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Recent advances in methods of synthesis and applications of bacterial cellulose/calcium phosphates composites in bone tissue engineering

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ABSTRACT

Bacterial cellulose (BC) is a nanofibrous biomaterial biosynthetized by a series of acetic bacteria with unique properties with application in many tissue engineering purposes. Calcium phosphates (CPs), mainly hydroxyapatite, are bioceramics that possess similar composition of host bones and are able to stimulate osteoconduction and osteointegration to living tissues.

Bacterial cellulose-calcium phosphates composites have caught the attention of researchers by their excellent mechanical properties and biocompatibility, being considered an excellent proposal to development of new synthetic grafts to bone tissue engineering. The minireview presented here focuses on various fabrication methods used to prepare and novel applications of BC-CPs composites and their applications in BTE.

Introduction

In the last decades, researchers of different fields, such as materials science and engineering, orthopedics and dentistry, have made efforts to develop new strategies in order to replace autologous, allografts and xenografts-based therapies for new viable alternatives to solve the problem of millions of people that suffer with trauma, tumors and bone related diseases. Despite having many advantages, as stimulate osteoconductive, osteoinductive and low immune responses, autologous bone grafts, considered gold standards in orthopedic and dentistry fields, provide a limited supply of implant required for surgery. Furthermore, a patient that requires this procedure is submitted to subsequent harvesting and insertion of material in the fractured area, that leads to considerable incidence of site morbidity associated with the harvested graft.[1-4]

Allografts and xenografts, although are commonly used as alternative techniques to overcome some disadvantages of autograft therapy, due to unlimited availability of material and no occurrence of donor sites morbidity (since the grafts are harvested from bone banks), are limited because of the possibility of infection and immunological rejection. Alloplastic grafts obtained from synthetic or natural biomaterials has been considered a promisor solution to obtain biocompatible and well-integrated materials with host bone, stimulating bone regeneration, that is the main goal of bone tissue engineering (BTE)[3,5-7].

Tissue engineering (TE) is a crucial subfield of regenerative medicine, whose major concern is manufacturing parts of body, such as tissues and organs *ex vivo*. Cells culture in scaffolds and the monitoring of their proliferation, differentiation and activity is an essential concept of TE. The main goal is to develop living constructs using biomaterials, cells and growth factors to restore, regenerate, preserve or enhance functions of damaged or lost tissues [8-10].

From cell cultures in scaffolds, the next step is to implant the bioengineered organs or tissues in humans to provide their integration and the biological environment to synthetize extracellular matrix (ECM). The main requirement of a TE scaffold is that degrades over time, while allows tissue regeneration. Several materials have been successfully used for TE and specifically in BTE applications.[11-12]

Bone is a hard tissue that support the structure of body. It presents a three-dimensional (3D) hierarchical structure compound by cells, non-collagenous proteins, hyaluronan, peptoglycans and nanocollagen fibers mineralized with hydroxyapatite (HA). HA, in particular, is the major bioactive inorganic material of bone and is able to support bone growth and osseointegration, being used in orthopedic, dental implants, spinal fusion and treatment of bone defects. HA/collagen composites have been traditionally employed to produce artificial bones.

Nevertheless, a more recent trend is to use other biocompatible materials to replace collagen, due to the possibility of cross-infection and poor definition of commercial sources[13-14].

Bacterial cellulose (BC), a renewable nanobiomaterial with a three-dimensional structure of cellulose fibers, may be considered a good candidate for this purpose, once it has been proved that BC scaffolds pores are able to support the ingrowth of human chondrocytes and human smooth muscle cells *in vivo* [13,15].

Some studies have described the manufacturing and applications of BC-CPs composites for bone tissue engineering purposes, especially by enhancing osteoblastic cell proliferation and differentiation. In this context, the present review focuses on studies related to different methods of synthesis and applications of BC/CPs composites in bone tissue engineering and hence in regenerative medicine in the last ten years[16].

Bacterial cellulose: a promisor material for TE

Bacterial cellulose (BC), also known as biocellulose or microbial cellulose is a biocompatible polysaccharide biosynthesized by several species of bacteria including those belonging to the genera *Komagataeibacter* (formerly *Gluconacetobacter*), *Aerobacter, Agrobacterium, Zooglea, Azotobacter, Achromobacter, Alcaligenes, Acantamoeba, Rhizobium, Pseudomonas, Salmonella* and *Sarcina*[17-18].

This biomaterial presents the crystalline form cellulose I and chemical structured formed by linked linear chains of β-1,4-glucopyranose residues, that are the same of plant-based cellulose[19-20]. However, bacterial cellulose is free of lignin and hemicelluloses and presents high purity and remarkable mechanical and physical properties such as high elasticity, durability, resistance to traction and high ability to retain and absorb water. BC is also biodegradable and easily purified using NaOH solution[21-22]. All those unique and advantageous properties are mainly derived from its ultrafine network structure (with approximately 1.5 nm in width) and enable BC to be applied in various fields such as medicinal (TE), environmental, food and cosmetics purposes[23].

Hydroxyapatite and other calcium phosphates for biomedical purposes

Calcium phosphates (CPs) are the main constituents of mineral phase of bones and teeth in vertebrates. For this reason, synthetic CPs have osteoconductive and osteoinductive properties and have been widely used for bone tissue regeneration and augmentation. CPs as biomaterials are generally classified according to composition as calcium hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$; alpha- or beta-tricalcium phosphate (α- or β-TCP), $Ca_3(PO_4)_2$; biphasic calcium phosphates (BCPs) for mixtures of HA and β-TCP; and unsintered apatites or calcium-deficient apatites (CDAs)[24-28]. Solubility and biological properties of those materials are extremely dependent on crystal size, ionic impurities, specific surface area, and both macroporosity and microporosity. Cell colonization, for example, is possible in those CPs materials since it is induced by associating CPs with organic substances which are calcined before sintering to achieve convenient porosity[29-31].

Despite having exceptional characteristics, such as a good integration with the host bone tissue and make the environment more favorable for bone regeneration, due to their cytocompatibility, synthetic CPs commonly present poor biomechanical properties arising from their weak tensile strength and inherent brittleness. To overcome this limitation and enable clinical applications, an alternative solution has been to add internal or external factors or even to associate synthetic or natural polymers with CPs forming composites[16].

BC-CPs composites for BTE applications: methods of synthesis and applications

BC fabrication, as noted above, is resulting of the activity some kind of Gram-negative acetic bacteria. There are two methods to fabricating BC depending on the purposes, namely as stationary and agitated culture. Pellicles are formed under static culture, by the accumulation of a gelatinous membrane at the air/liquid interface and fibrils or spherical-like particles are obtained under agitated culture conditions[19,32].

Considering the macroscopic morphology of the resulting composite, there are three pathways to prepare BC/HA composites, which can be extended to BC/CPs composites: (i) *in situ* growing of CPs into culture media; (ii) *ex situ* synthesis of CPs in BC fibers or for physical mixing of those two materials; (iii) synthesis of BC composite from BC solution. Fig. 1 presents a schematic representation of each approach for prepare those composites[16].

The *in situ* approach consists of adding the reinforcement CPs material into BC culture media at the beginning of BC cultivation in agitated or static conditions (Fig. 1a). This process has a great advantage of encaging materials that become part of the fibrils, modifying and enhancing substantially the physico-mechanical properties of BC fibrils, however presents a critical limitation when involves the incorporation of reinforcement materials that also have antibacterial activity against BC strains. From this point of view, CPs are suitable to be incorporated into culture media, since some studies have revealed that HA, for example, has been successfully suspended before BC cultivation without toxic effects against BC producer strains. Another disadvantage is related with the short time in which those particles remain suspended in BC synthesis media. To overcome this problem, the researchers have

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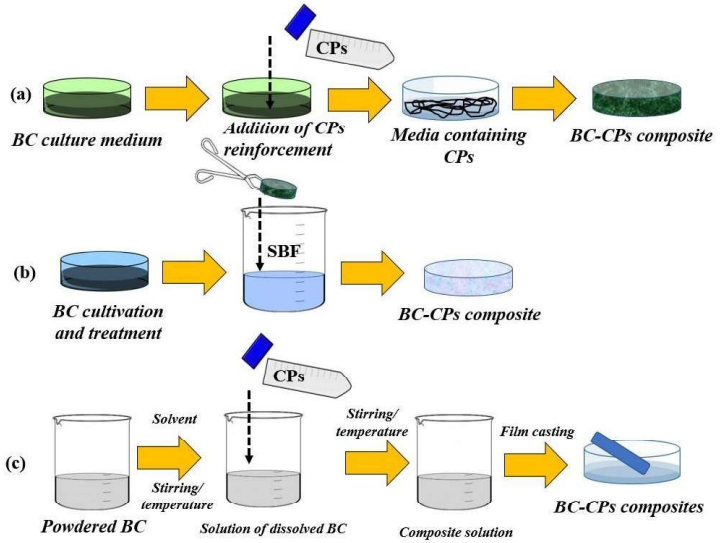

Fig. 1. (a) Schematic representation of BC composites produced through in situ synthetic strategy. Particles entrapped between growing BC fibrils. (b) Schematic representation of BC composites synthesized through an ex situ synthetic strategy. (c) Schematic representation of BC composites synthesized from dissolved BC solutions. The composite solution is casted to prepare BC films.

proposed strategies such as BC synthesis in agitated conditions or developing vessels equipped with spinning discs. Agitation culture, as discussed before, produces fibrils and cannot be applied to produce gels or sheets used in biomedical applications[16,33].

The *ex situ method*, as noted in Fig 1b, involves the incorporation of liquids or nanoparticles into BC structural matrix. The application of this method is successful when the reinforcement materials incorporated have suitable size and reactivity to the group OH of BC.

For this reason, only submicron and nanoparticles can be entrapped through hydrogen bonds with OH group in the BC matrix for this strategy, being an efficient and simpler method to obtain sheets and gels from static cultures.

The synthesis of BC composites from BC solutions is the best method to fabricate a large variety of different composites with more control of BC matrix composites and reinforcement materials. One of the most critical challenges is to find a suitable solvent to solve BC membranes and generally those solvents cause damages to BC structure. We summarized in Table 1 the most recent studies in which those strategies are employed to produce BC-CPs composites and the main findings of each one. These studies were found by combining the keywords "bacterial cellulose" and ("calcium phosphate" or hydroxyapatite) in *Web of Science* database in June 2018.

According to those reports, it is noted that in most studies, *ex situ* strategy has been chosen to prepare BC-CPs composites. In this approach, CPs are incorporated after BC cultivation process by soaking BC membranes in simulated body fluid (SBF) with an ion concentration equivalent to human blood plasma at physiological conditions of pH and temperature to stimulate biomineralization or in alternate solutions of Ca^{2+} and $PO4^{3-}$ ions[16,48].

Among the surveyed studies, Grande et al.[47] and Romanov et al.[44] were the only ones to relate the *in situ* addition of HA to BC culture medium in order to produce BC-HA composites. Both of them previously synthetized HA via a wet chemical precipitation and then the apatite was incorporated to the culture medium. However, considering that the viscosity of the culture medium was not ideal to suspend homogeneously HA particles, Grande et al.[47] modified it, by adding carboxymethylcellulose (CMC) at 1 or 2 % (w/v). The incorporation of CMC in the culture medium, during the formation of cellulose fibrils caused a decrease of almost 50% in average diameter of cellulose fibrils and an increase of 47.8% in pore size. Also, in accordance to the authors, an amount of 22% of HA was not entrapped in BC nanocomposite.

Romanov et al.[44], in turn, have suspended HA in BC culture medium without adjusting of viscosity and according to their results, have had the formation of partly textured HA on the surface of the cellulose fibrils. Actually, in this study, other two methods have been used to prepare BC-HA composites. In order to implement them, HA was suspended in the process of dispersion of BC nanogel films or synthetized in the suspended BC medium. All those methods of BC-HA have produced materials with potential biomedical applications, although their biological properties were not assessed.

Several studies have been carried out with BC membranes *in vitro* in preclinical assays for biomedical purposes. These studies have investigated the application of BC in drug, hormone and protein release systems, artificial skin, wound dressing, artificial cartilage, menisci, invertebral disks, dental implants valvular prostheses, artificial cornea and the urethrata[49-54]. Therefore, the researches involving the use of BC membranes for tissue engineering applications have increased considerably in number, over the last few years, according to the Fig. 2. These research works are based on bacterial cellulose in tissue engineering and bacterial cellulose/calcium phosphates composites.

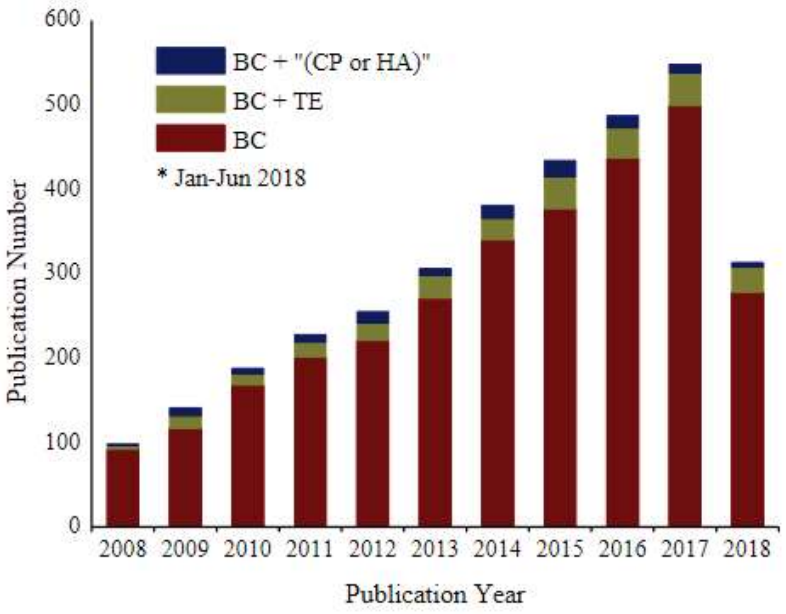

Fig. 2 - Annual publications of BC, BC with TE applications and BC-CPs composites since 2008 to June 2018. The search was carried out in *Web of Science*™.

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BC-CPs composites	Findings	Reference
BC-HA composites using silk fibroin as reinforcing phase	Silk fibroin from two different species was incorporated to BC membranes to improve mechanical properties of BC-HA composites. Bacterial cellulose-*Antheraea yamamai* silk fibroin/hydroxyapatite had advantages over Bacterial cellulose-*Bombyx mori* silk fibroin/hydroxyapatite and Bacterial cellulose/Hydroxyapatite on mechanical strength and *in vitro* cytocompatibility, being the most indicated for applications in BTE.	[34]
BC-HA composites produced *ex situ*	BC was firstly modified with PVP and after activated with $CaCl_2$ 0.1 $mol.L^{-1}$ for three days to be soaked with 1.5 SBF and produce BC-HA. In order to mimic cartilage tissue, BC was treated with chondroitin sulfate salt sodium (BC-GAG). Attachment and *in vitro* proliferation of osteoblasts and hACs were supported, respectively. *In vitro* studies revealed that those BC bilayer scaffolds accelerated the regeneration of articular cartilage and subchondral bone in a rat model.	[35]
BC-gelatin/HA composites	BC-gelatin/HA presented rougher surface topography, higher thermal stability and mechanical strength when compared with BC/HA composites and *in vitro* cell culture of rat bone marrow-derived mesenchymal stem cells cultured in BC-gelatin/HA revealed better adhesion, proliferation and differentiation than the cells cultured in BC-gelatin.	[36]
BC modified with chondroitin sulfate (added in culture medium).	BC was produced with addition of chondroitin sulfate with good *ex situ* calcium phosphate deposition. Those materialscan be applied to guided bone regeneration.	[37]
BC-β-TCP-HA based hydrogel scaffolds with addition of CMC or PVP to culture media.	BC based hydrogels were produced by adding polyvinylpyrolidone (PVP) and carboxymethylcellulose (CMC) to BC suspension and after that suspending β-TCP and HA. $CaCO_3$ was then incorporated in order to achieve biomineralization and an efficient interaction between the implant and the host bone. Hydrogels scaffolds were produced by solvent cast technique.	[38]
BC-HA composites with adjust of mechanical properties	BC gel-film was firstly produced. The BC-HA composites were prepared *ex situ*, increasing BC fraction to modify mechanical properties of the resulting composites. The authors proposed the use of those materials for bonereplacement/fillers.	[39]
Calcium phosphates grown on BC as template	In this report, the authors proposed the use of BC previously cultivated in static conditions as a template to grow CPs deposited by soaking/ultrasound technique. CPs were non-toxic and suggested to be applied as cements/fillers.	[18]
Modification of BC culture media with hyaluronic acid 1% (w/w) and *ex situ* biomimetic precipitation of CPs	BC fermentation medium was modified with hyaluronic acid 1% (w/w) and irradiated with gamma radiation in order to favor biomedical applications. After that, biomimetic precipitation of CP was performed from simulated body fluid (SBF), for perspectives in dental materials scaffolds applications.	[40]
3D nanofibrous BC-based templates with varying surface chemistry	BC pellicles were produced and its surface was modified by coating on it various materials. Calcium phosphate formation for each modified surface was investigated by X-ray absorption near-edge structure (XANES) spectroscopy.	[41]
BC-HA associated with bone growth peptides (OGP) or pentapeptide OGP (10-14)	BC-HA, BC-HA-OGP and BC-HA-OGP(10-14) were assayed to evaluate their potential in bone regeneration in critical- size calvarial defects. It was verified that BC-HA was efficient for bone regeneration in critical-size conditions.	[42]
Modification of BC culture medium by adding chondroitin and hyaluronic acid (1% w/w) and *ex situ* biomimetic precipitation of CPs	BC fermentation medium was modified with chondroitin and hyaluronic acid 1% (w/w). After that, biomimetic precipitation of CP was performed from simulated body fluid (SBF), for perspectives in dental materials scaffolds applications.	[43]
BC-HA composites synthetized by different techniques	BC-HA composites were produced by combined aggregation of HA and BC suspensions, introduction of the HA suspension in the culture medium (*in situ*) and synthesis of HA in the medium of dispersed cellulose.	[44]

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Sand Dollar skeleton coated by BC and CP	The skeleton of sand dollar (*Clypeaster subdepressus*), that is formed by interconnected pores, was coated by BC and then coated with calcium phosphates. Sand dollar skeleton provides a suitable geometry for bone regeneration, while BC/CP offers a bioactive surface for cell adhesion.	[45]
BC-CPs composites with CPs from different precursors	In this report, BC-CPs composites were synthetized by deposition of CPs previously synthetized by three different compositions. The introduction of a small number of Mg^{2+} ions (~5% wt), according to the authors, favored the crystallization process of CPs.	[46]
BC/ calcium deficient HA composite	Carboxymethilcellulose (CMC) was added to the BC culture medium to improve its viscosity and facilitate the posterior suspension of HA, before the formation of BC nanofibrils. HEK cells were cultivated, demonstrating its biocompatibility and cell viability.	[47]

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Conclusions

With the growing demand for new biomaterials for bone replacement and repair, the manufacturing of BC-CPs composites has been a viable alternative once those materials are able to mimic extracellular matrix of native bone, that is primarily compound by hydroxyapatite and fibrous collagen, namely an organic-inorganic composite. CP materials are bioactive and osteoconductive and if produced with suitable geometry and topography, have the potential to be osteoinductive, becoming closer to the "gold standard" autogenous therapy. In alignment to this, some studies have been translated into efforts to enhance biomechanical performance of those composites and also the rates of cell proliferation, migration, adhesion and differentiation, by associating other materials to BC-CPs systems.

References

1 Li J, Baker B A, Mou X, Ren N, Qiu J, Boughton R I and Liu H. Biopolymer/calcium phosphate scaffolds for bone tissue engineering. *Adv Healthcare Mater* 3:469-484 (2014).

2 .Lai G J, Shalumon K T and Chen J P. Response of human mesenchymal stemcells to intrafibrillar nanohydroxyapatite content and extrafibrilar nanohydroxyapatite in biomimetic chitosan/silk fibroin/nanohydroxyapatite nanofibrous membrane scaffolds. *International Journal of Nanomedicine* 10:

7 567-584 (2015).

3. Atak B H, Buyuk B, Huysal M, Isik S, Senel M, Metzger W, Cetin G. Preparation and characterization of amine functional nano-hydroxyapatite/chitosan bionanocomposite for bone tissue engineering applications. *Carbohydrate Polymers* 164: 200-213 (2017).

4. Duarte E B, Chagas B S, Andrade F K, Brígida A I S, Borges M F, Muniz C R, Souza Filho M S, Morais J P S, Feitosa J P A and Rosa M S. Production of hydroxyapatite–bacterial cellulose nanocomposites from agroindustrial wastes. *Cellulose* 22:3177–3187 (2015).

5. Lysenko O, Dubok O, Borysenko A and Shinkaruk O. The biological properties of the silver- copper-doped ceramic biomaterial. *J Nanopart Res* 17: 178 (2015).

6. Ahn S J, Shin Y M, Kim S E, Jeong S I, Jeong J O, Park J S, Gwon H J, Seo D E, Nho Y C, Kang S S, Kim C Y, Huh J B and Lim Y M. Characterization of hydroxyapatite-coated bacterial cellulose scaffold for bone tissue engineering. *Biotechnology and Bioprocess Engineering* 20: 948-955 (2015).

7. Ramani D and Sastry T P. Bacterial cellulose-reinforced hydroxyapatite functionalized graphene oxide:a potential osteoinductive composite. *Cellulose* 21:3585–3595 (2014).

8. Pandey A R, Singh U S, Momin M, Bhavsar C. Chitosan: application in tissue engineering and skin grafting. *J Polym Res* 24: 125 (2017).

9. Katari R S, Peloso A, Orlando G. Tissue engineering. *Adv Surgery* 48: 137-154 (2014).

10. Atala A. Regenerative Medicine and Tissue Engineering *Urol Clin N Am* 36 (2009) 199-209.

11. Antunes J C, Oliveira J M, Reis R L, Soria J M, Gómez-Ribelles J L and Mano J F. Novel poly(L-lacticacid)/hyaluronic acid macroporous hybrid scaffolds: characterization and assessment of cytotoxicity. *J Biomedical Mater Res A* 94A: 856-869 (2010).

12. Atala A. Bioengineering organs. *Current Opinion in Biotechnology* 20: 575-592 (2009).

13. Favi P M, Ospina S P, Kachole M, Gao M, Atehortua L and Webster T J. Preparation and characterization of biodegradable nano hydroxyapatite–bacterial cellulose composites with welldefined honeycomb pore arrays for bone tissue engineering applications. *Cellulose* 23:1263–1282 (2016).

14. Fragal E H, Cellet T S P, Fragal V H, Companhoni M V P, Ueda-Nakamura T, Muniz E C, Silva R and Rubira A F. Hybrid materials for bone tissue engineering from biomimetic growth of hydroxiapatite on cellulose nanowhiskers. *Carbohydrate Polymers* 152:734–746(2016).

15. Yin N, Chen S Y, Ouyang Y, Tang L, Yang J X and Wang H P. Biomimetic mineralization synthesis of hydroxyapatite bacterial cellulose nanocomposites. *Progress in Natural Science: Materials International*

9 21: 472-477 (2011).

10 16. Torgbo S, Sukyai P. Bacterial cellulose-based scaffold materials for bone tissue engineering. *Applied*

11 *Materials Today* 11,34-49 (2018).

17. Rajwade J M, Paknikar K M and Kumbhar J V. Applications of bacterial cellulose and its composites in biomedicine. *Appl Microbiol Biotechnol* 99:2491–2511 (2015).

18. Busuioc C, Stroescu M, Stoica-Guzun A, Voicu G and Jinga, S I. Fabrication of 3D calcium phosphates based scaffolds using bacterial cellulose as a template. *Ceramics International* 42, 15449-15458 (2016).

19. Qiu K and Netravali A N. A review of fabrication and applications of bacterial cellulose based nanocomposites. *Polymer Reviews*, 54:598–626 (2014).

20. Luo H, Zhang J, Xiong G and Wan Y. Evolution of morphology of bacterial cellulose scaffolds during early culture. *Carbohydrate Polymers* 111: 722–728 (2014).

21. Saska S, Barud H S, Gaspar A M M, Marchetto R, Ribeiro S J L and Messaddeq Y. Bacterial celulose-hydroxyapatite nanocomposites for bone regeneration. *International Journal of Biomaterials* 1- 8 (2011).

22. Tazi N, Zhang Z, Messaddeq Y, Lopes L A, Zanardi L M, Levinson D and Rouabbia M. Hydroxyapatite bioactivated bacterial cellulose promotes osteoblast growth and the formation of bone nodules. *Express*

24 2:61(2012).

23. Lima L R, Santos D B, Santos M V, Barud H S, Henrique M A, Pasquini D, Pecoraro E and Ribeiro S J L. Nanocristais de celulose a partir de celulose bacteriana. *Química Nova* (38) 9, 1140-1147 (2015).

24. Ficai D, Ficai A, Melinescu A and Andronescu E. Nanotechnology: a challenge in hard tissue engineering with emphasis on bone cancer therapy. Chapter 20. In: *Nanostructures for Cancer Therapy*. 513–539 (2017).

25. Samavedi S, Whittington A R, Goldstein A S. Calcium phosphate ceramics in bone tissue engineering: A review of properties and their influence on cell behavior. *Acta Biomaterialia* 9, 8037–8045 (2013).

26. Santos M V B, Osajima, J A, Silva Filho E C. Hidroxiapatita: suporte para liberação de fármacos e propriedades antimicrobianas. *Ceramica* 62, 256-265 (2016).

27. Feitosa G T, Santos M V B, Osajima J A, Barreto H M, Silva Filho E C. Hydroxyapatites obtained from different routes and their antimicrobial properties. *Materials Science Forum* 869 (2016).

28. Vieira E G, Vieira, T W S V, Silva M P, Santos M V B, Brito C A R S, Bezerra R D S, Fialho A C V, Osajima J A, Filho E C S. Tuned hydroxyapatite materials for biomedical applications. Chapter 6. In:*Biomaterials - Physics and Chemistry*, 87-104 (2018).

29. Verron E, Khairoun I, Guicheux J and Bouler J M. Calcium phosphate biomaterials as bone drug delivery systems: a review. *Drug Discovery Today* 15 (2010).

30. Parent M, Baradari H, Champion E, Damia C and Viana-Trecant M. Design of calcium phosphate ceramics for drug delivery applications in bone diseases: A review of the parameters affecting the loading and release of the therapeutic substance. *Journal of Controlled Release* 252: 1–17 (2017).

31. Denry I and Kuhn L T. Design and characterization of calcium phosphate ceramic scaffolds for bone tissue engineering. *Dental Materials* 32: 43-53 (2016).

32. Yan Z, Chen S, Wang S, Wang B and Jiang J. Biosynthesis of bacterial cellulose/multi-walled carbonnanotubes in agitated culture. *Carbohydrate Polymers* 74 659–665(2008).

33. Shah N, Ul-Islam M, Khattak W A and Park J K.Overview of bacterial cellulose composites: a multipurpose advanced material. *Carbohydrate Polymers* 98:1585– 1598 (2013).

34. Jiang P, Ran J, Yan P, Zheng L, Shen X and Tong H. Rational design of a high-strength bone scaffold platform based on in situ hybridization of bacterial cellulose/nanohydroxyapatite framework and silk fibroin reinforcing phase. *Journal of Biomaterials Science* 29: 107-124 (2018).

35. Kumbhar J V, Jadhav S H, Bodas D S, Barhanpurkar-Naik A, Wani M R, Paknikar K M and Rajwade J M. In vitro and in vivo studies of a novel bacterial cellulose-based acellular bilayer nanocomposite scaffold for the repair of osteochondral defects. *International Journal of Nanomedicine* 12:6437–6459 (2017).

36. Ran J, Jiang P, Liu S, Sun G, Yan P, Shen X and Tong H. Constructing multi-component organic/inorganic composite bacterial cellulose-gelatin/hydroxyapatite double-network scaffold platform for stem cell-mediated bone tissue engineering. *Materials Science and Engineering* C 78: 130–140 (2017).

37. Olyveira G M, Basmaji P, Costa L M M, Santos M L, Riccardi C S, Guastaldi F P S, Scarel-Caminaga R M, Capote T S O, Pizoni E and Guastaldi A C.Surface physical chemistry properties in coated bacterial cellulose membranes with calcium phosphate. *Materials Science and Engineering* C 75: 1359–1365 (2017).

38. Basu P, Saha N, Bandyopadhyay S and Saha P. Rheological performance of bacterial cellulose based nonmineralized and mineralized hydrogel scaffolds. AIP Conference Proceedings 1843: 050008 (2017).

39. Arkharova N A, Suvorova E I, Severin A V, Khripunov A K, Krasheninnikov S V, Klechkovskaya, V V. SEM and TEM for structure and properties characterization of bacterial cellulose/hydroxyapatite composites. *Scanning* 38: 757–765 (2016).

40. Olyveira G M, Santos M L, Riccardi C D, Costa L M M, Daltro P B, Basmaji P, Daltro G D and Guastaldi,A C. Physically modified bacterial cellulose biocomposites for guided tissue regeneration. *Science of Advanced Materials* 7: 1657-1664 (2015).

41. Luo H, Xiong G, Zhang C, Li D, Zhu Y, Guo R and Wan Y. Surface controlled calcium phosphate formation on three-dimensional bacterial cellulose-based nanofibers. *Materials Science and Engineering* C 49:526–533 (2015).

42. Pigossi S C, Oliveira G L P L, Finoti L S, Nepomuceno R, Spolidorio L C, Rossa Jr. C., Ribeiro S J L, Saska S and Scarel-Caminaga R M. Bacterial cellulose-hydroxyapatite composites with osteogenic growth peptide (OGP) or pentapeptide OGP on bone regeneration in critical-size calvarial defect model. *Journal of Biomedical Materials Research* A 103A: 3397-3406 (2015).

43. Olyveira G M, Santos M L, Costa L M M, Daltro P B, Basmaji P, Daltro G D and Guastaldi A C. Bacterial Cellulose Biocomposites for Guided Tissue Regeneration. *Science of Advanced Materials* 6: 2673-2678 (2014).

44. Romanov D P, Khripunov A K, Baklagina Y G, Severin A V, Lukasheva N V, Tolmachev D A, Lavrent'ev V K, Tkachenko A A, Arkharova N A and Klechkovskaya V V. Nanotextures of Composites Based on the Interaction between Hydroxyapatite and Cellulose Gluconacetobacter xylinus. *Glass Physics and Chemistry* 40: 367-374 (2014).

45. Barreiro A M, Recouvreux D O S, Hotza D, Porto L M and Rambo C R. Sand dollar skeleton as templates for bacterial cellulose coating and apatite precipitation. *J Mater Sci* 45:5252–5256 (2010).

46. Romanov D P, Baklagina Y U, Gubanova G M, Ugolkov V L, Lavrent'ev V K, Tkachenko A A, SinyaevV A, Sukhanova V E and Khripunov A K. Formation of organic–inorganic composite materials based on cellulose acetobacter xylinum and calcium phosphates for medical applications. *Glass Physics and Chemistry* 36: 484-493 (2010).

47. Grande C J, Torres F G, Gomez C M and Bañó M C. Nanocomposites of bacterial cellulose/hydroxyapatite for biomedical applications. *Acta Biomaterialia* 5: 1605–1615 (2009).

48. Lai C, Zhang S J, Wang L Q, Sheng L Y, Zhou Q Z and Xi T F. The relationship between microstructure and in vivo degradation of modified bacterial cellulose sponges. *J.Mater. Chem.* B,2015, 3: 9001-9010 (2015).

49. Hou Y, Wang X, Yang J, Zhu R, Zhang Z and Li Y. Development and biocompatibility evaluation of biodegradable bacterial cellulose as a novel peripheral nerve scaffold. *Journal of Biomedical Materials Research Part A* 106: 1288-1298 (2018).

50. Rashid E S A, Julkapli N M and Yehye W A. Nanocellulose reinforced as green agent in polymer matrix composites applications. *Polymers for Advanced Technologies* 29, 1531-1546 (2017).

51. Tronser T, Laromaine A, Roig A and Levkin. Bacterial cellulose promotes long-term stemness of mESC. ACS Appl. Mater. *Interfaces* 10 (19): 16260–16269 (2018).

52. Park M, Lee D, Shin S and Hyun J. Effect of negatively charged cellulose nanofibers on the dispersion ofhydroxyapatite nanoparticles for scaffolds in bone tissue engineering. *Colloids and Surfaces B: Biointerfaces* 130: 222–228 (2015).

53. Wan Y, Zuo G, Yu F, Huang Y, Ren K and Luo H. Preparation and mineralization of three-dimensional carbon nanofibers from bacterial cellulose as potential scaffolds for bone tissue engineering. *Surface & 22 Coatings Technology* 205:2938–2946 (2011).

54. Yang M, Zhen W, Chen H and Shan Z. Biomimetic design of oxidized bacterial cellulose-gelatin-hydroxyapatite nanocomposites. *Journal of Bionic Engineering* 13:631–640 (2016).