at the body sites need to be evaluated. Future studies might focus on electro-spinning techniques or deposition in layers to develop new nanocomposite materials.[77]

4. 3. Controlled Release Systems

Biofunctional molecules, such as cells, nucleic acids, proteins, or lipids can be successfully stabilized while maintaining their biofunctional properties by being preserved in controlled release systems that are stabilized using clay minerals.

Ruiz-Hitzky and coworkers[4] and Zafar and coworkers[80] reviewed biopolymer-clay nanocomposites for their application as drug or biomolecule delivery systems, which rely on their properties of bioadhesion, biodegradability, and cell uptake. These features contribute to maintaining a constant dosage of the bioactive substance within the therapeutic dosage throughout the treatment course.

An experimental study reported the use of nanostructured montmorillonite clay, for controlling lipase-mediated digestion of medium chain triglycerides, engineered by spray drying oil-in-water emulsions.[25] The performance of montmorillonite-lipid microparticles (75% w/w) under simulated intestinal conditions suggested their use a novel biomaterial and that encapsulation optimized lipase efficiency as a smart delivery system for lipophilic biomolecules.

In recent studies, a sustained release system, based on chitosan and montmorillonite prepared by ion exchange, for controlled oral mucosal administration of chlorhexidine (CLX) was proposed. *In vitro* release tests showed sustained long-term release without an initial burst release.[19]

Another approach involves the use of bioactive film-forming matrices with improved functionality as wound dressings; these matrices allow controlled release of biomolecules.[40] Bionanocomposites based on polysaccharide-clay minerals can be used to encapsulate biomolecules lacking cytotoxicity, increasing mucoadhesivity and stimulating cell proliferation.[80]

For bioactive film matrices, most studies have used lamellar silicates; however, recent studies confirmed that fibrous clay minerals are also promising for the development of bionanocomposites.[4,81] A challenge in films manufacture is the fabrication of the nanocomposite itself.[80] Improvement of the characteristics of the new materials based on fibrous clay minerals can be obtained by suitable dispersion of clay nanorods on the matrix using various disaggregation techniques, which represents the key to developing functional nanocomposites. [82]

5. Toxicity of clay minerals

Till date, studies elucidating the toxicological effects of clays at physiological concentrations are not conclusive. Importantly, the oral administration of MMT in rats at high doses (1000 mg/kg) did not lead to accumulation in any organ,[83] and cell viability and proliferation remained close to 100% for any concentration of MMT tested in ovarian Hamster cells.[84] However, low concentrations of MMT (5 µg/mL) in human intestinal cells led to an acute response, inhibiting cell proliferation after 24 h of incubation[83], and the same effect was observed in the HepG2 hepatic cell line.[85]

According to Mousa and coworkers,[86] this behavior is closely related to the flocculation of the clay, as well as the high concentration of salts in the culture medium, which contributed to the formation of agglomerates that accumulated around the cells, leading to cellular damage, such as blockage of membrane channels and alteration of cellular metabolism. Thus, it is appears that

the inhibition of cell proliferation is an indirect effect of clay aggregation rather than a cytotoxic effect of clay itself. This aggregation depends on the surface charge, ion exchange capacity, and the size and morphology of the particles. The authors concluded that the *in vitro* and *in vivo* cytotoxicity studies available clearly showed the biocompatibility of these compounds when they remained stable, i.e., without precipitation. According to the literature[6,16] these clay materials are inert. However, there is a lack of information concerning their biodistribution and clearance, and if this depends on whether the clays are surgically implanted or administered parentally.

The literature review on the toxicological effects of clays and clay minerals by Maisanaba and coworkers[87] provided conflicting information, wherein *in vitro* assays generally suggest that clays are cytotoxic, whereas *in vivo* experiments in rodents showed no systemic toxicity. However, the authors concluded that toxicity should be assessed on a case-by-case basis, because it depends on the modifiers used, experimental methodology to assess cytotoxicity, concentration range, purity of the sample, type of deposit used, and its geological formation conditions and time of exposition.

6. Conclusions

Clay minerals have technological advantages in medical sciences provided by their structural, morphological, and textural characteristics. Clay minerals also have several advantages in biosensor applications, controlled release systems, and tissue engineering, especially their biocompatibility and biodegradability. Notably, possible adverse effects of clay minerals on human health remain unclear and could be related to the presence of impurities in the sample, exposure time, or limitations of the experimental biological studies. Such inorganic nanoparticles, either lamellar or fibrous, are expected to be used in association with a wide variety of biomolecules in biotechnological applications. Recent research reinforces the promising potential of clay minerals in the development of biomaterials.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

REVIEW ARTICLE

From nano to macro: enabling nanotechnologies for human organ biofabrication (Electrospun Nanofibers and Hybrid Technique)

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ARTICLE INFO

Keywords:
Nanotechnology
Nanofibers
Nanoparticles
Tissue Engineering
Additive Manufacturing
Biofabrication
Electrospinning
Dual-Scale
Organ Printing

ABSTRACT

This review proposes to present how materials at nanolevel scale can contribute to the development of three-dimensional (3D) structures, human tissues, and organs which have macrolevel organization. Specific nanomaterials such as nanofibers and nanoparticles are presented and discussed in their application for biofabricating 3D human tissues and organs. The concept of self-assembling magnetic tissue spheroids as an intermediate mesolevel structure between nano and macrolevel organization and building blocks for biofabrication in dual scale level of complex 3D human tissues and organs is detached. The challenges and perspectives of employing nanomaterials and nanotechnological strategies in the biofabrication were also traced.

1 Introduction

It is essential to understand how recent advances in nanotechnology on nanolevel can enable and enhance progress in organ biofabrication on a macrolevel.

Biofabrication could be defined as an application principle of engineering and information sciences for automated robotic bioassembly of living 3D human tissue and organs (Mironov *et al*., 2009a; Guillemot *et al.*, 2010; Hinton *et al.*, 2017). In other terms, biofabrication is a biomedical application of additive manufacturing technology or computer-aided additive fabrication (Bracaglia *et al.*, 2017). The concept of biofabrication was reappraised and redefined by Groll *et al.* (2016) as the automated generation of biologically functional products with the structural organization of living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bioassembly and subsequent tissue maturation processes.

The emerging biofabrication research field is focused on using robotic automated engineering approaches in tissue bioassembly. Additive Manufacturing has transformed the global industry since automation and robotic techniques started to be employed with very high accuracy and repeatability. Further the hardware, software incorporates algorithms for optimizing the process assuring high quality products.

The recent state of tissue engineering and the biofabrication could be compared to the situation of the microelectronic industry before and after the introduction of automated robotic technologies for fabricating microchips and microprocessors. The robotization and automation helped to convert emerging promising technologies into economically feasible industries. Moreover, it is logical to predict that industrial scale engineering of complex human organs is practically impossible without advances in robotization and automation of biofabrication process.

Unquestionably, nanotechnology is one of the most relevant and rapidly emerging technologies of the XXI century (Roco, 2003). During the last decade, the exponential growth of nanotechnology applications in the area of tissue engineering had been strongly observed (Gyles *et al*, 2017; Rabionet *et al.*, 2017). Concerning published contents devoted to tissue engineering field in the last ten years, the number of publications has been also dramatically augmented (Mironov *et al*., 2008a,b; Mironov *et al*., 2009a,b; Lionetti *et al*., 2011; Rezende *et al.*, 2012; Perán *et al*., 2012). A quick systemic analysis consult on

ARTICLE HISTORY:
Received 10 September 2017; Received in revised form 03 October 2017;Accepted 26 January 2018
Available online 20 February 2018

PUBMED online database regarding the terms "nanotechnology" and "tissue engineering" showed that the number of publications of these fields is crescent over the years (Fig. 1).

The most considerable and non-trivial related to the interface of tissue engineering and nanotechnology is: "how can the use of materials (on nanolevel) enable biofabrication of human organs (on macrolevel)? ".

One of the most important points in the hybrid process is the wide range of usable materials. The materials can go from metals, ceramics, polymers (natural and/or synthetics) until composites mixing these groups. Looking at the biofabrication aspects ceramics and mainly polymers are highly used. On hybrid processes involving a 3D bioprinter and electrospinning as main topics in this paper, in general, the most common materials applied are alginate, collagen, chitosan, starchs as being the natural ones plus PCL (poly caprolactone), PLA (poly lactid acid), PLLA (poly L-lactic acid), PDLA (poly D-lactic acid), PLGA (poly lactide-co-glycolide) as the synthetic ones.

Therefore, the objective of this review is a presentation of how the most recent advances in the application of nanomaterials in tissue engineering can enable the robotic and automated biofabrication of 3D human tissues and organs which have macrolevel organization. The challenges and prospects of nanomaterials application in tissue engineering and nanotechnological strategies in organ biofabrication will also be discussed.

We would like especially to emphasize that this is not a comprehensive review about nanotechnology in tissue engineering, but rather focused attempt to explore the emerging potential of applications of nanotechnology to predominantly organlevel of tissue engineering or, more specifically, for organ biofabrication and how this nanoscale contribute to createthese three-dimensional structures that have a macroscale.

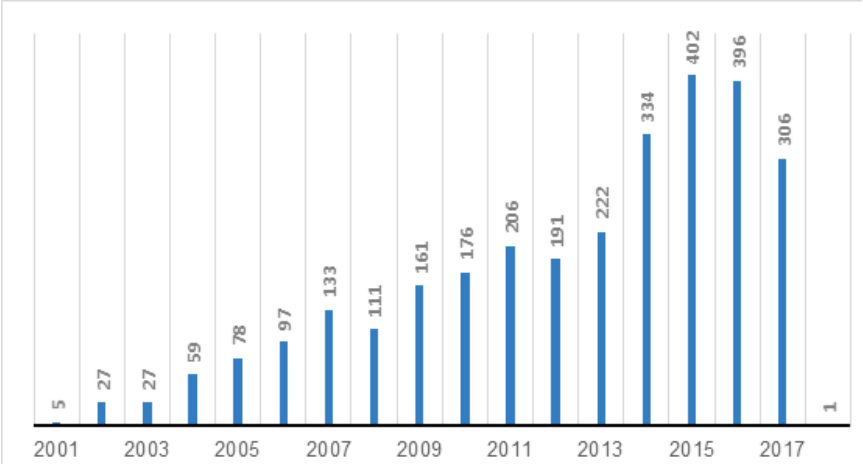

Figure 1 - A chart illustrating the times the terms "nanotechnology and tissue engineering" appear on PUBMED database

2. Nanofibers

The electrospinning technique has appeared as a relatively simple and scalable nanotechnological method for the generation of nanostructured scaffolds (Hidalgo *et al.*, 2013).

Electrospinning allows controlling the diameter of the spun fibers and can produce nanofibers and scaffolds that mimic the nanostructure of natural extracellular matrices (ECMs), as confirmed by a more efficient vascular cell attachment and spreading (Lee *et al*., 2007).

The general aspects of the electrospinning technology have been substantially reviewed elsewhere (Murugan and Ramakrishna, 2006; Barnes *et al.*, 2007; Murugan *et al*., 2007a; Mironov *et al*., 2008a,b,c, Hidalgo *et al.*, 2013; Braghirolli *et al.*, 2014). Considerable attention will be given on the usage of the electrospinning technology in the field of vascular tissue engineering (Kitsara *et al.*, 2017). A wide range of synthetic polymers has been successfully applied in the electrospinning technique. Furthermore, electrospinning of natural proteins, such as collagen and elastin, either alone or as in blends with synthetic polymers, has been published (Boland *et al*., 2004; Lee *et al*., 2007; Aguirre-Chagala *et al.*, 2017). However, the reported stability of vascular scaffolds created by electrospinning of merely natural proteins had not reached desirable levels yet (Ma *et al.*, 2008).

Although dense nanofiber meshworks provide excellent conditions for cell attachment and the spreading of endothelial cells on the luminal surface of the scaffold (Bondar *et al*., 2008), they also preclude effective cell migration into the scaffold and thus impede smooth muscle cell migration and the sequential formation of muscular layers inside the vascular tissue engineered constructs. Electrospinning allows the fabrication of a wide variety of nanofibers and nanostructured scaffolds with special characteristics and functionalities. The obtained nanofibers vary in shape, size and composition: they can be solid, composite, hollow, porous, decorated, helical and branched. This diversity of possible electrospun nanofibers offers interesting opportunities for the enhancement of vascular-scaffold functionality. For example, the hollow nanofibers and nanoshells created by a coaxial extruder, as well as composite-coated or decorated

auricular implant. Another promising direction is using drug-eluting nanofibers which will enable fabrication cost-effective cell-free hybrid dual scale scaffolds with optimal mechanical properties, superior biocompatibilities, and capacities for induced endogenous regeneration. The cell-free complaint electrospun scaffold has been shown to be promising in case of vascular tissue engineering (Wu *et al.*, 2012). Drug-eluting electrospun vascular scaffold has been also reported recently (Innocente *et al.*, 2009). It has been shown recently that cell-free bioprinted drug-eluting scaffold can induce the formation of cartilage *in vivo* (Lee *et al.*, 2010). Thus, cell-free drug-eluting dual scale biomechanically optimal and biocompatible scaffold fabricated with using hybrid FDM and electrospinning technology with capacities for induced endogenous regeneration could lead to the development of economically cost-effective implants and scaffolds. In case of using cell-free scaffolds or even non-biodegradable complaint and biocompatible implants fabricated by above described hybrid dual scale scaffold technology, it will be much easier to get required regulatory approval, and an accelerate desirable clinical and commercial translation.

Moreover, the other significant advantage that is achieved with this type of hybrid system is that different types of materials can be mixed, and 3D structures can be created where layers of materials can be deposited using the two techniques, that is, it can be used the material for FDM deposition and another for electrospinning. Also, a layer can be made by FDM and then place a layer using electrospinning and create sandwich-like structures, which may even allow obtaining systems with different rates of degradation (Fig. 4).

Other advantages that have been experienced through electrospinning are the incorporation of nanoparticles of inorganic compounds such as hydroxyapatite (HA), or metal particles such as silver (Ag). In the polymer solutions, these nanoparticles are dispersed, for which use can be made of some dispersing agent that does not allow their agglomeration, and they are dispersed through the fibers. In addition, given its nature, it is possible to increase the conductivity of the solution and achieve a good integration of the particles inside the fibers, as shown in Figure 5. In this figure, it can observe the external morphology of the fibers by SEM and the inner of these fibers by TEM showing the presence of the nanoparticles (HA and Ag) distributed around the fibers. This is evidence that through this technique, there are many hybrid type systems that can be obtained, in addition to the possible incorporation of drugs or biomolecules in the inner of the fibers as evidenced by several scientific papers reported (Balogh *et al*, 2015).

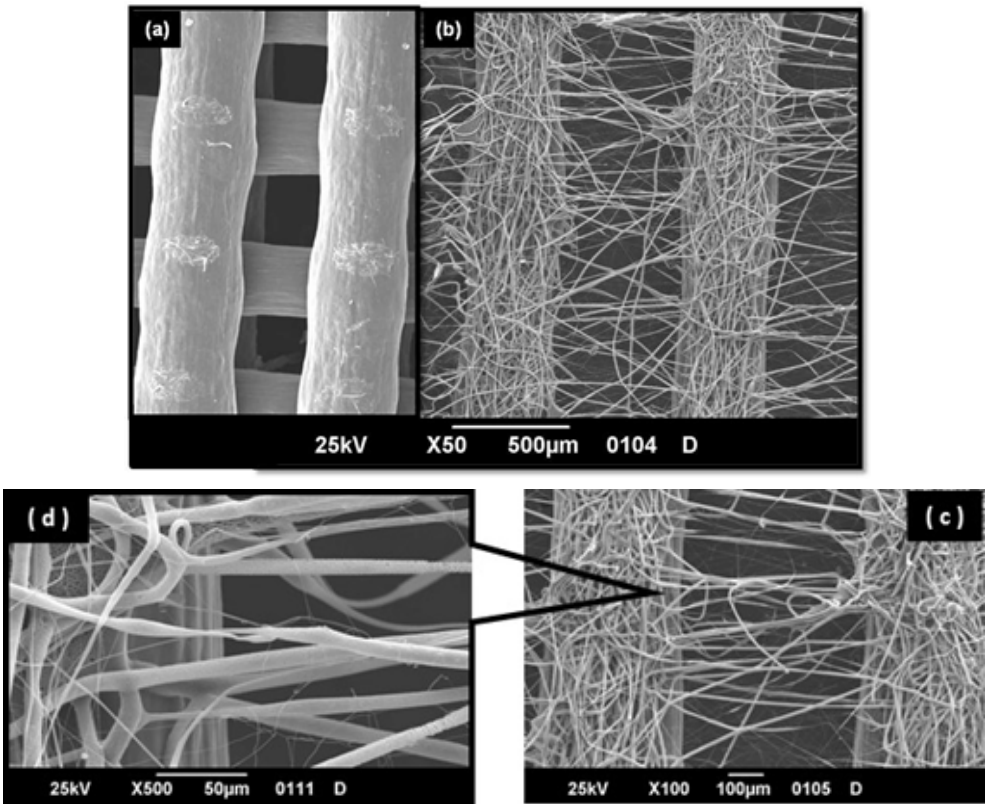

Figure 4 - SEM micrographs of the double scale scaffold. (a) PCL 3D scaffold obtained by FDM, where macropores are shown. (b) Image of the combined system after deposition of PLA (poly lactic acid) micro and nanofibers using electrospinning. These filaments show a preferred orientation and cover macropores created by Additive Manufacturing (FDM technique). (c) and (d) details show the good adhesion of the filaments deposited by electrospinning on the previously created scaffold by FDM. These structures allow not only the combination of scaffolding techniques, but also the combination of polymers. (Images supplied by B5IDA research group, Universidad Simon Bolivar, Venezuela).

nanofibers, could provide additional functionalities, including the capacity to release oxygen and to present growth factors and RGD peptides (Zhu *et al.*, 2017).

However, the full potential of electrospinning for the engineering of the full array of nanofibers with different functionalities remains to be explored. Significant progress has already been made related to the control of the fiber orientation (Murugan and Ramakrishna, 2007b), which is an important step toward the rational design of biomimetic vascular scaffolds. Nevertheless, in our opinion, controlling only the orientation of the nanofiber will probably not be enough. The recapitulation of the entire matrix architecture and the non-linear biomechanical behavior of the natural vascular wall are dramatically crucial. The most exciting advance in electrospinning is the successful one-step rapid fabrication of a vascular scaffold with integrated living cells (Stankus *et al.*, 2006). In these studies, the previously reported methods for the encapsulation of living cells were combined with the electrospinning of nanofibers into one procedure. Further optimization of this electrospinning strategy might offer the greatest potential for rapid biofabrication of vascular-tissue constructs and might eventually eliminate the need for time-consuming and expensive bioreactor-based cell seeding and scaffold cellularization. Although this impressive progress in vascular tissue engineering with the help of innovative electrospinning technologies has occurred, rapid cell integration into scaffolds and their optimal mechanical properties remain the main defiance.

The nanostructured electrospun matrices are an excellent substrate for fabrication of transplantable cell monolayer due to the biomimicking character of nanofibers; moreover, the resulted optimal conditions for cell attachment and spreading could compete with scaffold-free cell sheet technology (Hidalgo *et al.*, 2013). The potential functionalization of electrospun matrices and their transformation into drug-eluting scaffold provides another advantage (Zhan *et al.*, 2017).

On the other hand, dense electrospun nanostructured matrices usually do not present optimal properties for the cell invasion and effective cell seeding. Theoretically, it is possible to fabricate matrices with larger pores using additional spinning of sacrificial fibers or cryo-electrospinning (Kamoun *et al.*, 2017). However, the attempts to increase pore sizes and make adequate electrospun matrices for cell invasion and cell seeding could negatively impact on the material properties of matrices. The efficient solution of these two interdependent problems represents the main challenge in development and clinical translation and commercialization of electrospun technology in tissue engineering. The hybrid composite approach opens possible solution in the fabrication of electrospun matrices with desirable natural-like material properties. The development of electrospinning apparatus with two nozzles (for example, one for fabrication collagen mimicking nano and microfibers and one for fabrication elastin mimicking nano and microfibers) in combination with modified fiber collector with changeable diameter will allow fabricating wavy collagen mimicking nanofibers and biomechanically compliant composite vascular graft.

Coaxial and rotational electrospinning allows the biofabrication of a new vascular graft (Fig. 2). This type of equipment not only allows mixing different types of polymers, or to place layers of different types of polymers, but also allows obtaining different rates of degradation

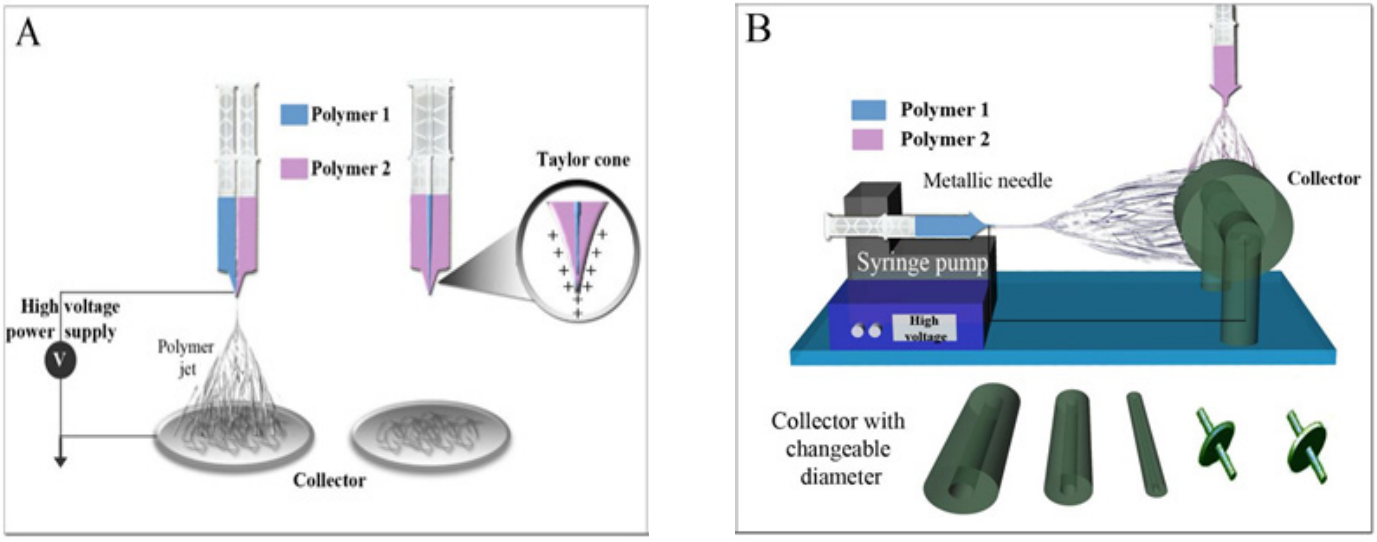

Figure 2 - (A) Schematic of side-by-side nozzle configuration. Two capillaries containing different polymer solutions are set side-by-side. As long as the two solutions have similar conductivities, a single Taylor cone will be developed, and a fiber jet containing both polymers will be produced. However, the relative amounts of each polymer can vary along the electrospun fiber. Also, schematic of coaxial nozzle configuration. A smaller capillary is set inside a larger capillary such that the long axes of the capillaries coincide. Different polymer solutions are passed through each capillary. At the tip of the capillaries the Taylor cone is formed and leads to the formation of fibers in which one polymer fiber is encapsulated within another (known as a core-shell morphology). (B) The rotational collector can be used with a unique polymer solution, but also permits to have two (or more) nozzles with different polymer solutions.

as well as the possibility of encapsulating different types of molecules (drugs, biomolecules, etc.). For example, the development of compliant composite drug eluting athrombogenic vascular graft with capacities of recruiting cells *in vivo* is one of the most promising approaches in vascular tissue engineering. Rolling, stacking and folding including cell-driven self-folding as a concept of 4D bioprinting (Li *et al.*, 2016; An *et al.*, 2016; Gao *et al.*, 2016) of electrospun matrices offer interesting opportunities for designing and engineering tissue engineering constructs of complex geometry (Cheng *et al.*, 2017, Mokhena and Luyt, 2017).

3. Hybrid fused deposition modeling and electrospinning nanofibrous technology

Combination of electrospinning with fused deposition modeling (FDM) opens a new opportunity for designing dual scale scaffold suitable for biofabrication of 3D tissue and organs (Fig. 3). Electrospinning dues to the biomimetic character of nanofibers provide superior matrices for cell attachment and biocompatibility, but material properties of electrospun matrices are inferior, and capacities for engineering 3D tissue constructs are limited. FDM allows fabricating 3D tissue engineered scaffolds of desirable patient-specific 3D shape and provides superior biomimetic material properties and better mechanical properties, but due to the large size of pores, the cell seeding is usually not optimal or time-consuming. Hybrid FDM and electrospinning dual scale scaffold technology eliminates limitation of both technologies and provides desirable synergistic beneficial effect (Park *et al.*, 2008).

One of the possible applications of hybrid technology is the fabrication of an auricular implant with optimal biomimetic material properties and enhanced biocompatibility. Patient specific computer-aided design of, for example, an auricular implant could be developed based on using laser scanning of the external ear (Wen *et al.*, 2008) and customized auricular implant could be fabricated by additive manufacturing technology such as a FDM and electrospinning will enhance the biocompatibility of the

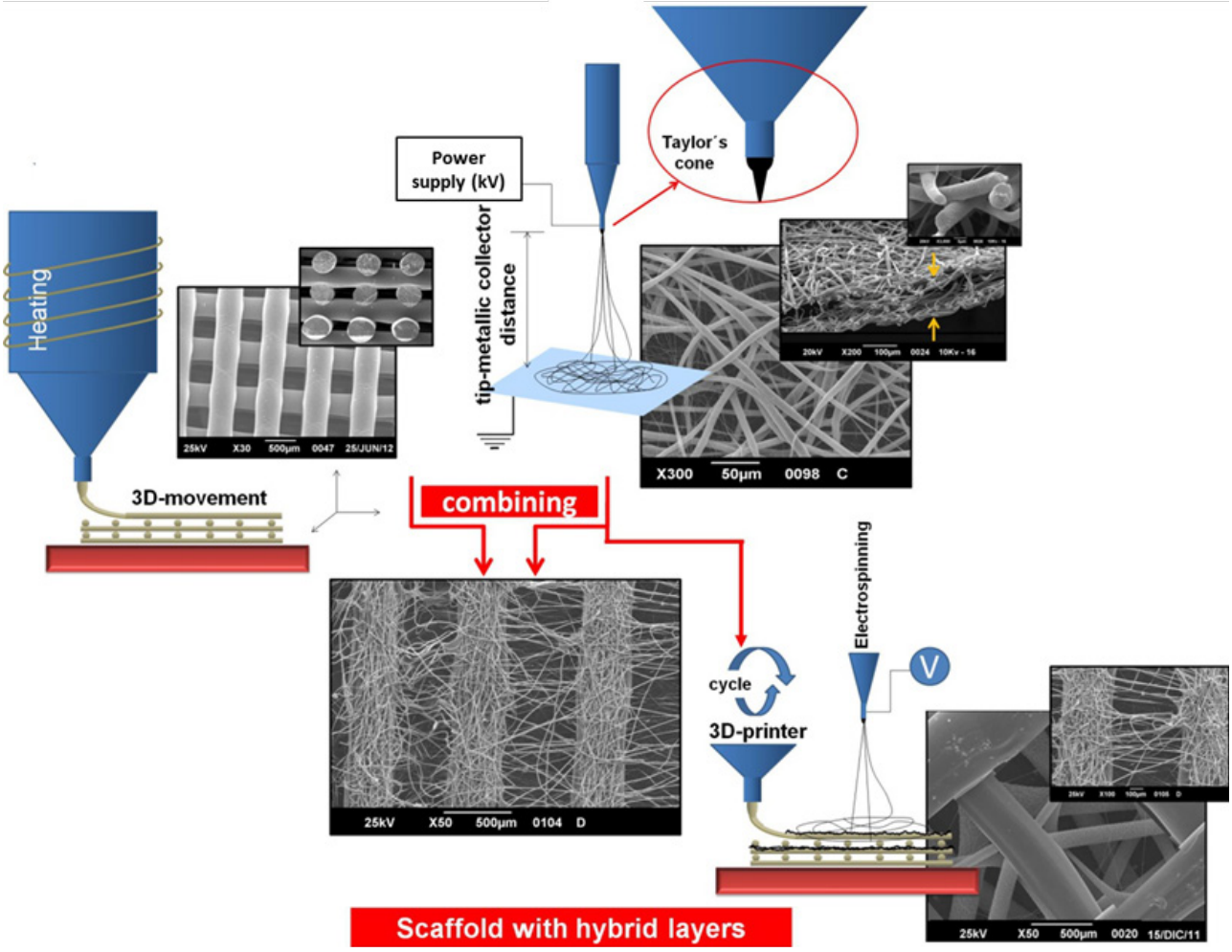

Figure 3 - Proposal of 3D dual scale polymer scaffolds (or hybrid scaffolds) produced by FDM 3D additive manufacturing technology and electrospinning (SEM micrographs supplied by B5IDA research group, Universidad Simon Bolivar, Venezuela) (Figure adapted from Sabino *et al.*, 2017).

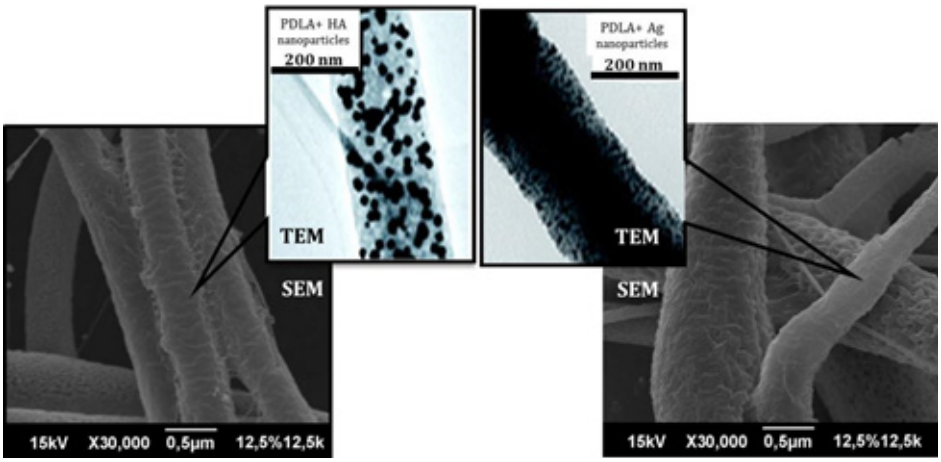

Figure 5 - Micrographs from Scanning and Transmission Electron Microscopy (SEM and TEM) of micro/nanofibers of PDLA (polymer D-lactic acid) charge with nanoparticles of hydroxyapatite (HA) and silver (Ag), obtained using electrospinning (images supplied by B5IDA research group, Universidad Simon Bolivar, Venezuela).

4. Conclusion and Future Perspectives

Nanotechnology applied in the tissue engineering field is rapidly evolving an ongoing process. As we tried to illustrate in this review paper, there is also strong potential for successful application of nanotechnology in human organ biofabrication which has macrolevel organization.

Nevertheless, the researchers on the intersection between the nanotechnology and the tissue engineering are still encountering some serious barriers. The first and most evident one is the toxicology of the nanomaterials.

Thus, the biocompatibility of nanomaterials for tissue engineering application must be seriously taken into account. The second challenge concerns the functionalization of the nanomaterials. Recent progress in designing drug-eluting nanofibers using coaxial electrospinning is a suitable example of evolution.

The third challenge is related to the design, synthesis and production of novel nanomaterials with biomimetic aspects. This trend is already evident in the fabrication of nanofibers using electrospinning technology. The development of composite nanomaterials is one of the most promising approaches in this direction.

The fourth challenge is the standardization of nanomaterials. In a wider view, to the achievement of the product certification, it is extremely necessary the standardization on certain stage of development of any emerging technology.

In the future, perspective functional biomimetic nanomaterials can enable regenerative medicine that means healing from inside and therefore decrease the necessity for tissue engineering *ex vivo*. Drug-eluting electrospun vascular graft is one possible example of this tendency.

Acknowledgments

The authors are grateful for The National Council for Scientific and Technological Development (CNPq) support by means of Regenerative Medicine project (CNPq 467643/2014-8), INCT-REGENERA (CNPq), and CTI Renato Archer CNPq PCI Program, further CERTBIO/ UFCG, Universidad Simón Bolívar and 3D Bioprinting Solutions.

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Journal homepage: http: www.journalamb.com/index.php/jamb

The II International Symposium on Medicinal Chemistry and Regenerative Medicine, held from November 22nd to 24th at, this event was directed to undergraduate and graduate students, professionals from the medical, pharmaceutical, biotechnology innovation management and entrepreneurs, as well as researchers involved in the areas of Medicinal Chemistry, Regenerative Medicine and Biotechnology.

The event aimed to promote the dissemination of new research and innovations that are at the frontier of knowledge in the area of Regenerative Medicine and Medicinal Chemistry and also to promote interaction with companies interested in these researches. Thus, as a result of the event, it is hoped to encourage discussion, sharing of knowledge, articulation of partnerships for new research projects and also generate a spark of ideas that can be led by future entrepreneurs.

In this second edition, a scientific session was held with the presentation of posters. The abstracts submitted and approved by the scientific committee are below.

Central themes

- Biopolymers
- Medicinal Chemistry
- Regenerative Medicine
- Innovation Management on Biotechnology

Biopolymers - Abstracts

International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Luminescent chitosan/sodium tripolyphosphate nanoparticles modified with [Eu(TTA)3(Bpy-si)] complex as new biomarkers

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ARTICLE INFO

Keywords:
Chitosan/sodium tripolyphosphate nanoparticles
Silylated Europium(III) complex
Luminescent biomarkers

ABSTRACT

Introduction and objectives: Chitosan (CS) is a versatile, biocompatible, non-toxic biopolymer extremely abundant in the biomass obtained by alkaline deacetylation of chitin, easily found in the exoskeleton of crustaceans, and in some types of fungi and marine squid. CS has been widely studied by the pharmaceutical industry as a potential agent for the healing of bones and tissues, in treatments for weight reduction and cholesterol, as antimicrobial agent and suitable matrix for coordination compounds as lanthanides ions aiming applications such as cell markers. In this way, the development of new coordination luminophores non-toxic and stable with good luminescence properties even in small concentrations is still a big challenge. In this work, we described the ionic gelation technique as method to obtain Chitosan/TPP nanoparticles (CS/TPP) modified with luminescent silylated [Eu(TTA)3(Bpy-Si)] complex. **Materials and Methods:** All nanoparticles were prepared by ionic crosslinking of CS dispersions with TPP aqueous solutions, according to the ionic gelation method. The CS solutions were prepared by dispersion of 30 mg of CS in 10mL of acetic acid solution 0.1M at room temperature and mechanical stirring. NaOH aqueous solution 1M was used to increase the pH up to 4.4. After that, TPP solution (10 mg TPP in 10 mL of water) was added dropwise to 10 ml of CS dispersion under stirring at room temperature for 1h. Simultaneously, 3 mg of [Eu(TTA)3(Bpy-Si)] complex were dissolved in 3 mL of water and added dropwise to CS/TPP solution under stirring at room temperature for 1h. Finally, the CS/TPP@[Eu(TTA)3Bpy-Si] obtained was dialyzed for 48h. **Results:** The nanohybrids were characterized by FTIR, SS-NMR and FE-SEM techniques that confirm the incorporation of the [Eu(TTA)3Bpy-Si] showing particles with average size of 84 nm. Luminescent properties were evaluated and the intensity parameters were calculated. These results showed that after luminophor grafting onto nanoparticles there was no significant decrease in its luminescent properties indicating that chitosan/TPP nanoparticles can be used as a good biomatrix. The luminescent nanohybrids suspension was evaluated in B16F10 cells strain by epifluorescence microscopy. By excitation at 470 nm, red emission of these nanosystems in the cell nuclei can be observed. **Conclusions:** Preparation of CS nanoparticles and CS@[Eu(TTA)3Bpy-Si] were successfully performed and confirmed by FTIR, SS-NMR, FE-SEM and luminescent measurements. The complex incorporation into the biomatrix was not affected by the acid pH of the CS nanoparticles. The choice of the CS as biomatrix appearing is a good choice to maintain the luminescent properties of the complex. CS@[Eu(TTA)3Bpy-Si] as new biomarkers was confirmed by Epifluorescence microscopy in melanoma cells showing red emission in cell nucleus region.

Financial support: CAPES

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Orodispersible films based on gellan gum and cashew gum intended for insulin administration: evaluation of transparency and erosion

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ARTICLE INFO

Keywords:
Orodispersible films
Cashew gum and gellan gum

ABSTRACT

Introduction: Orodispersible films (FOD) are intended for administration of drugs into the oral cavity. These films can be developed from polymeric materials, such as cashew gum (GC) and gellan gum (GG), since these materials show high mucoadhesive properties and rapid disintegration when placed in the oral environment. The development of these GG / GC-based FODs is in agreement with the need for an alternative route for the treatment of *Diabetes melittus*, since the subcutaneous route of insulin (INS) administration is an invasive route, causing discomfort, pain and local inflammation, decreasing patient adherence to treatment. The administration of INS through the FOD overcomes the drawbacks related to the enzymatic degradation of INS in the gastrointestinal tract, as well as its low intestinal permeability. **Objectives**: To analyze the degree of transparency of GG / GC FODs and its disintegration in artificial saliva. **Materials and Methods:** Polymeric films were obtained by the solvent casting method from GG: GC dispersions obtained in different proportions (1:2,5, 1:5, 2,5:2,5 and 2,5:1) and labeled as 1G / 2.5C, 1G / 5C , 2.5G / 2.5C and 2.5G / 1C, respectively. The FOD transparency measurements were performed on a Varian Cary 500 UV-Vis spectrometer at 200-800 nm interval. For the erosion test the FOD were cut and accurately weighed and then placed on the bottom of beakers containing artificial saliva solution at 37 ° C with 50 rpm stirring for 30 minutes. After the immersion time, the films were removed, oven dried at 60°C for 48 hours and weighed. Erosion was calculated gravimetrically. **Results:** The transparency measures in the visible region of the spectrum revealed that the films presented transmittance percentage ranging from 60 to 70%. The spectra showed that films that had a higher CG concentration had a lower transparency, which was attributed to the fact of GC did not completely disperse, blocking the passage of light. Likewise, films with a lower CG concentration had greater transparency as a consequence of a lower restriction on the passage of light. In the erosion test performed in simulated saliva solution, the 1G / 2.5C sample presented a lower percentage of erosion (19%), which is probably related to the higher concentration of GC, and it could be observed that in the 2.5G / 1C sample had a higher percentage of erosion (56%), with a lower GC concentration. **Conclusion:** We conclude that the degree of transparency of the FOD is a promising property, since it is a transparent solution and favors an application of the material in the biomedical area. According to erosion data, the 2.5G / 1C sample was more appropriate to use the FOD, since it presented a higher percentage of erosion in artificial saliva solution. Erosion testing is extremely important, since erosion also leads to a faster release of the incorporated drug, for mucoadhesive systems may be a good strategy for increasing permeation.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Antimicrobial biomaterial based on polysaccharide

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ARTICLE INFO

Keywords:
Gum
Biomembranes
Anti-Staphylococcal Activity

ABSTRACT

Introduction: The manufacture and use of biodegradable biomembranes from polymer with incorporation of bioactive drugs or molecules has aroused industry interest for the manufacture of dressings used in wounds and sutures. Among these polymers, polysaccharides like gums stand out because of the property of forming films or membranes. However, these polysaccharides are water-soluble and need to have improved physical properties for such an application. **Objectives:** With the current challenge of developing new biodegradable matrices for drug incorporation, this work has the objective of making membrane for application as an antimicrobial dressing. **Materials and Methods:** Three types of modified polymer were developed and characterized by FTIR, elemental analyses. **Results:** Characterization by FTIR showed a presence of bands between 1560 and 1490 cm^{-1}, characteristics of amines functional groups. In addition, the modified gum exhibits anti-staphylococcal activity.

Financial support: FAPESP-PPSUS

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Assessment of mutagenicity of polymer films of Allium Cepa L. With application for food packaging

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ARTICLE INFO

Keywords:
Food packaging
Mutagenicity
Ames test

ABSTRACT

Introduction: Nowadays, most of the food packaging systems are based on petroleum-derived synthetic plastics, whose production has increased exponentially over the past two decades. Nevertheless, the utilization of these materials involves a serious environmental problem and high recycling costs. In order to deal with this issue, current research focuses on the development of biodegradable materials from renewable sources. So, polymer films of *Allium cepa* L. are being produced for this purpose. However, because it touches the food and chemicals can migrate into the food, their safety should be regulated. **Objectives:** Thus, the aim of the present study was to determine the mutagenic effects of the onion-based washed and not washed films (*Allium cepa* L.) with application for food packaging. **Materials and Methods:** The films were produced in BioSmart Nanotechnology Company and gently donated by Dr. Diógenes dos Santos Dias. Mutagenic activity was evaluated by the *Salmonella*/microsome assay (Ames test), using the *Salmonella typhimurium* tester strains TA98 and TA97a (detect frameshift mutations), TA100 (detect base-pair-substitution mutations) and TA102 (normally used to detect mutagens that cause oxidative damage and base-pair-substitution mutations), with (+S9) and without (–S9) metabolization, by the preincubation method. **Results:** The results showed only signs of mutagenicity of the not washed films with the largest mutagenic indexes of 1.9. The washed films did not induce an increase in the number of revertant colonies relative to the negative control, indicating absence of mutagenic activity, under the conditions used. **Conclusion:** The detection of genotoxicity is highly advisable, so as to avoid the risk of genotoxic exposure to mutagens and carcinogens. These results contribute to valuable data on the safe use these materials for commercial purposes. However, further investigations exploiting mutagenesis mechanisms should be conducted.

Financial support: Uniara and Prosup (Brazil)

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Citotoxicity and mutagenicity studies of tempo-oxidized cellulose nanofibers

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ARTICLE INFO

Keywords:
Biomaterials
Cellulose
Nanofibrillar cellulose
Mutagenicity
Cytotoxicity

ABSTRACT

Introduction: Nanocellulose is described as a product or extract of a native cellulose composing a material with a nanomeric structure. Despite being considered the most attractive renewable material for advanced applications due to its unique physical and mechanical properties, little is known about the mutagenic potential and cytotoxic effects of nanoscale cellulose. Thus, the evaluation of mutagenicity and cytotoxicity in substances with promising applicability within tissue engineering, such as cellulose, is necessary owing to the fact that further tissue damages can be avoided. **Objectives:** Therefore, the objective of this study was to evaluate the mutagenic activity of TEMPO-oxidized cellulose nanofibers (ToCNF) by the Ames test, a widely used assay to detect mutations at the gene level, and its cytotoxic potential by the MTT assay. **Materials and Methods:** ToCNF was produced in UNIARA's BioPolMat laboratory and gently donated by Prof. Eliane Trovatti, PhD. In this study, the Ames test was performed with changed strains of *Salmonella typhimurium* (TA98, TA97a, TA100 and TA102) and for the MTT assay were employed a normal cell line (GM-07492) and a cell line with metabolism profile of carcinogens (HepG2). **Results:** According with the results obtained in this report, the modified cellulose did not induce any statistically significant difference neither in the number of revertant colonies of *S. typhimurium* nor of the cell viability when compared to their respective negative controls in both experiments. **Conclusion:** The absence of mutagenicity and cytotoxicity is extremely relevant because it provides reliable data to support future clinical researches. However, further toxicological tests are needed to ensure its safe use.

Financial support: Uniara and Fapesp (Brazil).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Rheological behavior of sodium alginate hydrogel containing bacterial cellulose

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ARTICLE INFO

Keywords:
Bacterial cellulose
Hydrogel
Sodium alginate
Rheology

ABSTRACT

Introduction and objectives: Hydrogels are three-dimensional hydrophilic networks with capable of absorbing large quantities of water or biological fluids. Hydrogels are biodegradable and biocompatible with long-term stability, ease of biochemical modifications of formed structures, and enables the incorporation of organic/inorganic products. Various polymeric materials are used to form hydrogels; however, the use of polysaccharides, such as the use of alginate hydrogels has been increased due to the biocompatibility, biodegradability, immunogenicity, and non-toxicity properties. The possibility of incorporation of bacterial cellulose (BC) nanofibers into alginate hydrogels is very interesting in several areas of knowledge, such as medicine, since the structure of BC is a viable matrix to assist the treatment of dermal lesions and it has been used as a temporary substitute for skin, burns, ulcers, grafts, as a wound cover and to aid in dermal abrasions. The aim of this work was to develop alginate hydrogels containing BC nanofibers and also investigate the rheological behavior of the developed hydrogels which impact when applied to the skin. **Materials and Methods:** Hydrogels of sodium alginate with calcium were developed using different polymeric materials and also it was incorporated different concentrations (0.5% and 1% in relation to the dry mass) of BC. Rheological behavior was evaluated with parallel plate rheometer (Anton Paar) was used to measure the complex viscosity (η*) the storage modulus (G') and the loss modulus (G") as a function of frequency. Tests used 25mm diameter plates at a temperature of 32°C. The range of frequencies used was 0.01 to 500 rad/s[1]at 2%strain, which proved to be in the linear viscoelastic range according to a prior amplitude sweep test. The gap between plates was 1.00 mm. Flow curve was also analyzed with shear rate range from 0 to 100Pa / s for the ascent ramp for 120 seconds and from 100 to 0Pa /s for the descent ramp for 120 seconds. **Results and Conclusions:** The results obtained in this study demonstrate that alginate hydrogels with CB presented a thixotropic behavior, facilitating the application of the product under the skin with a pleasant sensorial. Results of the and frequencys weep shows that Alginate/BC hydrogels has adequate interaction among the components indicating a stable structure which makes it difficult to separate the constituents of the formulation besides being indicative of greater stability of these hydrogels.

Financial support: SevenIndústria de produtos biotecnológicos Ltda.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Cytotoxicity, antimicrobial activity and morphology of bacterial cellulose with chitosanfilm loaded with ciprofloxacin

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ARTICLE INFO

Keywords:
Bacterial cellulose
Chitosan
Wound healing

ABSTRACT

Introduction and objectives: Treatment of skin lesions is a great clinical importance, justifying the high investments in new products that reduce the time of healing and increase the patient comfort. As a result, different products and patches are market, however, most are imported and make the treatment expensive. In this context, biopolymers gain prominence in the industry due to its efficient, abundant and low cost, such as bacterial cellulose and chitosan. In the present work, the objective was to analyze the cytotoxicity, antimicrobial property and morphology of the film produced by bacterial cellulose and chitosan associated with ciprofloxacin. **Materials and Methods:** Cytotoxicity assay was performed by the MTT reduction method ({3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide), which allows measuring the amount of viable cells based on the principle reduction of MTT salt by mitochondrial enzymes to formazan (ISO 10993-5), in human fibroblast cells GM07492.Inhibition halos against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were determined by using modified disk diffusion method according to international clinical standards (CLSI/NCCLS), replacing disks for empty and ciprofloxacin loaded BC/chitosan films in the agar plate surface (Mueller Hinton agar) and inoculated with bacteria (0.5 McFarland scale). SEM experiments were carried out using samples previously coated with evaporated carbon. The images were obtained using the JEOL T-300 microscope operating at 2 kV. **Results:** Cytotoxicity assay demonstrated that the negative control group and CB determined the same pattern of cell viability, evidencing the absence of toxicity from the extract of the CB membranes analyzed. Ciprofloxacin loaded BC/chitosan samples exhibited a significant but slight decrease in the metabolic activity of cells. In contrast, it does not characterize a cytotoxic influence, considering the percentage of viability exhibited in the analyzes (greater than 80%).Antimicrobial activity tests, the ciprofloxacin loaded BC/chitosan film demonstrated activity for both bacteria tested, in evidence against *Pseudomonas aeruginosa* that showed higher activity than against *Staphylococcus aureus*. The results also showed the antimicrobial activity of chitosan against *Pseudomonas aeruginosa*, evidenced in the small inhibition halo formed when inoculated the BC/chitosan film, without ciprofloxacin. Analysis of SEM images revealed surface and transversal section morphology of films. The surface was homogeneous and characteristic of chitosan. From the transversal sectional images, was observed the chitosan in the center of film immersed in the bacterial cellulose membrane, and at the extremities of the film. **Conclusions:** Generally, the film did not prove to be toxic, in addition, it presented antimicrobial activity against the tested microorganisms and had its morphological structure characterized. Presented research work will open new prospect for the development of composites that could be used as wound dressing and them potential applications in tissue engineering.

Financial support: CAPES.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Effect of palygorskite clay on the release properties of metronidazole from bacterial cellulose membranes

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ARTICLE INFO

Keywords:
Palygorskite
Metronidazole
Bacterial Cellulose

ABSTRACT

Introduction and objectives: Bacterial cellulose (BC) is synthesized by different species of bacteria and shows many advantages in relation to plant cellulose. In pharmaceutical field, BC has been successfully exploited in the design of controlled drug delivery systems due to its well-organized 3D network of fibers. Although the highly porous structure of BC can be used successfully for the preparation of new nanocomposites, it promotes the fast release of the drug in a short time (burst release), which can cause the side and toxic effects, constituting a pharmacologically dangerous and economically inefficient. In order to overcome this drawback, the aim of this work was to explore the high adsorptive capacity of paligorskite (PAL) due to the high density of silanol groups on the external surface and high internal area as a strategy for the effective entrapment of the metronidazole (MTZ). PAL is a natural clay that presents fibrous morphology with 2:1 crystalline structure. **Materials and Methods:** PAL was dispersed in an aqueous MTZ solution (10 mg/mL) in different proportions (1:1, 7:1 and 15:1) under magnetic stirring at 750 rpm, 30 °C for 72 h. BC membranes synthesized by *Komagataeibacter rhaeticus* in Hestrin and Schramm culture medium were allowed to swell in PAL-MTZ solutions until complete absorption. *In vitro* MTZ release was carried out using USP type V dissolution apparatus (paddle over disk) in a Hanson Research (New Hanson SR-8 Plus) dissolution station, using phosphate buffer (0.1M; pH 6.0) according to sink conditions, at 37 ± 0.5 °C under 50 rpm rotation speed. **Results:** Release profiles showed that MTZ directly incorporated in BC (0 % PAL) depicted a burst effect of release (62%) in the first 30 min, which is probably attributed to the high porosity of BC, as well as to the drug molecules adsorbed on the membrane surface, allowing their free diffusion. After 180 min of test, 82 % of MTZ was released. Samples containing 1:1 and 7:1 PAL:MTZ were not effective in the release control, showing a release profile similar to that of the control sample. However, the sample prepared with the highest ratio of PAL (15:1) allowed the prolongation of release rates, so that after 180 min of test, only 60 % MTZ was released. According to the kinetic study, the mathematical model that best correlated was that of Weibull, with parameter b>1, revealing that the MTZ release was governed by a complex mechanism, involving diffusion, swelling and erosion. **Conclusion:** The set of results indicate that the strategy proposed to overcome the fast release of drugs from BC matrices was very efficient, suggesting its use as an important technological platform for controlled release.

Financial support: PNPD/CAPES.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Hybrid bacterial cellulose - pectin films for transdermal delivery of bioactive molecules

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ARTICLE INFO

Keywords:
Bacterial cellulose
Transdermal delivery
Antimicrobial patch
Polymeric film
Levofloxacin

ABSTRACT

Introduction and objectives: Novel biopolymeric films based on bacterial cellulose (BC) modified with high methoxylated pectin (HMP) were developed for transdermal drug delivery. **Materials and Methods**: The ability of films to incorporate an antibiotic, levofloxacin (Levo), was analyzed. Incorporation efficiencies (EE) were determined using films with different proportions of HMP (from 0.1% to 2.0%) with a maximum drug payload of 6.23 mg/g. **Results:** Characterization studies revealed the existence of a cooperative network between both polymers and deep structural changes in BC matrix. Besides, HMP presence decreased water loss in the BC film from 93% to 75% after 90 min. Additionally, film incorporation capacity of macromolecules, using Human Serum Albumin (HSA) as a model protein, was studied. HMP presence enhanced in more than 3.5 times the EE of HSA and no pH dependence was observed. Release kinetics of both molecules showed hyperbolic profiles with sustained release. On independent experiments, HMP presence generated around 50% decrease on both macromolecules release rates. Additionally, the incorporation of HSA into BC-HMP matrix exhibitied a modulation on Levo release profile. The antimicrobial activity of Levo released from the BC-HMP-HSA films was confirmed using *Staphylococcus aureus*. In-vitro studies revealed no apparent cytotoxicity of the released compounds in mammalian CHO cells. **Conclusion:** As a conclusion, on this work the versatility of bacterial cellulose material was tested by *in situ* modification with an additive biopoolymer. The hybrid material exhibited proper characteristic for its application as a transdermal graft with antibiotic properties.

Financial support: The present work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 0498), Universidad Nacional de La Plata (Grant X545, I159) and Agencia Nacional de Promoción Científica y Técnica (ANPCyT, PICT2011-2116) of Argentina.LC Bartel had a return fellowship of the Alexander von Humboldt Foundation. Also, we want to thank CPKelco (Buenos Aires, Argentina) for the pectin samples.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Biocomposites based on tpp crosslinked chitosan / bacterial cellulose as a potential strategy for Ciprofloxacin release

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ARTICLE INFO

Keywords:
Acterial cellulose
Chitosan
Ciprofloxacin

ABSTRACT

Introduction and Objectives: Bacterial cellulose (BC) presents high crystallinity, fibers of nanometric size gives it a greater water hold capacity and not contain lignin, pectin and hemicellulose in its structure. The polymer has been studied, produced and applied in several areas. Chitosan is a polysaccharide obtained from the N-deacetylation of chitin, consisting of polymeric (1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose units. Because of the biocompatibility, non-toxicity, biodegradability, and intrinsic antibacterial properties, chitosan is considered as a versatile material with potential biomedical applications. Therefore, the aim of this work was to use bacterial cellulose crosslinked with sodium tripolyphosphate (TPP) and chitosan loaded with ciprofloxacin and to evaluate the antimicrobial capacity and the in vitro release study of ciprofloxacin. **Materials and Methods:** The commercial kitLIVE / DEAD BacLight® were used for microbiological assays and the bacteria *Pseudomonas aeruginosa* and *Staphylococcus. aureus* were incubated at 24 hours to allow biofilm formation. Subsequently, biofilms were completely covered with empty and ciprofloxacin loaded BC/Chitosan films for 10, 30 and 60 min. Controls with untreated bacteria (Live) and HClO treated biofilm (Dead) were performed. Then, were observed in a Leica DM 2500 epifluorescence microscope (Germany) equipped with UV filters (495–505 nm) at 400X to determine the viability of the bacteria. Ciprofloxacin release was evaluated in phosphate buffer (10.0mM pH 5,8). Briefly, one film (10 mm) was incubated in 20 mL buffer at 37°C. Samples were taken at different times, and ciprofloxacin was measured at the maximum absorbance wavelength (277 nm). **Results**: For both *Pseudomonas aeruginosa* and *Staphylococcus. aureus*, a reduction in the bacterial population was observed after 30 and 60 minutes of contact with the bacteria, increasing as time passed. The release profile of ciprofloxacin showed a gradual release in 15 min (37%) and 25 min (52%) until a burst in 50 min (80%) and follow constant. After this quickly release, significant percentages of the amounts of drug released up to 300 min were not observed, suggesting a prolongation of the release, which could be exploited for pathologies in which an initial loading dose is required, followed by maintenance of the dose of the antibiotic. **Conclusions:** The rapid release verified by the study suggests that the system provides an enough drug for its effectiveness, corroborating with the antimicrobial activity test.

Financial support: CAPES

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Synthesis, pressing and characterization of bacterial cellulose produced by *Komagataeibacter Rhaeticus*

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ARTICLE INFO

Keywords:
Bacterial Cellulose
Hydraulic Press
Central Composite Design

ABSTRACT

Introduction and Objectives: Cellulose is a homopolymer of D-glucopyranose residues linked by β-(1 →4) glycosidic linkages and is metabolized in plants, animals and secreted by specific genera of bacteria. Bacterial cellulose (BC), presents high crystallinity, fibers of nanometric size which gives it a greater water hold capacity and differs from vegetal cellulose as it does not contain lignin, pectin and hemicellulose in its structure. The polymer has been studied, produced and applied in several areas. However, about BC produced by *Komagataeibacter rhaeticus*, there are few reports in the literature regarding its characteristics, either after a process of membrane pressing. Therefore, the objective of this work is the characterization of the morphological structure and mechanical and thermal characteristics of the BC after pressing process of hydraulic press and drying. **Materials and Methods:** After being purified, the membranes were pressed by a hydraulic press, evaluating the variables: pressing time of 10, 20 and 30 seconds and forces of 1, 2 and 3 tons, according to central composite design to verify the influence of the press in parameter morphological, mechanical and thermal.SEM experiments were carried out using samples previously coated with evaporated carbon. The images were obtained using the JEOL T-300 microscope operating at 2 kV. Mechanical properties of membranes were evaluated using texture analyzer TA-XT2 (Stable Micro Systems). Force displacement curves were recorded until the film rupture and used to determine the puncture strength (Ps), elongation at break (Eb), perforation energy (Ep). Thermogravimetric analyses were performed using an SDT Q600 (TA Instruments, USA), at 20 °C/min under nitrogen atmosphere (30 mL/min). **Results:** SEM images shows that the pressed samples presented more compacted fibers, and tended to align in the same direction as the increase of the force and time of pressing. It was also observed a lower porosity when compared to CB without treatment. In the mechanical analysis, BC with treatment presented a progressive puncture strength, according to a gradual increase of force and pressing time, however, this value was lower with BC without treatment. In the elongation at break, BC without treatment presented lower value when compared to the membranes with treatment that presented greater elongation. All samples showed a similar thermal behavior, the curves obtained displayed two mass losses. The first one, a small mass loss related to loss of surface water (~ 3.7 - 5.5). The second mass loss event was attributed to the sample decomposition process of cellulose (~74 - 84 %). **Conclusions:** The data obtained so far show that the use of the BC treatment press can change its morphological structure beyond the mechanical and thermal properties.

Financial support: CAPES

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Babassu Mesocarp (*Orbignya phalerata*) Modified With Phthalic Anhydride For Applications In Electrochemical Sensors Of 5-Fluorouracil Chemotherapeutic

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ARTICLE INFO

Keywords:
Babassu Mesocarp
Gold Electrode
Sensor
5-Fluorouracil
Electrochemistry

ABSTRACT

Introduction and Objectives: 5-Fluorouracil (5-FU) is a broad-spectrum drug used in the treatment of neoplasms such as glioblastoma and several other cancers, including head and neck cancer, gastrointestinal tract cancer, and breast cancer. On the other hand, there is no knowledge of a level of exposure to 5-FU that is considered safe, for example, for those who are not in chemotherapy treatment. The occupational exposure to 5-FU, even for a short time, as is the case of healthcare professionals who administer these drugs, can cause adverse effects such as skin rashes, nausea, hair loss, allergic reactions, damage in DNA, etc. Thus, it is very important to develop low-cost sensors capable of detecting 5-FU in different samples and at low concentrations. In this perspective, the objective of this study was the development of electrochemical sensors for detection of 5-FU, from the use of a polymer extracted from babassu mesocarp (BM), which was chemically modified with phthalic anhydride (BMPA) to improve its solubility and electrochemical properties. **Material and Methods:** The reaction for BMPA synthesis was based on literature. A flexible gold electrode (FEAu) was constructed for this study, in which the cost of the electrode was estimated at approximately 0.027 US dollars. The FEAu was modified with a micro droplet (10 µL) of a solution containing BM or BMPA at 1.0 mg/L. The electrochemical assays were performed in a conventional electrochemical cell, using FEAu/BMPA as working electrode. **Results:** The modification in babassu mesocarp with phthalic anhydride was confirmed by FTIR, XRD, TG/DTG, Zeta Potential, and SEM analysis. The modification caused a very positive effect on the electrochemical behavior of the polymer, since the BMPA showed a more reversible redox process and with greater electrochemical stability in relation to BM. The current of oxidation process of 5-FU had an increase of 276% when FEAu/BMPA electrode was used. Also was observed a displacement in the oxidation potentials of BMPA in presence of 5-FU, suggesting strong interaction between them. After construction ofa calibration plot for 5-FU using FEAu/BMPA electrode, the analytical sensitivity and the limit of detection for 5-FU were estimated at 8.8 µA/µmol/L and 3.4×10^{-7} µmol/L, respectively. **Conclusions:** Electrochemical sensors developed from babassu mesocarp may be an economically viable alternative for monitoring of the 5-FU antineoplastic, since in addition to being sensitive to this drug they are constructed of a natural polymer, renewable and widely abundant in nature.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Development of sensor device based on purified palygorskite associated with antimicrobial peptide DRS 01

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ARTICLE INFO

Keywords:
Fibrous Clay minerals
Attapulgite
Pithecopus hypochondrialis
Nanostructured Films
Layer-By-Layer

ABSTRACT

Introduction and Objectives: Biomolecules immobilization is a promising approach in development of sensor devices. Antimicrobial peptides (AMPs) are part of the innate immune system of several organisms with firmly established antibiotic potential that can be used as recognition elements for target substances in biosensor devices. Clay minerals are inorganic solids of crystalline structure with morphology and unique physicochemical features, such as adsorptive and thermal properties. In this sense, these inorganic systems have emerging as suitable matrices for anchorage of organic molecules. This work reports the purification, characterization and application of nanocrystals of palygorskite (PAL), a fibrous clay mineral from Guadalupe (state of Piauí), for immobilization of the peptide Dermaseptin 01 (DRS 01) bylayer-by-layer (LbL) technique to develop an electro active film for applications as biosensor device. **Materials and Methods:** The natural PAL was submitted to physical and chemical purification processes for the enrichment of its adsorptive properties and the concentration of clay-mineral. Structure, chemical composition and morphology of PAL were investigated by X-Ray Diffraction (XRD), Fourier-Transform Infrared spectroscopy (FTIR), X-Ray Fluorescence spectrometry (XRF), Scanning Electron Microscopy (SEM) and Transmission Electron Microscope (TEM). LbL films based on PAL and DRS 01 were prepared by alternating immersion of Indium tin oxide (ITO) substrates in dispersions of 1 mg/mL of PAL and DRS 01 for five minutes. Films were electrochemically characterized by Cyclic Voltammetry (CV) in 0.1 mol L^{-1}phosphate buffer (pH 7.25), UV-Visible spectroscopy, FTIR attenuated total reflection (FTIR-ATR) and Atomic Force Microscopy (AFM). **Results:** PAL was purified with enrichment of its properties as confirmed by XRD and FTIR techniques with pronounced reduction of quartz peaks. The results for ITO/DRS 01 and ITO/PAL/DRS 01 films showed an oxidation process at +0.77 V, confirming that DRS 01 maintained its electro active behavior, when together with PAL. The results of CV showed differences in the current density of 1.82 μA cm^{-2} for the film containing unpurified PAL (ITO/PAL-IN/DRS 01) to 2.63 μA cm^{-2} in the film containing purified PAL (ITO/PAL/DRS 01). The 3-bilayer ITO/$(PAL/DRS\ 01)_3$ film showed an increase in current density values that was around 4.60 μA cm^{-2} compared to the film with a single bilayer. **Conclusions:** The purification of clay mineral played an important role in the electrode response. The nanostructured film developed emerges as a low-cost platform, versatile and easy to prepare, even on other substrates, for biodetection of pathogens in clinical, environmental and pharmaceutical analysis, as well as other biotechnological applications.

Financial support: CAPES

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Scaffolds of pla (POLYLACTIC ACID) obtained by additive manufacturing functionalized with calcium polyphosphate coacervate for application in tissue engineering

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ARTICLE INFO

Keywords:
Tissue Engineering
PLA
Coacervate
Scaffolds

ABSTRACT

Introduction: Tissue Engineering have been gaining prominence, since it has a wide range of applications. The general purpose is development of biological substitutes for repairing and/or replacement of damaged tissues. There are three basic elements for tissue engineering: Cell, which is responsible for formation of new tissue; Biocompatible Polymer Matrix, which provides an appropriate environment and support for cell growth; and the Growth Factors, which are biologically active molecules that stimulate and define the cell differentiation. 3D printed poly-acid lactic scaffolds could be a promising technology for tissue engineering applications. In order to improve cell adhesion and proliferation on PLA scaffolds, 3D samples have been modified with polyphosphate coacervate, which is a rich gel-like containing mainly phosphorus and calcium elements. **Objectives:** The aiming of this work is surface modification of 3D-PLA scaffold using polyphosphate coacervate as coating and modifier agent. **Materials and Methods:** Commercial filaments of PLA were used to build 3D-scaffolds (10 mm of diameter and 5 mm of height) by UP-3D plus 3D printer. 3D-PLA scaffolds were submerged for 24 h in polyphosphate coacervate at room temperature. PLA-Coacervate samples were frozen and lyophilized. **Results:** SEM images indicate that the polyphosphate coacervate clusters were within PLA 3D structures. The layer-by-layer scaffold structure was kept intact. EDS data indicates presence of oxygen, phosphorus, sodium, chlorine and calcium, the main components of polyphosphate coacervate and PLA. Structural and thermal analyses confirmed that the coacervate polyphosphate was incorporated in PLA scaffolds. **Conclusions:** Cytotoxicity and cell adhesion preliminary tests suggest the possibility to use this new scaffold in medical applications.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Filaments for 3d printing based on polymeric blends of poly-hydroxybutyrate / starch for applications in tissue engineering

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ARTICLE INFO

Keywords:
3D printing
Polymeric blends
Poly-hydroxybutyrate and starch

ABSTRACT

Currently have been growning the interest in biopolymers for the production of biomaterials using 3D printed, since they are biodegradable, biocompatible and non-toxic. Among all natural polymers, poly-hydroxybutyrate (PHB), which is a polymer produced from bacteria *Alcaligenes eutrophus*, it is a renewable, linear, semi-crystalline resources and belonging to the class of poly-hydroxyalkanoates. The main disadvantage of this biopolymer is the high cost in production and some deficiencies in their properties such as low mechanical resistance and thermal instability. To address these deficiencies is possible the association of PHB with natural additives, such as starch, cellulose and others natural polymers. In this work, polymers blends filaments based on different proportions of PHB/ starch have been prepared using a homemade extruder of single screw. The obtained filaments have been characterized as Scanning Electron Microscopy (SEM), thermogravimetric analysis (TGA), mechanical tests of tensile and impact, and biodegradation test. The filaments have also been tested using 3D printed fused deposition modeling (FDM) method in order to produce prototypes that can be applied as a biomaterial in tissue engineering.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Regulatory process of a bioceramic laboratory by the health surveillance guideliness

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ARTICLE INFO

Keywords:
Regulatory Guidelines
Health Surveillance
Ceramic Biomaterials
Laboratory Management

ABSTRACT

Introduction: Biomaterials production companies, specially, bone grafts based in bovine bone mineral matrix (CaP), needs approval and certification by the competent health organizations, in order to commercialize safely in the Brazilian market. Thereby, they depend on the guidelines and its complementary rules of the collegiate board of directors in the Nacional Health Surveillance Agency (ANVISA) and Health Ministry (MS) at national level; in a state level, the standards are secured by the State Secretary of Health (SES/SP) and municipal by the Health Surveillance (VISA). **Objectives:** The purpose of this abstract is to understand which are the regulatory standard to manage a laboratory. By them, develop all quality system (SQ) documents in accord to the internal processes of fabrication and operation. All these documents are necessary to have a Technical Authorization Report (LTA), allowing a company located in São Carlos/SP to commercialize its products. **Materials and Methods:** To do so, the SQ management must be written in accord on the guidelines of the collegiate board resolution (RDC) nº 16/2013 (Technical Regulation of good manufacturing practices for health products), RDC nº 02/2010 (Management of health technologies in health facilities) and RDC nº 306/2004 (Technical Regulation for the management of waste of health services). These are the main national guidelines and, using them as base; it was written (i) Manual of Good Fabricating Practices (BPF); (ii) the Standard Operating Procedures for Specific Processes (POP-PE), (iii) of Equipment (POP-EQ), (iv) and of Good Fabricating Practices (POP-BPF); (v) the Solid Waste Management Plan (PGRSS) and the Equipment Management Plan (PGE). **Results:** From these documents, a physical adaptation project of the company has been created to suit the pattern of sections, people, processes and raw material flow required by the VISA in the RDC nº 50/2002 (Technical Regulatory to plan, program, elaboration and evaluation of physical projects of health care establishment). All documents were filed to VISA and, in a preview analysis, it was opened the regulatory process. In the end of the physical reform, all the documents are going to be reviewed and the VISA will perform the audition of the regulatory process. With the LTA approved, different compositions of CaP will be produced and offered in the Brazilian market. **Conclusions:** The importance of this work was to solve an existing problem inside the company, and to report in a scientific base the regulatory process, so that future entrepreneurs can use this method.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Towards an academic spin-offs maturity model

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ARTICLE INFO

Keywords:
Academics spin-offs
Maturity model
Entrepreneurship

ABSTRACT

The university has been challenged to include entrepreneurship inside the undergraduate and graduate programs, as a way to motivate new venture companies and innovation. This challenge is being faced by universities around the world. In Brazil, the teaching of entrepreneurship, in most institutions, follows the traditional method. This work aims to present an academic spin-offs maturity model, based on the model of Fiates et al. (2008), which is a representation of a business acceleration model. The Fiates model considers 5 fundamental factors to describe the spin-off maturity levels, which are: the entrepreneur, the product, the capital, the market and the management. The proposal of this research aims to incorporate new dimensions for Fiates' maturity model. The research project foresees a review of the spin-offs maturity models, cited in the literature, and case studies of academic spin-offs for the identification of new dimensions of spin-off maturity model. The proposal model will have validated from comparative analysis. The paper discuss how to organize the teaching of entrepreneurship, in order to meet all the maturity levels of the academic spin-off, combining theory and practice, from the prospecting and technological application. This project will generate practical results, such as actions to foster academic entrepreneurship and strengthen students' entrepreneurial training. In addition, the results can be to contributing in the field of entrepreneurial university theory.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Regulatory process of a bioceramic laboratory by the health surveillance guideliness

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ARTICLE INFO

Keywords:
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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Technological forecasting on additive manufacturing for bone tissue engineering in Brazil

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ARTICLE INFO

Keywords:
Additive Manufacturing
Bone Tissue Engineering
Scaffolds

ABSTRACT

Introduction: Biomaterials are biological or synthetic materials used as technologies that will interface with a biological system, with the aim of repairing tissues or compromised functions in the organism of humans and other animals. With the advent of 3D printers, biomaterials have reached better performance levels due to the possibility of producing parts on a small scale and using geometrical forms little used in the existing techniques. **Objectives:** The present study describes the beginning of a technological forecasting study on additive manufacturing used in bone tissue engineering in the Brazilian context. **Materials and Methods:** To this end, a search was made in the Web of Science database, using keyword combinations of three complementary topics: (i) bone tissue engineering, (ii) materials (in this case, as bone grafting is the main goal, calcium phosphates were used such as Hydroxyapatite) and (iii) additive manufacturing. The results were processed using the VantagePoint v. 5.0 software in order to carry out a bibliometric analysis of the records retrieved. **Results:** A total of 720 articles from 44 countries and 695 institutions were retrieved and analyzed. Regarding countries, the main agents in the scene of tridimensional printing in bone grafting are China (24.1%), the USA (20.8%) and Germany (13.6%), while Brazil is in 18th place, with 14 articles published (1.94%). In Brazil, the beginning of research into this technology was in 2008 at the State University of Campinas (UNICAMP - SP) and reached its peak in 2013 with 4 publications, and the Federal University of Rio Grande do Sul (UFRGS - RS) continues to be the main producer of knowledge. Of the 14 articles published in Brazil, 35.7% were with international participation from Belgium, Japan, Portugal, Spain, Switzerland, the USA, Germany and Italy. **Conclusions:** This data describes Brazil as not being influencing country on this technique. Furthermore, it has been shown to be an emerging process in countries that have an established tradition in technological production. It should be observed that, while the first studies of using 3D printing in bone grafting dates from the end of the 1980s, in Brazil, it only began 20 years later. Therefore, this study shows a delay in Brazil regarding this technology application. In order to gain more conclusions about this topic worldwide and in Brazil, other routes of datamining must be followed. This should be done in future work considering other databases, particularly in the areas of engineering and biomedicine, as well as using specific patent databases.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Science awareness coffee: a contribution to the development of the entrepreneurial ecosystem with researchers from Araraquara / SP.

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ARTICLE INFO

Keywords:
Academic entrepreneurship
Innovation in biotechnology
Entrepreneurial ecosystem

ABSTRACT

Introduction: Between 1987 and 2008 there was a growth of more than one thousand percent in the number of doctorate graduates in Brazil. However, in order to reach proportions similar to that of developed countries, a 4.5-fold increase in the participation of PhDs in their population would be necessary. On the other hand, there is an alarming fact: formation of cadres to meet the demands of the postgraduate degree itself no longer accounts for absorbing the picture formed. In 2007 and 2008, 39.8% of the recent doctors "did not were found as employees". In view of this state of affairs, an increasingly promising alternative is entrepreneurship, although what has been seen is that the higher the degree, the lower the level of entrepreneurship. It seems that undertaking is a synonym of abandoning the academic career, but in several centers of academic excellence, actions are being put into practice to bring the academy closer to the market: Leaders in innovation fellowships of the University of Oxford, Babson College and also the creation of several Science Parks in all the state of São Paulo. The process of creation of a company has a complex character and is linked to a set of social, cultural and economic factors.To raise the success rate of academic entrepreneurship activities it is necessary to establish motivation mechanisms for students with Entrepreneurship potential. Entrepreneurial capacity refers to a type of human capital that comprises the set of knowledge resources and skills, which are essential for an opportunity of achievement, combined with the motivation to do so. **Objectives:** Aiming at the development of low cost exploratory strategies to foster sustainable scientific entrepreneurship, we proposed a series of meetings called "ConsCiência Coffee" – or Science awareness Coffee. **Materials and Methods:** Researchers from the Araraquara/SP region meet monthly to choose and discuss topics of common interest to improve their entrepreneurship skills. Some of the questions include: "How much is my idea, innovation or company worth?"; "What is the best legal and tax aspect to initiate an enterprise", "What is the bureaucratic way to open a company?""How can CANVAS structure and Business Plan be applied to each innovation?". **Conclusions:** Based on these questions, approaches have been promoted to various partners, such as CANVAS workshops and the construction of various public communication tools on academic entrepreneurship, projects and innovations, as well as the possibilities of new partnerships, expanding the local entrepreneurial ecosystem.

Financial support: CAPES.

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Regenerative Medicine - Abstracts

International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Biomodulatory influence of low-intensity laser therapy and serum rich in growth factor inhuman fibroblasts cells

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ARTICLE INFO

Keywords:
Tendon
Tendinopathy
Platelet-rich plasma
Low-intensity laser therapy

ABSTRACT

Introduction: Low-intensity laser therapy (LILT) and serum rich in growth factor (SRGF) derived from the human platelet-rich plasma technique (hPRP) are clinically used as biostimulants agents in tissue repair. The use is justified by the ability to stimulate cell proliferation and differentiation, the synthesis of extracellular matrix components and local neoangiogenesis. On the other hand, the effectiveness of these techniques, as well as administration parameters, are not properly established. **Objectives:** Thus, the present study aimed to characterize, in an *in-vitro* condition, the biomodulatory influence of both techniques on human fibroblast cells. **Materials and Methods:** Samples of blood from a male volunteer were centrifuged for fractionation of platelet-rich plasma and activated with calcium chloride (10% m/v) to obtain SRGF. Human fibroblasts (line GM07492) were seeded in 24-well plates ($5x10^4$ cells) in DMEM culture medium supplemented with 2.5% SRGF or fetal bovine serum (FBS). The groups related to LILT evaluation were submitted to laser radiation at wavelengths (λ) of 685 and 830nm. Doses of 0.3, 0.6, 0.9, 1.2 and 1.5 J/cm^2 with power density of 18 mW/cm^2 were evaluated. The cell viability were determined by the MTT-Formazan colorimetric assay 24 hours after irradiation and expressed as percentage of viability of control group (DMEM+2.5% FBS). The results were established by descriptive statistical procedures and variance analysis (ANOVA), complemented by Fisher's post-test. **Results:** Comparison of cell viability between the group using FBS and SRGF showed no statistical differences, indicating similarity in the viability potential between the growth factors sources. Regarding LILT, both λ showed biomodulatory potential on human fibroblast viability, although the effective dose, type and intensity of modulation were significantly different. Radiation at λ 685 nm provided a 40% increase in viability at dose of 0.6 J/cm^2. The λ of 830 nm resulted in a 60% increase in viability at the dose of 0.3 J/cm^2 and a reduction of 15% and 10% at the doses of 0.9 and 1.2 J/cm^2, respectively. This biomodulatory influence was not modified by human PRP-derived growth factors. **Conclusions:** These results demonstrate a λ and dose-dependent biomedulatory potential of LILT on human fibroblasts in vitro, which was not influenced by the action of SRGF derived from hPRP technique.

Financial support: CAPES

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Low-level laser therapy increased the number of ovarian follicles in the polycysticovaries-induced rats

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ARTICLE INFO

Keywords:
Laser therapy
Polycystic ovary
Ovarian follicle
Biotechnology

ABSTRACT

Introduction: Rats induced to polycystic ovaries (PCO) present ovarian alterations in both folliculogenesis and steroidogenesis. There is a reduction in the number of follicle and corpora lutea in addition to appearance of folliclecysts. Hyperandrogenemia is a consequence of steroidogenic alterations which induces irregular estral cycles. Low-level laser therapy (LLLT) and Light emitting diodes (LEDs) therapies have been investigated and used in clinical practice related to biomodulating influences on cellular functions both *in vivo* and *in vivo*. **Objectives:** The objective of this study was to analyze the LLLT effects on the number of the ovarian follicles in PCO-laser treated rats. **Materials and Methods:** Forty-five female adult Wistar rats weighing 250g-300g were divided into control (n= 15), PCO (n= 15) and PCO/laser (n= 15). PCO rats were induced by injection of estradiol valerate (EV) (2 mg/kg/wb, i.m., one-step). After PCO induction rats were divided into 30, 45 and 60 days after injections. The animals were manually contained and irradiated with laser at the wavelength of 808nm, with power between 60 mW, in a dose of 3 Joules (J)/ point, for 18 seconds on the dorsal region, performing a transillumination on each ovary 3 times a week, totaling 6 J of energy per irradiated animal/per day of application. After sacrifice the ovaries were collected for preparation and subsequent analysis of the histological slides. The results are presented as mean ± SEM. Significant statistical differences among the means of the treatment groups were decided for p-values<0.05. This study was approved by local Animal Care and Use Committee (CEUA–UNIARA, protocol nº 019/16). The results are reported as the means ± SEM. **Results:** The results showed that the largest number of follicles was observed in the control group 30 days (8 ± 1) and the lowest number was observed in the PCO group 60 days (1.66 ± 0.57). The 60-day PCO/laser group (6.66 ± 0.57) presented a higher number of follicles compared to the PCO group and equal to the control group. **Conclusions:** The results allow us to conclude that the use of LLLT increased the number of ovarian follicles in the PCO-induced rats apparently restoring the ovarian activities of folliculogenesis.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Resistance exercise can promote morfofunctional changes in ovary polycystic-induced rats

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ARTICLE INFO

Keywords:
Polycystic ovary
Resistance exercise
Metabolism
Body weight
Ovary weight

ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome characterized by an ovulation, clinical and/or biochemical signs of hyperandrogenism and abnormal ovary morphology. Rats induced by polycystic ovary (PCO) presents structural and functional ovary modifications that compromise the estrous cycle. The induction of PCO in rats using Estradiol Valerate (EV) is an interesting model because it develops ovarian alterations similar to PCOS in women. **Objectives:** The objective of this study was to identify possible changes in body weight, ovarian weight, gonado somatic index (IGS) and maximal voluntary carrying capacity (MVCC) at the end of the training in PCO-induced rats submitted to resistance exercise. **Materials and Methods:** Forty female adult Wistar rats weighing 200g-300g were divided into 2 group: 30 days (n= 20) and 45 days (n= 20) after PCO induction, and these groups were divided into 4 sub-groups: control (n= 5), PCO (n= 5), control/training (n= 5) and PCO/training (n= 5). The PCO induction was performed with a single dose of EV (2 mg/ 0.2 mL/rati.m.). The resistance exercise chosen was stair climbing (3 times/week) with loads added to the tails of the rats. The data were analyzed using ANOVA and Tukey's test. Significant statistical differences among the means of the treatment groups were decided for p-values<0.05. This study was approved by local Animal Care and Use Committee (CEUA–UNIARA, protocol nº 025/16). **Results:** For the 30-day group: the left ovary weight was higher for the control group (0.09708 ± 0.015 g) compared to the PCO/training group (0.06904 ± 0.0123 g). For the 45 day group: the body weight of the control group (367.2 ± 18.3g) was greater than the PCO group (274.8 ± 18.47 g) and the PCO/training group (290.8 ± 20.52 g). The PCO/training group had a lower body weight than the control group. For the 45-day group: the right ovary weight of the control group (0.0584 ± 0.0129 g) was greater than the PCO group (0.0276 ± 0.0063 g). There was no statistical difference for either IGS or MVCC. **Conclusions:** The PCO-induced rats by EV is a viable model for studies that correlate ovarian and metabolic alterations. This PCO-induced model is compatible with metabolic and reproductive characteristics of human PCOS and can be used for the study of physical exercise intervention on endocrine-reproductive mechanisms. In addition, resistance exercise can modulate adaptations in the body and ovarian masses of PCO-induced rats.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Development and evaluation of lamelar denso scaffold for tissue engineering

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ARTICLE INFO

Keywords:
Acute myocardial infarction
Tissue engineering
Lamellar scaffold
Biopolymers
Collagen

ABSTRACT

Introduction: Acute myocardial infarction (IAM) continues being responsible for the reduction in life expectancy and large numbers of deaths worldwide. Tissue engineering for cardiac tissue regeneration has been an alternative to restore the structure and mechanical functionality of the heart. Scaffolds are porous three-dimensional support, temporary, used to mimic the structure of the extracellular matrix and stimulating specific cellular responses at the cellular level / molecular organic tissue for regeneration. **Objective**: To project, to development and to evaluate dense lamellar scaffolds with potential use in tissue engineering. **Materials and Method**: The hydrogels, for fabrication of scaffolds, were prepared by mixture of collagen, chitosan and Poloxamer 407. Scaffolds for AMI were produced by plastic compression (using hydrostatic press). Briefly, cast highly hydrated hydrogels were transferred to a porous support comprising (bottom to top) absorbent paper blot layers, a steel mesh and two nylon meshes. The matrices were freeze-dried, resulting in cross-linked collagen-chitosan scaffolds. The samples were evaluated by swelling efficiency, porosity, interconnectivity and pore size, Fourier transform infrared spectroscopy and physiomechanical properties. **Results**: The physical mechanism controlling solute uptake was observed as anomalous transport, with the n values equal 0.79. The scaffold showed high interconnectivity of the porous with 71.68% of the porosity. The pore dimensions estimated from SEM microphotographs for scaffold was in the range of 15 – 25 μm. The FTIR spectrum of the blend polymers shows the characteristic bands of the parent molecules. No additional bands were identified indicating that did not have chemical interaction between the polymers used in the formulation. The results of physiomechanical properties like as elasticity, flexibility, drilling, traction and mucoadhesion were 0.608 ± 0.044 N, 0.635 ± 0.029 N, 2.001 ± 0.022 N, 1.806 ± 0.058N and 0.648 ± 0.040 N, respectively. **Conclusion:** The lamellar scaffolds obtained by the blend of collagen and chitosan, by plastic compression, showed promising feature in the application of the tissue engineering.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Characterization of collagen and evaluation of potential use as gel in tissue engineering

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ARTICLE INFO

Keywords:
Collagen
Biopolymers
Tissue engineering

ABSTRACT

Introduction: Collagen is the most abundant protein in the extracellular matrix and has been considered to be a group of proteins with a fibrillar structure, which contributes to the extracellular scaffolding. In the presence of acid, alkalis and saline aqueous solutions a considerable increase in the amount of water absorbed by the collagen is reported. The isoelectric point of the collagen is in the range of 6.5 to 8.5, and any deviation from this pH (i.e. change in the isoelectric point) may cause non-specific swelling. **Objective:** To investigate the difference between collagen fiber (CF) and powder (CP), in different pH, with potential use as biomaterials in tissue engineering. **Materials and Method**: The CF and CP characteristic were evaluated using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). The gels were obtained in different collagen concentration (0.50, 0.75 and 1.0 %) and mediums with pH 7, 4 and 2 (water, acetic acid and sodium phosphate, respectively).The gels were evaluated by water holding capacity (WHC) and gelation temperature (Tsol-gel) between 8-38°C. **Results:** Thermoanalytical curve showed three thermal transition, next to 85°C (loss of water and denaturation of collagen protein), 227°C and 275°C (degradation) for both samples (CF and CP). The chemical groups showed by CF and CP were compatible with literature. Collagen gel has Tsol-gel behavior at low temperatures, that is, with the increase in the temperature occurs the decrease of the gel viscosity, therefore, the gel collagen shows Tgel-sol (transition temperature gelled/solution). The average value for WHC of the CP and CF was 92% of initial mass of the gel. As concentration as medium were factors that influence the Tsol-gel of the gels. For gels containing 1% of collagen did not occur Tsol-gel, the preparation keep itself gelled in all temperatures. CF showed Tgel-sol at 23°C to 0.75% in water, 17°C to 0.5% in sodium phosphate medium and 33°C to 0.5% in acetic acid. CP in water showed Tgel-sol at 22°C and 31°C for 0.50% and 0.75%, respectively. CP in sodium phosphate showed Tgel-sol at 11°C and 21°C for 0.50% and 0.75%. In acetic acid medium the Tgel-sol was observed only to the 0.50% concentration of the CP. **Conclusion:** The medium used to prepare the CP and CF gels influenced significantly the Tgel-sol behavior. The concentration of CP or CF can be modulated in an attempt to achieve the Tgel-sol proper. The CP and CF gel shows potential use in tissue engineering as injectable gel and in the scaffold fabrication.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

PLA (polylactic acid) scaffold printed by 3d tecnology and functionalized by plasma of oxygen

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ARTICLE INFO

Keywords:
Polylacticacid
3D printing
Plasma treatment

ABSTRACT

Introduction: The purpose of tissue engineering is to repair, replace, create or regenerate tissues and organs. Tissue engineering depends on a triad composed of cells, scaffolds or structures, polymers, and growth factors. Nowadays, it is necessary to use the biomaterial that allows tissue engineering to build and shape through 3D technology to support cells, repair organs with biocompatibility, and chemical and physical properties that allow the surface to work. Among the polymers, we can use PLA (polylactic acid). PLA has physical properties as well as biodegradability and biocompatibility. Thus, the PLA has characteristics necessary for the manufacture of a scaffold. The scaffold is a support or structure that allows there generation of tissues. To create a scaffold, we use rapid prototyping. This new field of research is a versatile technique for generating large quantities of shapes and sizes of polymers. Three-dimensional (3D) printing, also known as the manufacture of additives (AM), extrudes the polymeric material into layers. Rapid prototyping (RP) represents the direct manufacturing of pieces per layer, guided by digital information from a computer-aided design (CAD) file without any specific part tool. To promote and increase properties such as hydrophilicity, adhesion, and proliferation, on the surface of the scaffold, we must work the surface with oxygen plasma. **Objectives:** The goal of this study is to develop polylactic acid scaffolds printed by 3D printing (PLA) by superficially functionalizing the scaffold with oxygen plasma for required fixation and growth of osteocyte cells. **Materials and Methods:** To achieve the scaffold we used FDM 3D printer (Fused Deposition Modeling), called (Boa Impressão 3D), model Stella, Curitiba-PR, Brazil. The scaffolds were modeled (10 mm in diameter x 1 mm in height) in Autodesk Inventor CAD software and exported in the format STL. The Movitech filament with a diameter of 1.75 mm was used for the extrusion of PLA. To increase the roughness, we used the functionalization of the oxygen plasma on the surface of the scaffold. The system consists of a stainless steel reactor (~ 5.2 x 10-3m^3) containing two parallel circular electrodes with 11.9 cm in diameter, separated by 5cm. **Results:** We evaluated the contact angle (water) and it showed 38.95°; 16.5 °; 13.31°; 8.63 °; 29.88° for the times of 0,5 min, 1 min, 5 min, 10 min and 20 min respectively. Analyzing these results from the contact angle, were alized that the functionalization through oxygen plasma provided greater hydrophilicity to the PLA surface. We used AFM atomic force microscopy to investigate surface roughness and reached up to a 450% increase on the scaffold compared to an untreated scaffold. We submitted the functionalized scaffold to the proliferation and viability test with 1x10^5 Osteo-1 cells seeded in DMEM environment for 72 hours. We achieved 89 % of proliferation. We also noticed that increased roughness, caused by the oxygen plasma, increased cell adhesion and proliferation on the scaffold surface. **Conclusions:** It is understood that the scaffold obtained by extrusion of 3D printing with PLA and the functionalization with oxygen plasma promotes a better control of shape and size of the organ or tissue to be built, and imitates the extracellular matrix.

Financial support: CAPES

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

The use of stem cells as a promising method for cardiac regeneration: current scenario and future prospects

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ARTICLE INFO

Keywords:
Stem Cells
Heart
Regeneration

ABSTRACT

Introduction: Once cardiovascular diseases are the main cause of global mortality, there is high interest in the development of therapies for the reduction of the impact of these pathologies. The use of Stem Cells (SC) as a therapeutic method is a recent tactic for cardiac regeneration, justifying reviews about its current and future use. This technique is promising because it is able to provide cardiomyocytes after ischemic events, induce revascularization of the damaged area, and prevent deleterious pathological remodeling. **Objectives:** Analyze, through specialized literature review, the current scenario and future perspectives of the use of SCs for cardiac regeneration focusing obstacles and strategies of study. **Materials and Methods:** Articles from the last 6 years (2011 to 2017) were searched in the databases MEDLINE (accessed via PubMed), SciELO, LILACS, Scopus and Cochrane Library in English and Portuguese. The descriptors were: Stem, Cell, Cardiac, Heart, Regeneration, Repair and Therapy. Initial selection of articles was carried out based on the titles and abstracts and, after verification of the appropriate content, the complete text was searched. Thus, 55 articles that approached the theme were obtained. After reading, 23 texts that fully answered to the objectives of this research were selected. The most recent articles, with the highest impact factor and level of evidence were prioritized. Case reports and opinion articles were excluded. **Results:** The current scenario of SC implantation for cardiac regeneration is still little explored. The reviewed articles lists as difficulties in the study of the subject: heterogenic groups and methodologies, low long - term clinical follow - up of the volunteers and restricted number of works due to difficult access to suitable material and case – control groups. Not all types of SCs have already been studied. As obstacles to therapeutic applications, studies found that undifferentiated SCsmay induce teratomas and karyotype anomalies, genetic and immune rejection, immature phenotype (implanted cells tend to be more similar to their fetal counterparts), heterogeneity of SCs subtypes, and the difficulty of producing sufficient cells for effective repair. As control strategies, SC reprogramming techniques with non-viral vectors are being employed to avoid possible teratogenic events, addition of retinoic acid and manipulation of the Wnt differentiation pathway to obtain specific subtypes of cardiomyocytes and culture systems based on bioreactors to obtain adequate amount of cells. Combined gene and cell procedures are being used and developed in order to increase the survival of implanted SCs, such as cardiac patches in vitro, engineered heart tissue and injectable scaffolds. **Conclusions:** SC technology is an attractive approach to the generation of cardiac tissue, but studies face obstacles during research process that may affect its results. Long-term follow up studies and the development of combined gene and cell procedures can change this scenario.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Influence of type 2 diabetes mellitus on cardiac regeneration after ischemic cardiomyopathy

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ARTICLE INFO

Keywords:
Diabetes
Heart
Regeneration

ABSTRACT

Introduction: Cardiovascular pathologies are the main cause of worldwide mortality. These diseases represent an estimated 80% of all deaths in diabetic patients. Once type 2 diabetes mellitus (T2DM) is an expanding global health problem, there is high interest in the understanding of the *physiopathological* influence of this condition on heart regeneration after ischemic events as well as development of appropriated therapies. **Objectives:** Analyze, through specialized literature review, the possible interventions against the negative effects of T2DM, focusing its *physiopathological* influence, on cardiac regeneration after ischemic cardiomyopathy. **Materials and Methods:** Articles from the last 7 years (2010 to 2017) were searched in the databases MEDLINE (accessed via PubMed), SciELO, LILACS, Scopus and Cochrane Library in English and Portuguese. The descriptors were: Type 2, Diabetes, Mellitus, Cardiac, Heart, Regeneration, Repair and Therapy. Initial selection of articles was carried out based on the titles and abstracts and, after verification of the appropriate content, the complete text was searched. Thus, 35 articles that approached the theme were obtained. After reading, 17 texts that fully answered to the objectives of this research were selected. Most recent articles, with the highest impact factor and level of evidence were prioritized. Case reports and opinion articles were excluded. **Results:** The elevated prevalence of cardiac disease with T2DM lead to studies of stem cell (SC) mediated heart repair in a limited number of patients and pre-clinical diabetic models. Some studies showed that mesenchymal stem cells (MSC) infused into diabetic rats with diabetic cardiomyopathy improved their heart function, lowered serum glucose and increased serum insulin levels compared with the control diabetic group (possibly through angiogenesis and attenuation of cardiac remodeling). It was noticed that MSC therapy promoted effective cardiac nerve sprouting, enhanced cardiomyocyte proliferation and increased endothelial cell incorporation into neovascularization, reducing heart complications in the presence of T2DM. However, studies also pointed that, in specific situations, T2DM inhibited the multipotency of MSCs and impaired their sufficiency to increase blood flow recovery. Hyperglycemia also showed deleterious effects on the role of endothelial progenitor cells in vascular and tissue repair, as well as affected the migration of cardiac SCs. On possible strategies to prevent or reverse the deleterious diabetic effect on SCs and cardiac regenerative therapies, articles suggest control of hyperglycemic state, reversal of oxidative stress, cardiac niche enhancement (to recover the cardiac homing capacity of SCs) and molecular modulation of specific targets (such as p38 MAPK and ERK1/2). **Conclusions:** The association of T2DM and cardiac diseases is relevant and a global health problem. The understanding of its *physiopathological* aspects can lead to better treatment options. Cardiac regeneration after ischemic cardiomyopathy with SCs is a promising therapy, still in development, that will require adaptations when applied to T2DM patients.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Magnetic resonance imaging parametrization for threedimensional biomodels printing of patella joint cartilage

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ARTICLE INFO

Keywords
Magnetic resonance imaging
Parameterization
3D printing of biomodels
Patellar cartilage

ABSTRACT

Introduction: One of the most important causes of reduced body movement is osteoarthrosis (OA), the knee joint is the most involved and a fundamental component of this joint is patellar cartilage. Cartilaginous lesions lead to OA, fast or insidious, resulting from genetic, traumatic, vascular and metabolic alterations, leading to the irregularity of its surface and reduction of its thickness, aspects to be studied in detail. The spatial view of cartilage defects, as well as the monitoring of disease progression are of fundamental importance for the effectiveness of the treatment. It is not common to surgeons the three-dimensional (3D) idea of parts of the human body in the study and planning of conservative (clinical) and surgical treatment. **Objectives:** The objective of this research is to establish a higher quality parametrization for the acquisition of magnetic resonance imaging in order to obtain reliable three-dimensional biomodels of articular patellar cartilage through 3D printing technology. **Materials and Methods:** For this, a healthy individual, female, 25 years old and with no orthopedic antecedents or complaints compatible with patellar chondropathy was evaluated. The patient underwent magnetic resonance imaging of the knee in Magneton Essenza 1.5T equipment (SIEMENS®) coupled to a 14-channel radio-frequency coil. Axial images were established from the acquisition sequences T2-Gradiente, Proton Density (DP), T1- SpinEco, T1-Vibe and T2-GradienteEco (T2-GRE). The images were processed in the software InVesalius® and Magics® for the construction of three-dimensional biomodels in virtual environment. The recommended acquisition parameters for the generation of 3D biomodels of the patellar articular cartilage were established through a comparative analysis between the different acquisition sequences, considering the time spent in the image processing and the accuracy achieved in the virtual biomodels. **Results:** The results showed that all acquisition sequences allowed the generation of the 3D biomodels. In contrast, the processing parameters, the time spent performing the processing and the accuracy reached in the biomodels were significantly different among them. The best performance was achieved with the T1-Vibe acquisition sequence, which provided the shortest processing time and the highest accuracy of the three-dimensional biomodel. **Conclusions:** It is concluded that the parameters associated with this acquisition sequence are recommended for obtaining magnetic resonance imaging for the preparation of three dimensional biomodels for 3D printing. New research will be conducted to investigate the influence of other acquisition parameters, such as the field strength of the main magnet and the technical characteristics of the radiofrequency coil.

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▷Medicinal Chemistry - Abstracts

International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Evaluation of the kinetic profile of the release of tetracycline in simulated gastric fluid and simulated intestinal fluid from biopolymer microparticles

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ARTICLE INFO

Keywords:
Alginate
Chitosan
Drug Delivery System
Tetracycline
Polyelectrolytes

ABSTRACT

Introduction and Objectives: The aim of this paper was to prepare and characterize biopolymeric microparticles (MP) with bioadhesive properties to promote the sustained antibiotic release. The development of such devices aims to prevent early disruption of treatment protocol with antibiotics caused by lack of adherence from the patient, whose disruption is one of the causes of bacterial resistance increase. **Materials and Methods:** MP were prepared through mechanical shearing of chitosan (CH), sodium alginate (SA), and tetracycline hydrochloride (TC), whose amount followed an experimental design 2^3, whereby an influence of each component was observed in properties such diameter (D), encapsulation efficiency (%EE), and loading percentage (%LP). Diameters were measured on the Malvern Mastersize 2000 equipment. It was obtained particles with diameters between 4.5 and 8.5 μm were obtained. %EE was calculated through the relation between the difference of the total mass of TC (TM_{TC}) and the free mass of TC (FM_{TC}) and TM_{TC}. And %LP was calculated through the relation between the difference of the TM_{TC} and the FM_{TC} and the total mass of polymer. FM_{TC} was obtained through the liquid extracted by centrifugation at 10000 RPM for 10 minutes of 500 μL of MPs suspension placed in a ultrafiltration device Amicon® Ultra 0,5 MWCO 100K. TC was quantified by High performance liquid chromatography (HPCL) according to the Pharmacopoeia method of United Stated (USP 30 – NF 25, 2007). **Results:** %EE differed between 56% and 87% and %LP between 19% and 87%. Reagents and microparticles were characterized as to main functional groups identification by Fourier Transform infrared spectroscopy (FTIR); as to thermal stability by Differential Scanning Calorimetry (DSC). DSC tests indicated that preparations have less thermal stability with respect to pure reagents. FTIR spectra were recorded in the Fourier transform spectrometer, Agilent Cary, model 630 FTIR, with module ATR. FTIR spectra indicated the interaction between the CH, SA, and TC functional groups, which intensity differed according to the ratio of these compounds and affected both, loading percentage and encapsulation efficiency, as well as the release profile. Sustained release profile of TC in simulated body fluid with different PHs (simulated gastric fluid (SFG) and simulated intestinal fluid (SFI) were observed by 2h and 48h, respectively. These results suggest that there is a decrease of the burst effect in the TC release in both SFG and SFI, avoiding that the initial dose reaches a toxic level. Release profile in SFG is different than the observed in SFI, expected result, once the matrix constitutes of polyelectrolytes, which are sensible to the PH of solution. **Conclusions:** These results indicate that some microparticles are promising to a final formulation.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Cytotoxic and mutagenic effects of new Cu(II) complex as potential antituberculosis agent

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ARTICLE INFO

Keywords:
Cu(II) complexes
Ames test
Micronucleus test
Cytotoxicity

ABSTRACT

Introduction: Since the discovery of the cytotoxic and antitumor activities of cisplatin, medicinal chemistry has seen impressive advances in the bioinorganic chemistry, with a growing interest in the study of the biological activities of metal complexes. In this context, copper (Cu) has a number of qualities that have made it attractive for such research, as it operates as a first-row transition metal, is the third most abundant trace metal in the human body (behind iron and zinc), is important for plants, animals and fundamental to the performance of several enzymes involved in energy metabolism, respiration, and deoxyribonucleic acid (DNA) synthesis. Previous studies showed that Cu(II) complexes, formed from the interaction of Cu(II) ions with biologically active ligands, have shown excellent antimicrobial activity against *M. tuberculosis*. **Objectives:** In view of this antimycobacterial activity and its potential as a lead compound for drug development, the aim of the present study was to investigate the mutagenic activity of Cu (II) complex with isoniazid [$Cu(NCO)_2(INH)_2$]_$4H_2O$ (I3)], by the Ames test and the micronucleus assay, to assess the safe use of this complex in the treatment of tuberculosis, besides the cytotoxic activity in a normal cell line (GM-07492 - human lung fibroblasts) and a cell line with metabolism profile of carcinogens (HepG2) by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. **Materials and Methods:** The complex was synthetized in the Faculty of Pharmaceutical Sciences of Araraquara and provided by Dr. Patrícia Bento da Silva. The Ames test was performed using TA98, TA100, TA97a and TA102 strains of *Salmonella typhimurium*, as sensitive indicators of DNA damage, in the absence (-S9) and presence (+S9) of metabolic activation system in five concentrations, varying from 15.6 to 250 µg/plate. The micronucleus assay was performed in HepG2 cells in 3 different concentrations of I3, varying from 125 to 500 µg/mL. **Results:** The results obtained by Ames test showed that I3 induces only signs of mutagenicity in strains TA100 and TA97a tested in presence of metabolic activation. Similarly, it was observed a chromosomal damage in HepG2 cells with significant increases of micronuclei and nuclear budsand. With respect to cell viability, the complex induced a concentration-dependent decrease, but even so, the cytotoxicity (IC50) was higher than 500 µg/ml, indicating low toxicity. **Conclusions:** Further investigation is necessary to permit its more effective and safer use, beyond to clarify the mechanisms and the conditions that mediate the biological effects of Cu (II) complexes, before considering them as therapeutic agents.

Financial Support: UNIARA and FAPESP (2017/06317-7).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Nuclease activity of a copper complex with sulfameter

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ARTICLE INFO

Keywords:
Copper
Sulfonamide
Nuclease activity

ABSTRACT

Introduction: Copper complexes have a remarkable redox chemistry that has been explored for the development of artificial nucleases. The DNA damage is usually correlated to the anticancer and antibacterial activity of these copper compounds. **Objectives:** In this work, we report the nuclease activity of a copper(II) compound containing the sulfonamide sulfameter and 1,10-phenantroline as ligands. **Materials and Methods:** The copper compound was synthesized with 1:2:1 (copper(II) : sulfameter : phenantroline) ratio and characterized with chemical, spectroscopic and crystallographic techniques. For the evaluation of nuclease activity, two experiments were performed. Firstly, the plasmid pGEX4T-1 was incubated with the copper(II) complex and submitted to agarose gel electrophoresis. On the second experiment, ascorbic acid was added to the reaction mixture containing the plasmid and the compound. Copper(II) nitrate was also analyzed for comparison. **Results:** Results demonstrated that the complex alone alters the electrophoretic pattern of the plasmid, indicating some interaction, but no significant nuclease activity. Addition of ascorbic acid resulted in no visible band in the gel, indicating complete degradation of plasmid DNA at the complex concentration of 10 μmol·L^{-1}. Copper(II) nitrate, however, only altered the percentage of population of DNA in the supercoiled and partially supercoiled forms, but no complete degradation was observed up to the concentration of 20 μmol·L^{-1}. **Conclusions:** These results showed that the copper-sulfonamide is a promising compound for further biological studies.

Financial Support: FAPESP (grants 2015/25114-4, 2015/09833-0 and 2015/20882-3), CNPq (grant 442123/2014-0) and CAPES.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Palladium(II) and platinum(II) complexes with hydrazide derivative of nalidixic acid: synthesis and characterization

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ARTICLE INFO

Keywords:
Platinum(II)
Palladium(II)
Nalidixic acid hydrazide
Spectroscopic analysis

ABSTRACT

Introduction: Platinum-based drugs are one of the most effective classes of antitumor agents. Nowadays, the Pt(II) complexes approved as anticancer agents are cisplatin, carboplatin and oxaliplatin, and it is estimated that they are used in 50% of all cancer treatments. A great effort has been made to develop new metal complexes with tumor-inhibiting properties better than cisplatin and its derivatives in efficacy, selectivity, reduced toxicity and improved pharmacology. Despite the endeavors to treat cancer more effectively, the pace of drug development is far from the current increasing rate of cancer incidence and mortality. To improve this scenario, one strategy of drug discovery is to combine the cytotoxic properties of a metal ion with the desired features of a specific ligand that have favorable safety profile and known pharmacokinetics. Nalidixic acid (1-ethyl-7-methy-1,8-naphthyridine-4-one-3-carboxylate, nx) is considered the precursor of the antibacterial quinolone series and is a interfacial inhibitor of DNA gyrase protein in bacteria. The inhibition of such enzyme causes a preferential, rapid and reversible inhibition of DNA synthesis. **Objectives:** In the present work, a modification of nx structure into a carbonyl hydrazide (hzd) was made and the obtained hydrazide was combined with Pt(II) and Pd(II) ions to further evaluate its antitumor activities. **Materials and Methods:** The hzd was synthesized from nalidixic acid following the procedure previously reported by our research group, and both complexes were synthesized by very similar routes, in which 0.50 mmol of hzd in methanol is reacted with 0.50 mmol of K_2PtCl_4 or K_2PdCl_4 in water, heated at 50°C for 2 hours under stirring and then stirred for additional 16 hours. **Results:** For both complexes, insoluble yellow solids were obtained, separated by filtration and washed with water and methanol. Calcd. for Pdhzd or $Pd(C_{12}H_{14}N_4O_2)2Cl_2$ (%): C, 43.03; H, 4.21; N, 16.73. Found (%): C, 44.22; H, 4.39; N, 16.84. Calcd. for Pthzd or $Pt(C_{12}H_{14}N_4O_2)2Cl_2$ (%): C, 38.00; H, 3.72; N, 14.77. Found (%): C, 37.53; H, 3.70; N, 14.26. Nitrogen solid state nuclear magnetic resonance spectroscopy indicated that coordination of the ligand to the metal centers occurs by the NH_2 group of hzd as the signal of the nitrogen atom of free ligand is shifted by -40.3 and -37.7 ppm in the Pdhzd and Pthzd complexes, respectively. **Conclusions:** This hypothesis is also supported by the infrared spectroscopic data of the complexes, where changes in the stretching modes of the NH2 group are observed. Biological studies are in progress.

Financial Support: CNPq Grants #442123/2014-0 and #140707/2013-1, FAPESP Grant # 2015/25114-4

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Synthesis and cytotoxic activity on Pd(II) complexes containing thiosemicarbazide

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ARTICLE INFO

Keywords:
Pd(II) complexes
Thiosemicarbazide
IR and NMR spectroscopy
Medicinal chemistry
Antitumor activity

ABSTRACT

Introduction: The interest on introduction of transition metal ions aiming at designing new chemotherapeutic agents has been prompted by the discovery of medicinal uses of cisplatin in treatment of several types of human neoplasms. Particularly, palladium(II) compounds have attracted considerable attention because of the analogy between the coordination chemistry of Pd(II) and Pt(II) complexes. In order to increase the kinetic stability of Pd(II) compounds, N,S-chelating ligand have been successfully employed to enhance the activity of these species in the cellular medium. Thiosemicarbazide is a N,S-chelating ligands capable to coordinate via sulfur and nitrogen atoms, affording a stable five-memberedring. For this reason, this ligand would be of interest to prepare new biologically active Pd(II) complexes. **Objectives:** Thus, we present here in the synthesis and cytotoxic evaluation against of the complexes of the type [PdX(tscz) PPh_3]X, where tscz = thiosemicarbazide, PPh_3 = triphenylphosphine and X = Cl^-(**1**) or I^-(**2**). **Materials and Methods:** Compound **1** was obtained from the reaction between [$PdCl_2(CH_3CN)_2$] and thiosemicarbazide (tscz), with further addition of triphenylphosphine. Complex **2** was synthesized by substitution of the chlorido group by iodide. The compounds were characterized by elemental analysis, IR spectroscopy, and ^1H and ^{13}C NMR spectroscopy, differential thermal analysis (DTA), and thermogravimetry (TG) and conductivity measurements. The cytotoxic activities of the complexes have been evaluated in vitro by MTT assay against two cell lines: human lung fibroblast (MRC-5) and human lung carcinoma (A549). For comparison purposes, the cytotoxicity of cisplatin, a standard metal-based antitumor drug, was also evaluated under the same conditions. **Results and Conclusions:** According to the cytotoxicity data, the free ligand tscz is considered inactive. The cytotoxicity of **1** and **2** against A549 cells was 10.46 and 11.27 μg/mL, respectively, being the value found for cisplatin 36 μg/mL. With regard to MCR-5 cells, both Pd(II) complexes displayed similar IC_{50} values (**1**: 3.06; **2**: 3.17 μg/mL), being more toxic than cisplatin (9.3 μg/mL).

Financial support: We thank FAPESP, CNPq and the Institute of Chemistry.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Flow cytometry studies over NCI/ADR-RES tumor cells of a silver(i) complex with 5-fluorouracil: preliminary results

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ARTICLE INFO

Keywords:
Flow Cytometry
Metal Complex
Anticancer Activity

ABSTRACT

Introduction: In our previous work, we showed the antiproliferative activity of a silver(I) complex with 5-fluorouracil (Ag-5fu) against multi-resistant ovarian tumor cells (NCI/ADR-RES) when compared to free 5-fluorouracil (5fu), silver nitrate ($AgNO_3$) and cisplatin. The enhanced activity of Ag-5fu appeared to be a synergistic effect between 5fu and $AgNO_3$, when comparing their antiproliferative profiles. **Objectives:** Therefore, for a better understanding of the role of 5fu and silver(I) in the anticancer activity of Ag-5fu, we performed flow cytometry studies over NCI/ADR-RES tumor cells, comparing the effects of Ag-5fu, 5fu and $AgNO_3$ on cell cycle arrest and apoptosis induction. **Materials and Methods:** The multi-resistant human ovarian tumor cell line NCI/ADR-RES was obtained from the National Cancer Institute (Frederick-MA, USA). Stock cultures were grown in complete medium (RPMI 1640 supplemented with 5% FBS and 1% penicillin/streptomycin) at 37°C with 5% CO_2. Solutions of the compounds 5fu, Ag-5fu and $AgNO_3$ were prepared in complete medium and different concentrations were used for the assays. For cell cycle arrest: 0.75 μg mL-1 and 1.5 μg mL-1 for 5fu and $AgNO_3$, and 1.5 μg mL-1 and 3.0 μg mL-1 for Ag-5fu. For apoptosis induction (nexin): 1.5 μg mL-1 for 5fu and $AgNO_3$, and 3.0 μg mL-1 for Ag-5fu. The chosen concentrations were based on the metal:ligand proportion of the Ag-5fu complex (1:1 in mass). Cells were seeded in 6 and 12 well plates ($5x10^4$ cells/mL) for cell cycle and nexin assays, respectively, for 24h prior to treatment with the compounds. Flow cytometry experiments were performed in a Guava EasyCyte Mini Flow Cytometer (Millipore, MA, USA) using Guava Cell Cycle and Nexin reagents (Merck Millipore) according to manufacturer's instructions. For all assays cells without treatment were used as negative controls. For cell cycle arrest cells were treated for 36h, while for nexin assay cells were treated for 18h. **Results:** Cell cycle assay showed the arrest in G1 phase by 5fu and Ag-5fu, but no for $AgNO_3$, which did not arrest cell cycle in 36h. Nexin assay showed the induction of apoptosis for Ag-5fu and $AgNO_3$, but not for 5fu, which did not cause cell death for 18h of treatment. The Ag-5fu complex showed 11% cells marked only with annexin-V and 49% cells double marked with annexin-V and 7-AAD, in contrast to 8% and 28% for $AgNO_3$, respectively. **Conclusions:** These results show that in the Ag-5fu complex cell cycle arrest is caused by 5fu, while cell death seems to be triggered by apoptosis and caused by silver(I).

Financial support: CAPES, FAPESP (Grant #2015/25114-4) and CNPq (#442123/2014-0).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Complexes of V, Pt and Pd with amino acids and a-hydroxycarboxylic acids

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ARTICLE INFO

Keywords:
Flow Cytometry
Metal Complex
Anticancer Activity

ABSTRACT

Introduction: Complexes of Pt(II), Pd(II) and V(IV,V) show cytotoxic activity on tumor cells, but their low selectivity causes acute side effects. There are many works published in the literature that report that the structural modification of the complexes by using modified ligands is an effective method to enhance their activity as chemotherapeutic agents and decrease the side effects. **Objectives:** The main goals of the present work are the synthesis of new complexes of V, Pt and Pd with amino acids, amino acid derivatives and α-hydroxycarboxylic acids and to test the *in-vitro* cytotoxic activity. **Materials and Methods:** The amino acids and the α-hydroxycarboxylic acids were used in their anion form. In general, the complexes were prepared in aqueous solution under reflux by using the molar proportion 1M:1L and 1M:2L. Complexes of V with glutamic acid, lysine, 2,2-bypiridin, cysteine, gabapentin, phthalic, mesaconic and orotic acids were obtained and are being characterized. Complexes of Pt with 5-FU (5-fluouracil) and phthalic and mesaconic acids were obtained using the same procedure and were partially characterized. These complexes were tested with mouth and neck cancer cell lines. Complexes of Pt(II) and Pd(II) with gabapentin and orotic acid were obtained and are being tested for mutagenic and cytotoxic activity on different cell lines. **Results:** Preliminary results show that the cytotoxic activity of the Pt-5-FU complex is lower than cisplatin and higher than free 5-FU. Pt complexes with phthalic and mesaconic acids did not show any cytotoxic activity. This fact can be explained by the oxidation of Pt(II) to Pt(IV) during the synthesis and a new synthetic method has been tested. **Conclusions:** Additional studies will be necessary to elucidate the structure of the complexes and the cytotoxic activity for different types of cancer cells.

Financial support: To CAPES and FUNADESP for the fellowships.

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Study of the mutagenic potential of a new platinum complex by ames test

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ARTICLE INFO

Keywords:
Metal Complex
Mutagenicity
Ames Test

ABSTRACT

Introduction: Over the years there has been a continuous interest in the chemistry of metal complexes, because of their key role in clinical therapy in biological applications of metal coordination compounds of biologically active ligands. Transition metals are particularly suitable for this purpose because they can adopt a wide variety of coordination numbers, geometries and oxidation states in comparison with other main group elements. However, one of the most important negative biological effects is the damage to DNA, since increases in DNA damage are associated with higher incidence of cancer and other different undesirable health consequences, including infertility and genetic disorders. **Objectives:** Whereas, carcinogenicity and mutagenicity are among the toxicological effects that cause the highest concern for human health, the aim of this study was to investigate the mutagenic activity of the platinum complex with mesaconic acid (Pt-Mesac) by Ames test, a widely used assay that detects mutations at the gene level through strains genetically modified of the *Samonella typhimurium* bacteria. **Materials and Methods:** Pt complex was produced and provided by the doctoral student Filipe Payolla, under the responsibility of Prof. Dr. Antônio Carlos Massabni. The Ames test was performed using TA98, TA100, TA97a and TA102 strains of *S. typhimurium*, as sensitive indicators of DNA damage, in the absence (-S9) and presence (+S9) of metabolic activation system in five concentrations, varying from 12.5 to 100 μg/plate. **Results:** The results obtained showed that Pt-Mesac was not mutagenic under the conditions used, because did not induce any increase in the number of revertant colonies relative to the negative control. **Conclusions:** The absence of mutagenic effect by Pt complex against *S. typhimurium* bacterial strains in the Ames test is highly relevant. However, further pharmacological and toxicological investigations are necessary to determine the mechanism(s) of action to guarantee their safer and more effective application to human health.

Financial support: Funadesp and Fapesp (Brazil).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Synthesis, characterizationand antibacterial activities of a new Au(III) complex with hydrochlorothiazide

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ARTICLE INFO

Keywords:
Au(III)
Hydrochlorothiazide
Metal complex
Antibacterial activities

ABSTRACT

Introduction: The discovery of antibiotics can be considered one of the most significant achievements of modern science for the control of infectious diseases. However, the microbial resistance to antibiotics in use nowadays turns important the search for new compounds with better therapeutic efficiency and lower potential of development of bacterial resistance. One of the strategies to defeat the multiresistance is to combine metal ions, such as silver and gold, with ligands that already possess biological activities and further evaluate their efficacies as novel antibacterial agents. Gold compounds, in special, have been considered as antibacterial agents since the discovery of the antimicrobial activities of potassium dicyanoaurate(I) by Robert Koch in the end of the 19th century. Nowadays, gold compounds are mainly used as antiarthritic compounds, with emphasis to auranofin. Hydrochlorothiazide (HCZ), 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7sulfonamide 1,1-dioxide, a thiazide diuretic, is often used in combination with other agents in the treatment of hypertension and patients with ischemic diseases. In the view point of coordination chemistry, HCZ can be considered versatile ligand, being able to coordinate to metal ions such as Au(I,III). **Objectives:** Based on these considerations, a new Au(III) complex with HCZ, hereby identified as Au-HCZ, was prepared and evaluated about its antibacterial activities. **Materials and Methods:** The Au-HCZ complex was synthesized by the reaction of HCZ with $Li[AuCl]_4$ in an aqueous/alcoholic solution at pH 10. Elemental analysis indicated a 1:1 metal:ligand composition. Anal. calc. for $AuC_7H_7Cl_3N_3O_4S_2$ (%) C, 14.9; H, 1.25; N, 7.44. Found (%) C, 14.4; H, 1.40; N, 6.80. The FTIR spectrum of the complex was evaluated in comparison to that of free HCZ. Changes in the absorption bands of the vibrational modes of the N-H groups suggest nitrogen coordination to Au(III). Antimicrobial activity of Au-HCZ was preliminarily evaluated by disc diffusion and further confirmed by minimum inhibitory concentration (MIC) assays against bacterial strains *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. **Results:** The MIC assay demonstrated the inhibitory activity of the complex against the considered strains with values of 2.13 mmol L^{-1} for *S. aureus*, 1.07 mmol L^{-1} for *E. coli* and 1.07 mmol L^{-1} for *P. aeruginosa*. **Conclusions:** Further studies are envisaged to confirm the potential of application of the Au-HCZ complex for treatment of bacterial infections.

Financial support: Brazilian agencies FAPESP (grant # 2015/09833-0, 2015/25114-4), CAPES and CNPq (grant # 442123/2014-0) and FUNADESP (National Foundation for the Development of Private Higher Education grant no. # 2700316).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Physical-chemical characterization and cellular viability study of *baccharis dracunculifolia* plant extracts

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ARTICLE INFO

Keywords:
Vegetable extract
Baccharis dracunculifolia physicochemical characterization

ABSTRACT

Introduction and Objectives: *Baccharis dracunculifolia* (BD), also known as field rosemary, occurs naturally in the South, Southeast and Center-West of Brazil, mainly in cerrado regions. Young BD leaves are formed by tectonic and glandular trichomes, and contain volatile and aromatic oils. Those oils give BD the typical aroma of the green propolis, produced by the insect *Apis mellifera*. Diverse therapeutic activities are available in the literature, and it is possible to highlight the anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, cytotoxic, hepatoprotective and antimutagenic activities. Development of new DB-based systems requires knowledge about its chemical profile and cell viability. Physical-chemical characterization of BD vegetal extracts (VE) and cell viability tests, through MTT assay, are the main focus of this work. **Materials and Methods:** VE is provided by Ciclo Farma Indústria Química Eireli chemical industry, located in Serrana – SP – BR. Determination of flavonoids were performed by UV/Vis spectroscopy, and the same extracts have also been subjected to fibroblast cell viability assays. **Results and Conclusions:** Up to the present moment, the presence of phenolic compounds and flavonoids, such as, caffeic acid, coumaric acid, Artepelin C, quercetin, rutin can be confirmed in the BD extracts. Cytotoxicity of VE is dose-dependent, and can be tuned by BD concentration.

Financial support: Fapesp

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Search for high-added value compounds from soya crops agricultural waste

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ARTICLE INFO

Keywords:
Isoflavones
Soy
Agricultural waste

ABSTRACT

Introduction: It is estimated that a quarter of the approximately 140 billion tons of agricultural biomass produced per year worldwide is produced in Brazil, and that more than 30% of the total waste produced in this country are agricultural wastes. According to the United Nations, there is the need to intensify research on technologies for converting agricultural waste into useful resources to society. Furthermore, the search for compounds of interest in waste by means of advanced value-added strategies based on green technologies, before adding them to low value-added products, has been seen as a business opportunity. Soya crops corresponds to almost 60% of total cultivated are in Brazil having a strong impact in Brazilian GDP, but also in the generation of agricultural waste in Brazil. Soybeans are known to be one of the main sources of isoflavones, a class of flavonoids with innumerable properties for human health. Isoflavones rich extracts of soy beans are largely commercialized for the treatment of women who are going through climacteric. **Objectives:** This work aimed to investigate the potential of soya agricultural wastes (stems, leaves and twigs of soybeans) as raw materials for the preparation of phenolic rich extracts based on green solvents. **Materials and Methods:** Samples were extracted by dynamic maceration and analysed by HPLC-DAD/UV. **Results:** Overall, the greener ethanol and acetone provided better extractions than those observed for the reference solvent acetonitrile, an undesired chemical from the sustainability point of view. Isoflavones were found in stems and leaves together with other flavonoids, whereas the twigs showed to be a rich source of flavones. **Conclusions**: These residues showed potential to be raw materials for the production of flavonoid rich extracts rather than simply waste. The green solvents ethanol and acetone were able to replace acetonitrile for such extractions.

Financial support: São Paulo Research Foundation (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support (grants #16/08179-8 and #45398 2014-5).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Curcumin-cinnamaldehyde hybrids against *Xanthomonas citri* subsp. *citri*

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ARTICLE INFO

Keywords:
Xanthomonas citri subsp. *citri*
Curcumin
Cinnamaldehyde

ABSTRACT

Introduction: Citrus canker is one of the most aggressive citrus diseases, affecting the cultivation of citrus plants in some tropical areas. This disease is caused by *Xanthomonas citri* subsp. *citri* (Xac) and occurs in major orange juice producing countries, including Brazil and the USA. Chemical control of this disease has become ineffective, due to irrational use of cupric compounds. Recently, it was identified copper-resistant genes in Xac, which difficulted its eradication. Nevertheless, efforts are needed to identify and develop innovative anti-Xac compounds. Molecular hybridization is a widely used medicinal chemistry tool for design of bioactive compounds, we purposed hybridization using curcumin and cinnamaldehyde. Curcumin and Cinnamaldehyde are natural products used in cooking and medicine that exhibit broad spectrum antibacterial action against Gram-positive and Gram-negative species. **Objectives:** The objective of this work was to evaluate anti-Xac activity of curcumin-cinnamaldehyde hybrids (CCH). **Materials and Methods:** Firstly, a series of CCH (**1**-**12**) was synthesized and their structures were confirmed by 1 H and 13 C NMR. The minimum inhibitory concentration (MIC) of compounds against Xac was performed by the Resazurina Microtiter Assay. **Results:** Among evaluated compounds, **12** showed greater potency, with MIC value of 42.94 μg mL-1. Its minimum bacterial concentration (MBC) was determined at 100 μg mL-1 and compound **12** inhibit Xac growth. Thus, **12** was selected for Xac cell treatment evaluation by measuring the cell multiplication capacity in artificially inoculated plant tissues through the pathogenicity assay. In planta assay was performed on sweet orange "Pera Rio" (*Citrus sinensis*). Seedlings were contaminated by Xac and subsequently treated by **12** at concentrations of 2xMIC and MIC. Canker incidence was evaluated weekly. Treatments performed with **12** inhibited the Xac growth in both concentrations. **Conclusions**: In conclusion, curcumin-cinnamaldehyde hybrids are promising compound against Xac. Furthermore, design by molecular hybridization was effective because curcumin and cinnamaldehyde separated were not able to demonstrate significant bacterial death.

Financial support: Coordination for the Improvement of Higher Education Personnel (CAPES), Brazilian Council for Scientific and Technological Development (CNPq) and the Sao Paulo Research Foundation (FAPESP).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Bioinspired surgical clip and coated with natural drugs from the Amazon

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ARTICLE INFO

Keywords:
Surgical clip
Coating
Drugs from Amazon

ABSTRACT

Introduction: The suture is the closing of edges of a wound to approach tissues and to seal blood vessels. The use of ant mandible to suture wounds has been reported approximately 1,000 years BC, quoted in the Indian medical text *Charaka Samhita*. The specie used in this process was the *Atta laevigata* ant, because it has adequate size for manipulation, design and its biomechanics are perfect for opening and closing the mandible and to approach the edges of the wound. In addition, the species releases through the mandible a substance that works as an antibacterial agent for the injury, making this natural system self-sufficient in the suture. Based on this knowledge, it was developed the surgical clip bioinspired (MU9102934-1). The new mechanic system facilitates the handling both in its placement of the clip and also in its removal from the skin, which is less traumatic and efficient for the patient than the traditional clamping devices. The surgical clip will be coated with *Carapa guianensis* oil, popularly known as Andiroba and *Pterodon emarginatus* oil, popularly known as Sucupira. These oils are extracted from medicinal plants of the Amazon and they are traditionally used by Riverine peoples. The natural oils present efficient analgesic, anti-bacterial, anti-inflammatory, anti-fungal and anti-allergic properties and they were also found to be effective in the healing process. **Objectives:** The objective of this work is to coat the surgical clip with natural antibacterial oils improve its performance in the healing process. **Materials and Methods:** In this work, the coatings using the solution composed by natural oils and acetone were dip coated on the surface of the material AISI 420 after different intervals of time of immersion and drying. In addition, the natural oils characterization was carried out using the Fourier Transform Infrared Spectroscopy- FTIR. The adhesion of the oils to the clip surface was evaluated by morphological analysis: Electron Microscope Scanning - SEM and Microscope of Atomic Strength - AFM, including the verification of the *in vitro* cytotoxicity potential of the surgical clip coated with drugs. **Results:** The adherence results of the solutions on the AISI 420 samples surface were similar after 30s, 1min and 1.5min of immersion, as well as after 2min, 4min and 6min of drying, respectively. In addition, the coated samples proved to be effective on bioassays conducted with bacteria and fungi. **Conclusions**: The results support the traditional use of natural oils as antibacterial agents. They can be considered promising coatings on the bioinspired surgical clip.

Financial support: Foundation of Research Support of the Amazonas State - FAPEAM

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Study on the mutagenic potential of kaurenoic acid, a bioactive diterpenoid present in Copaiba oil, by Ames test

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ARTICLE INFO

Keywords:
kaurenoic acid
Mutagenicity
Ames test

ABSTRACT

Introduction: Plants that belong to *Copaifera* spp. are rich in kaurenoic acid, a diterpene that showed a wide variety of interesting biological activities, including antiparasitic and antimicrobial effects, anti-inflammatory action, and cytotoxicity against human cancer cells and hemolytic effects against mouse erythrocytes. However, previous studies showed that exposure of V79 cells to higher concentrations of kaurenoic acid caused significant increases in cell damage index and frequency. **Objectives:** To complement the results of the literature, their mutagenic activity was assessed by Ames test in this study. This assay detects mutations at the gene level through strains genetically modified of the *Samonella typhimurium* bacteria. **Materials and Methods:** In this study, it was performed using TA98, TA100, TA97 and TA102 strains of *S. typhimurium* in the absence (-S9) and presence (+S9) of metabolic activation system, in five concentrations, varying from 25 to 200 µg/ plate, established in previous cytotoxicity studies. **Results:** The results obtained showed that kaurenoic acid was not mutagenic under the conditions used, because did not induce any increase in the number of revertant colonies relative to the negative control. **Conclusions**: The absence of mutagenic effect against *S. typhimurium* bacterial strains in the Ames test is highly relevant. However, in light of the above and of the findings of the literature, it is necessary to clarify the conditions and the mechanisms that mediate the biological effects of kaurenoic acid before treating it as therapeutical agent, because the balance between the therapeutic vs. the toxicological effects is an important parameter in assessing its applicability in relation to phytotherapeutic potential.

Financial Support: UNIARA and FAPESP

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Evaluation of the Cytotoxic activity of standardized extracts of *myrcia bella* cambess. (Myrtaceae)

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ARTICLE INFO

Keywords:
Myrcia bella
Cytotoxicity
MTT assay

ABSTRACT

Introduction: Plant-based systems continue to play an essential role in healthcare, and their use by different cultures has been extensively documented. Moreover, medicinal plants have historically proven their value as a source of molecules with therapeutic potential, and nowadays still represent an important pool for the identification of novel drug leads. Myrcia bella, a common and important species in many savanna fragments, distributed in the state of Sao Paulo, has potential use in the traditional medicine for treatment of diabetes mellitus. However, little is known about their undesirable properties such as mutagenicity, carcinogenicity and toxicity. **Objectives:** Thus, the aim of this study was to investigate the cytotoxicity of standardized extracts (70% ethanol) of leaves of M. bella. **Materials and Methods:** Cytotoxicity was assessed by changes related to metabolic functions of mitochondria detected by a colorimetric method known as MTT (tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) in a normal cell line (GM-07492 - human lung fibroblasts) and a cell line with metabolism profile of carcinogens (HepG2 - human hepatocellular carcinoma). **Results:** According to the results, M. bella induced a statistically significant reduction on cell viability of the HepG2, in all concentration tested compared to the negative control. In the treatments with the GM-07492 cultures, M. bella revealed lack of cytotoxicity; cell viability of the extract was greater than 80% **Conclusions**: The results obtained in this study are extremely relevant because it provides reliable data to support future clinical researches. However, further toxicological tests are needed to ensure its safe use.

Financial support: Uniara and Fapesp (Brazil).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Toxicity evaluation of cinnamylideneacetophenones against human cervical cancer cells positive for HPV16 and HPV18

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ARTICLE INFO

Keywords:
Cinnamaldehyde
Antitumorals
Cervical Cancer

ABSTRACT

Introduction: Human Papilloma Virus (HPV) is the main causative agent of cervical cancer, including HPV16 and HPV18 types, which have been found in 99% of cases. In worldwide, cervical cancer is the most common cause of cancer related deaths among women. In Brazil, cervical cancer is the third most frequent tumor, with 5.430 cases of deaths reported by INCA. It was estimated 16.340 new cases reported in 2016. Thus, there is a need for innovative compounds against cervical cancer. In this context, screening of natural compounds constitutes an valuable alternative for the discovery of antineoplastic agents. Cinnamaldehyde is the main chemical constituent of essential oil from *Cinnamomum cassia*. This compound has demonstrated to be an cytotoxic agent against several human cancer cells. **Objectives:** Thus, the objective of this work was to evaluate toxicity of named as cinnamylideneacetophenones (cinnamaldehyde derivatives) against human cervical carcinoma cells positive for HPV16 (CaSki ATCC CRM-CRL-1550) and HPV18 (HeLa ATCC CCL-2). **Materials and Methods:** Cells were treated with substances **1** – **4** and cinnamaldehyde, which was used as a positive control, in concentrations ranging from 0.75 to 800 μmol L^{-1} to derive IC_{50} (concentration capable of inhibiting 50% of the cells). Cellular viability was evaluated after 48 h by MTT assay. This methodology evaluates cell metabolic activity, measuring on spectrophotometer the reduction tetrazolium bromide in formazan by activity of mitochondrial dehydrogenases. The experiments were performed in triplicate and in three independent assays. Statistical analyses were performed by one-way ANOVA with Tukey's post hoc test using Graph-Pad Prism 7.0 software. **Results:** Out of 4 compounds, **2** showed the highest toxicity against CaSki and **4**, demonstrated toxicity against HeLa, exhibiting IC_{50} values of 37.78±8.20 and 59.13 ± 2.41 μmol L^{-1} respectively ($p<0.01$ vs. control). Cinnamaldehyde was not able to inhibit cell growth (IC_{50} >100 μmol L^{-1}). It is suggested cinnamylideneacetophenones **2** and **4** were more potent than cinnamaldehyde. The presence of hydroxyl group at 3' position on ring A, increased cytotoxicity. **Conclusions**: In conclusion, **2** and **4** demonstrated superior cytotoxicity, when compared to cinnamaldehyde. These compounds are hits for development of new agents useful for cervical carcinoma treatment.

Financial support: Capes, Fapesp, CNPq, PROPg-Unesp, PROPe-Unesp

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Synthesis, antibacterial and antitubercular activities of cinnamaldehyde derivatives

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ARTICLE INFO

Keywords:
Cinnamaldehyde
Antimicrobial
Antibacterial

ABSTRACT

Introduction: Cinnamaldehyde (CMD) is the majority component in cinnamon bark oil, and it is responsible for its spicy and sweet. The spectrum of antibacterial activity of CMD has been extensively reported and includes effects against Gram-positive, Gram-negative and mycobacteria species. The mode of action of CMD involves bacterial multi-targets, including membrane disruption, cell division inhibition, and reactive-oxygen species induction. CMD possess aliphatic aldoxyl group, which is an undesirable functionality due to its high reactivity. **Objectives:** Herein, we designed and synthesized a series of derivatives with the replacement of aldoxyl group by acetophenone moiety. **Materials and Methods:** Their antibacterial and antitubercular activities were evaluated against Gram-positive and Gram-negative species, as well as *Mycobacterium tuberculosis.* In addition, hydrophilicity of all compounds was measured by HPLC-PAD experiments, which enabled calculation of partition coefficients (log *Po*/w). Synthesis of derivatives was achieved by aldol condensation between CMD and corresponding acetophenone under basic catalysis, at room temperature. For antibacterial and antitubercular evaluations were used *Staphylococcus aureus* ATCC 14458 *(Sa)*, *Streptococcus mutans* ATCC 25175 *(Sm)*, *Streptococcus sanguinis* ATCC 10557 *(Ss), Pseudomonas aeruginosa* ATCC 15442 *(Pa), Escherichia coli* ATCC 10536 *(Ec), and M. tuberculosis* ATCC 27294 *(Mt)*. MIC and MBC were determined by broth microdilution method, in 96-well microtiter plates. We calculated the log *Po*/w by using HPLC-PAD method suggested by OECD protocols. **Results:** Phenolic derivatives **2** and **3** exhibited MIC and MBC values of 19.5 to 78.1 μg/mL against *Sa, Sm, and Ss.* Also, amino- (**4**) and vanillyl- (**7**) derivatives exhibited activity against *Sm* and *Sa* (MIC = MBC = 78.1 μg/mL). Derivatives bearing hydrophobic and electron-with drawing substituents were inactive against *Sa*, *Sm* and *Ss*. These results indicate electron-donating and hydrophilic groups enhanced antibacterial activity. Bioactive derivatives against Gram-positive species were not able to inhibit Gram-negative species growth. Compounds **1**, **6**, **9**, and **17** presented potent effects against *Mt* (13.2 μg/mL ≤ MIC ≤ 23.4 μg/mL). The electronic nature of substituent was not relevant for antitubercular activity. Anti-*Staphylococcus* and anti-*Streptococcus* compounds exhibited log *Po*/w values ranging from 2.5 to 3.3. Among these, **2** and **3** were the most potent, with log *Po*/w values of 2.7 and 2.6, respectively. The four most active anti-*Mycobacterium* compounds presented log *Po*/w values ranging from 3.2 to 3.5. Highly hydrophobic compounds displayed log *Po*/w > 3.5 and were not able to act against *Sa*, *Sm, Ss,* and *Mt*. Preliminary structure-activity relationship (SAR) investigations suggested hydrophilicity is central parameter for antibacterial and antitubercular activities of CMD derivatives. **Conclusions**: Furthermore, our results corroborated the potential of CMD as privileged starting material and template for synthetic collection of hits.

Financial support: CAPES, CNPq and FAPESP

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Antimycobacterial activity of dehydrozingerone derivatives

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ARTICLE INFO

Keywords:
Tuberculosis
Mycobacterium tuberculosis
Dehydrozingerone
Derivatives, antimycobacterial
Tuberculostatic
Hydrophobic

ABSTRACT

Introduction: Tuberculosis, an infectious disease of worldwide distribution, is considered a serious public health problem. The causative agent of tuberculosis is *Mycobacterium tuberculosis*, an aerobic bacterium, which has not capsule and spores. Emergence of resistant strains of *M. tuberculosis*, HIV co-infection and existence of latent intramacrophagic bacilli led to the search for innovative drugs, allowing greater adherence of the patient to treatment. Dehydrozingerone (DZG) is a phenolic compound from ginger rhizomes (*Zingiber officinale*). DZG has demonstrated several biological activities, such as: cardiovascular protection, anti-inflammatory, antitumoral, antimutagenic, antioxidant, antidepressive, anti-Alzheimer, antimalarial, antifungal, antibacterial and hypoglycemic. **Objectives:** This study aimed to synthesize and evaluate tuberculostatic activity of 10 DZG derivatives against *M. tuberculosis*. **Materials and Methods:** DZG derivatives were synthesized by aldol condensation reactions between DZG and benzaldehyde derivatives substituted by electron acceptor and donor groups, as well as hydrophilic and hydrophobic groups. For synthesis of DZG, it was used aldol condensation reaction between vanillin and acetone. DZG derivatives were evaluated *in vitro* against*M. tuberculosis* H37Rv strain (ATCC 27294) by broth dilution method coupled with the *Resazurin Microtiter Assay* (REMA). Compounds were tested in concentrations ranging from 25.0 to 0.09 μg mL^{-1} for determination of minimum inhibitory concentration capable of inhibiting 90% of *M. tuberculosis* growth (MIC_{90}). Rifampicin was used as reference tuberculostatic. **Results:** Derivatives **2**, **3**, **5**, **7**, **9** and **10** demonstrated antimycobacterial activity with MIC_{90} values ranging from 8.0 to 2.4 μg mL^{-1}. In general, it was observed that presence of *para*-substitutions on ring B by hydrophobic groups enhanced antimycobacterial activity, and eletronic nature of substituent was not relevant for tuberculostatic activity. In addition, it was evident that the cyclization, which promotes relative conformational restriction, triggered a decrease toward antimycobacterial activity. Therefore, structure-activity relationship data were observed, highlighting the influence of different hydrophobic groups on tuberculostatic activity. **Conclusions**: Furthermore, DZG derivatives corroborate the importance of natural products for the design of innovative tuberculostatic agents.

Financial support: FAPESP, CAPES, CNPq, PROPe – UNESP, PROPG – UNESP

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Synthesis and anti-*staphylococcus aureus* activity of aminochalcones

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ARTICLE INFO

Keywords:
Antimicrobial
Aminochalcones
Antibacterial
Staphylococcus Aureus

ABSTRACT

Introduction: Bacterial resistance is a natural phenomenon, which has been enhanced after use of antibiotics, generating superbugs, which are resistant to several drugs. The indiscriminate use of antibiotics, slow rate diagnoses and poor hospital hygiene are factors of bacterial resistance. Thus, the discovery of innovative antibacterial agents is necessary. Chalcones are privileged scaffolds in Medicinal Chemistry, which have exhibited broad spectrum of pharmacological activities, such as: antibacterial, antifungal, antimalarial, antiviral, antioxidant and anti-inflammatory. **Objectives:** The objective of this work was to synthesis and evaluation of 2'-, 3'- and 4'-aminochalcones against *Staphylococcus aureus.* **Materials and Methods:** A series of aminochalcones was synthesized by aldol condensation Claisen-Schmidt reactions between aminoacetophenone derivatives and benzaldehyde derivatives. Their antibacterial activity was tested against methicillin-sensitive *S. aureus* ATCC 25923 (MSSA) and methicillin-resistant *S. aureus* ATCC 33591 (MRSA). Bacterial susceptibility activity was expressed as values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Chalcones were tested in concentrations ranging from 0.48 to 62.5 μg mL^{-1}. Vancomycin was used as reference antibiotic. **Results:** Allaminochalcones substituted with amino group at 3' position of ring presented antibacterial activity. Among these, aminochalcone **3e** (3,4-Cl_2) exhibited the highest antibacterial potency against MSSA, displaying MIC value of 1.95 μg mL^{-1}. 2'-aminochalcone **2b** (4-Cl) demonstrated the highest anti-MRSA potency, demonstrating MIC value of 7.8 μg mL^{-1}. 4'-aminochalcones were not able to inhibit MSSA and MRSA growth. Aminochalcone **3e** was twice less potent than vancomycin, displaying MIC value of 1.95 μg mL^{-1} against MSSA. Furthermore, **3e** was eight times less active against MRSA (MIC = 7.8μg mL^{-1}). **Conclusions**: These results suggested 2 'and 3'-aminochalcones are promising *hit* antibiotics with biological potential similar to vancomycin.

Financial support: Capes, CNPq and Fapesp

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

Synthesis and trichomonicidal activity of hydroxychalcones

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ARTICLE INFO

Keywords:
Trichomonas
Trichomoniasis
Hydroxychalcones

ABSTRACT

Introduction: Trichomoniasis, caused by *Trichomonas vaginalis*, is the most common non-viral sexually transmitted disease (STD) around the world. For the trichomonias is treatment, nitro-compounds, metronidazole and secnidazole are the most prescribed, although there are several resistant strains and severe adverse effects. Thus, the search for new trichomonicidal agents is urgent. **Objectives:** In the present work, hydroxychalcones were designed, evaluating importance of hydroxyl position on rings A and B, as well as ring B substituted by groups recommended by the Manual Method of Topliss. **Materials and Methods:** Chalcones were synthesized via aldol condensation reaction of Claisen-Schmidt. The structures of substances were confirmed by Nuclear Magnetic Resonance. The trichomicidal activity of the hydroxychalcones was evaluated against strains of *T. vaginalis* (ATCC 30236) with the concentration determination capable of inhibiting 50% growth (IC_{50}). These substances were submitted to toxicity tests against human vaginal epithelial cells (HMVII, ECACC 92042701) and the selectivity index values (SI) were established by the ratio of the lethal concentration (CL_{50}) and IC_{50}values ($SI = CL_{50}/ IC_{50}$). **Results:** Chalcones with hydroxyl on ring A exhibited activity against *T. vaginalis* higher than those with hydroxyl on ring B, being the first used for bioactivity optimization steps. Among these, the 4'-hydroxychalcone and 3'-hydroxychalcone showed higher antiprotozoal activity, displaying IC_{50} values of 27.5 and 49.4 μM, respectively. On the other hand, 2'-hydroxychalcone exhibited IC_{50} of 76.4 μM. However, hydroxychalcones did not show selectivity and were toxic to host cells, with SI of values ranging 0.5 to 1.1. **Conclusions**: The hydroxyl group at 3' and 4' position were crucial for trichomicidal activity. With draw and donating groups donors maintained and / or decreased potency for the 3' - hydroxychalcone and 4'-hydroxychalcone frameworks.

Financial support: CNPq, CAPES, FAPESP, PROPe-UNESP, PROPG-UNESP

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

In vitro bacterial reverse mutation assay: mutagenicity study of oleoresin of *copaifera langsdorffii*

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ARTICLE INFO

Keywords:
Copaifera langsdorfii
Mutagenicity
Ames test

ABSTRACT

Introduction: In recent times, many studies have been directed towards the identification of natural products with therapeutic properties, given the increased use of plant products for cultural, medicinal and social purposes. These natural products can be used directly or may be extracted to identify new bioactive compounds. So, it is essential to evaluate their biological effects to minimise the potential risks to human health. In this context, studies concerning genotoxicity may indicate the safety and effectiveness of herbal health products. **Objectives:** Thus, the aim of this study was to investigate the mutagenic activity of oleoresin extracted from the trunk of *Copaifera langsdorffii* by the Ames test. **Materials and Methods:** In this study, the Ames test was performed using TA98, TA100, TA97 and TA102 strains of *Salmonella typhimurium* in the absence (-S9) and presence (+S9) of metabolic activation system, in five concentrations, varying from 0.5 to 4.0 mg/ plate. **Results:** The results obtained showed that *C. langsdorffii* oleoresin did not induce any increase in the number of revertant colonies relative to the negative control, indicating the absence of mutagenic activity. **Conclusions**: The absence of mutagenic effect by this oleoresin against *S. typhimurium* bacterial strains in the Ames test is highly relevant, and is a positive step towards ensuring its safe use in medicine. However, further pharmacological and toxicological investigations are necessary to determine the mechanism(s) of action to guarantee their safer and more effective application to human health.

Financial support: Uniara and Fapesp (Brazil).

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International Journal of Advances in Medical Biotechnology

Journal homepage: http: www.journalamb.com/index.php/jamb

In vitro mutagenicity of *murraya paniculata* assayed by bacterial reverse mutation (Ames) test

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ARTICLE INFO

Keywords:
Medicinal plants
Murraya paniculata
Ames test
Mutagenicity

ABSTRACT

Introduction: The use of medicinal plants as alternative remedies has been increasing over time. The species of the genus *Murraya* has been traditionally used as an analgesic and local anesthetic for the treatment of eczema and rheumatism. *Murraya paniculata*, belonging to the Rutaceae family, has a great diversity of secondary metabolites. So, it has aroused interests in the research on its chemical, biological, chemosystematic and pharmacological aspects. However, despite being a natural extract and known by its popular use, little is known about its mutagenic potential. It is essential the evaluation the mutagenicity of extracts used by the population with therapeutic efficacy, since the cancer and other pathologies can come from mutations in the DNA. **Objectives:** Thus, the aim of the present study was to determine the mutagenic effects of the ethanolic extract of the leaves *M. paniculata* by the Ames test. **Materials and Methods:** The extract was produced and provided by the doctoral student Celia Magaly Casado Martin in the Laboratory of Pharmacognosy of the Faculty of Pharmaceutical Sciences of Araraquara (UNESP), under the responsibility of Prof. Dr. André Gonzaga. The Ames test uses bacteria as sensitive indicators of DNA damage, and a rat liver homogenate (S9 microsomal fraction) for metabolic conversion of carcinogens to their active mutagenic forms. In this study, the Ames test was performed using the preincubation methodology with TA98, TA100, TA97 and TA102 strains of *Salmonella typhimurium* in the absence (-S9) and presence (+S9) of metabolic activation system, in five concentrations, varying from 1.25 to 20 mg/ plate. **Results:** The results obtained showed that *M. paniculata* acted directly and its mutagenicity increased with metabolic activation. According to the strains involved, *M. paniculata* induces substitution of base pairs (TA100), and, at a much higher rate, frameshift mutations (TA98 and TA97a). **Conclusions**: Considering that the extract is a complex mixture of several unknown organic compounds, the mutagenicity observed may be explained in part by a synergy between compounds present in the extract. So, this plant should be used cautiously for medicinal purposes.

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