Abstract

Hydroxyapatite stands out between biomaterials due to its properties of osteoconduction and osteoinduction, being adequate to be used in bone grafts. The high stability and flexibility of the structure allows for several biomedical applications, for example, the use as polysaccharide based on the scaffold formulations and the cationic substitutions occurring through the doping of the material using metals, which may enhance biological characteristics, such as improving the action of combating bacterial infections in situ. This study was a research of articles and patents, without and with time restriction (2007–2017), which contain information about hydroxyapatite in the tissue engineering, biomedical, doped with cerium and its properties of antibacterial activity. There were also searches of products and companies that commercialize these types of materials aimed at tissue engineering area. Scopus was used for searched of articles and were EPO, USPTO, and INPI used for patents, and to search for products and companies were used search engines. Few papers were found to associate all the keywords, but the ones found are recent works, thus showing a new area with potential to be investigated.

Keywords: hydroxyapatite, scaffold, doping, antibacterial activity
1. Introduction

Tissue bioengineering is the science that involves the applications of engineering and health sciences to assist and accelerate the regeneration of defective and damaged tissues in the human body. It aims to create and improve new therapies and to develop new biomaterials that can be used to restore, improve, or prevent worsening of compromised tissue function such as in situations with the loss of tissue integrity resulting from trauma, developmental deformities and diseases [1, 2].

In the case of loss or compromise of the bone tissue, several natural or synthetic biomaterials such as polymers, ceramics, and metals or their composites have been investigated and used as a substitution alternative in different ways. The main alternative for damaged or lost bone tissue replacement is the autogenous bone graft. This is the first alternative to be used for the regeneration of the bone tissue due to its osteogenic properties [3].

However, grafts have limited the availability due to the need for surgical procedures with possible local infections, rejection by the transplanted organism, and progressive reabsorptions of the material. As a result, scientific research is developing new biomaterials for its replacement. Synthetic grafts can be an interesting alternative, due to intrinsic characteristics such as biocompatibility and chemical similarity with the bone tissues of living beings, allied with their properties of osteoconduction and osteoinduction [4–8].

Bioceramics is the class of ceramics used for repair and replacement of diseased and damaged parts of musculoskeletal systems. They are the most widely used materials in the class of traumas such as calcium phosphates, hydroxyapatite (HAp) (Ca_{10}(PO_4)_6(OH)_2), octacalci-umphosphate (CaH_2(PO_4)_{0.5}H_2O), calcium pyrophosphate dihydrate (Ca_3P_2O_7.2H_2O), and β-tricalcium phosphate (Ca_3(PO_4)_2) [9]. Calcium phosphates are classified according to a molar ratio of calcium and phosphorus Ca/P ranging from 0.5 to 2.0. HAp is the most widely used component in biomedical applications for phosphates and is the main component of the bone and is known for its excellent cellular and tissue affinity. HAp is widely used in tissue regeneration and biomedical applications in the form of coatings on metal implants, bone and nerve tissue graft production, drug release agents, wound protection, cell culture substrates, enzymatic immobilization, bone prosthesis or graft coatings, due to their excellent biocompatibility, osteoconduction property, and similarity with the inorganic component of the natural bone [10–12].

This biomaterial has the ability to establish chemical bonds with the living tissue of the bone due to its structure and chemical composition that are similar to apatite, which is found in the human skeleton. In addition, the biocompatibility and bioactivity of HAp can promote the proliferation of osteoblasts that are new bone-forming tissue. Other studies with these materials also cover the areas of biology, chemistry, materials engineering, and so on [13–15].

Porous three-dimensional scaffolds based on HAp are the ideal materials mostly used in modeling, reconstructing, and forming new bone tissues. Scaffolds adapt indirectly to the tissue and favor tissue differentiation, migration, and proliferation or osteoblastic formation [16]. However, it is not possible to use the HAp alone as scaffolds due to mechanical defects.
The combination of biodegradable polymers and bioactive inorganic materials ultimately improves the mechanical properties, biocompatibility, and cellular affinities of individual components [17].

Biocomposites based on natural biopolymers are being studied and associated with HAp due to the biocompatible and biodegradable behavior of some of these natural polymers. This new generation of biomaterials combines with bioactive properties that resemble the natural function of bone, triggering tissue regeneration mechanisms in vivo [18, 19]. Biocomposites HAp-biopolymers that often closely resemble the position and structure of mineralized tissues provide excellent mechanical properties and favorable biological properties, proving to be an ideal candidate for tissue engineering as well as orthopedic and dental applications [20].

Anionic polysaccharides, such as alginate, hyaluronic acid, silk fibroin, cellulose and natural gums, and others, such as chitosan, are excellent alternatives for improving the biocompatibility of HAp when it is in association. These biocomposites are potential models for the mineralization of HAp because its anionic surface can bind the Ca\(^{2+}\) ions, besides controlling the nucleation and the growth of the crystal reduce the interfacial energy between the crystal and the surface. Several materials composed of HAp can be prepared using polysaccharides in the form of scaffolds for biomedical applications and bone tissue engineering [20, 21].

Many surgical procedures involve the formation of a chemical interface of the biomaterial/bone type and, consequently, the biological fixation, which the living bone structure penetrates the free space of the biomaterial, causing the permanent fixation of the bone. However, these procedures may lead to problems of bacterial infections that are difficult to control during the postoperative period, and consequently, the excessive use of antibiotics may not provide sufficient protection, causing the loss of bone material and generating resistant strains of bacteria, which are difficult to treat [22–24]. The defense mechanisms activated by the immune system can be reinforced through the introduction of antibacterial agents that have biological interaction with the biomaterial. One of the alternatives is the substitution capacity of HAp ions by doping, and these ions have antibacterial properties: silver (Ag\(^{+}\)), cupper (Cu\(^{2+}\)), zinc (Zn\(^{2+}\)), selenium (SeO\(_{3}^{2-}\)), strontium (Sr\(^{2+}\)), lanthanides (Ce\(^{3+}\), Ga\(^{3+}\), Sm\(^{3+}\)), and so on [25–28].

The sites that have the ions that compose Hap (Ca\(^{2+}\), PO\(_{4}^{2-}\), OH\(^{-}\)) can be occupied by ions of similar size and charge. This ability to incorporate ions through the doping is an alternative that is based on the fact that the introduction of small amounts of some ions can cause changes that improve the biological, physical-chemical, mechanical, and antimicrobial properties of the material [26, 29–33]. Thus, this study aims to present a search for articles and patent of technological inventions, which include information about hydroxyapatite in relation to its applications in the field of tissue engineering and doping with cerium ion for biomedical applications.

This work was conducted with the help of scientific articles and technological innovation patents and products present in the market. The articles in the SCOPUS database were used, and the keywords used were as follows: hydroxyapatite, scaffold, polysaccharide, doped, cerium, and antimicrobial activity. These keywords were combined with each other, and quotation marks were used for searching compound words.
Keyword research related to study topics was based on the information contained in the abstract, keywords, and titles. For the search of patents for technological innovation, the research was conducted in patent database: European Patent Office (EPO), United States Patent and Trademark Office (USPTO), and Brazil’s National Institute of Industrial Property (INPI).

Searches for products and companies were done by using search engines (Google, Bing, Yahoo, Bing, and Ask), and the keywords were as follows: hydroxyapatite, polysaccharides, biopolymers, scaffold, tissue engineering, odontology, osteoporosis, tooth, bone, cell growth, bone graft, and implants.

Access of both articles and patents was realized in May 2017, using the same fields of research and the same keywords for the search of articles. In the case of INPI, we used the words also in Portuguese. The researches of articles and patents were conducted in two ways: with and without time restriction from 2007 to 2017, and the search for products marketed was in September 2017 using the information provided in the catalogs and websites of the companies found in the database.

2. Results and discussion

2.1. Search for articles in the SCOPUS database

The investigation of the number of articles published revealed that the words are being combined, there is a decrease in the number of publications found, and, in some cases, there is no publication related to these words. The results were obtained using the separate and combined keywords such as hydroxyapatite, scaffolds, polysaccharide, doped, cerium, and antibacterial activity are shown in Table 1.

In Table 1, comparing the publication time of articles and analyzing the data, with and without time restriction between 2007 and 2017, it was observed that most of the publications are concentrated in this period. This shows that studies on the material have been increasing over the last decade. When using the combination of words such as hydroxyapatite and scaffold and polysaccharide and doped and cerium and antibacterial activity, which are the main keywords for this work, it is noted that no related article was found in the databases researched. The results show the specificity because there are no articles dealing with related words or the themes proposed by this manuscript.

The expression of words such as hydroxyapatite and scaffold and polysaccharide (Table 1) was found in 74 works between 1960 and 2017 and was found in 66 works between 2007 and 2017. However, only 46 articles are relatively of experimental scientific research. Thus, only the number of articles related to the abovementioned keywords (about 85.71%) was published in the last decade. In other words, this topic has been receiving more attention in the last decade from the global scientific community.

When combining the keywords such as hydroxyapatite and scaffold and polysaccharide and doped, two articles were found but only one of these is effectively experimental scientific
research; the other is a review. The article entitled “Bioactivation of knitted cellulose scaffolds by strontium” was published in the year 2008 by Brandt, Muller and Greil, researchers from the Materials Science Department of the University of Erlangen-Nuremberg, Germany. The article discusses the use of the properties of strontium (Sr$^{2+}$) in the treatment against osteoporosis, its anabolic and nonresorptive activity. The material used was in the scaffold form, which was prepared using a HAp doped with Sr$^{2+}$ plus doped cellulose composition. The study evaluated the kinetics of Sr$^{2+}$ release during static exposure to simulated body fluid to evaluate the precipitation of carbonated hydroxyapatite under conditions that simulate the inorganic part of human blood plasma.

The keywords hydroxyapatite and doped and cerium, which form the starting material for the scaffolds composition, according to the study of the articles found for the combinations (Tables 1 and 2) important information could be verified as method of synthesis, microorganisms used in antibacterial tests. In most of the articles, the goal is to develop a material with antibacterial activity and stimulate the formation of new bone tissues from the synthesis of hydroxyapatite doped with cerium. One of the articles exposes the association of cerium with strontium-doped hydroxyapatite in order to improve biological properties and antibacterial activity. Table 2 shows some of these articles and describes a relationship between the use of the synthesized materials and their applications, and Table 3 shows their respective objectives.

| Keywords                                      | Publications (1960–2017) | Publications (2007–2017) |
|----------------------------------------------|--------------------------|--------------------------|
| Hydroxyapatite                               | 49,983                   | 26,970                   |
| Scaffold                                      | 98,299                   | 81,265                   |
| Polysaccharide                               | 136,782                  | 60,187                   |
| Doped                                        | 333,539                  | 187,096                  |
| Cerium                                       | 73,813                   | 47,834                   |
| Antibacterial activity                       | 79,671                   | 48,238                   |
| Hydroxyapatite and scaffold                  | 5,191                    | 4,463                    |
| Hydroxyapatite and scaffold and polysaccharide| 74                       | 66                       |
| Hydroxyapatite and doped                     | 1,219                    | 1,089                    |
| Hydroxyapatite and doped and cerium          | 24                       | 20                       |
| Hydroxyapatite and doped and antibacterial activity | 71                       | 70                       |
| Hydroxyapatite and scaffold and polysaccharide and doped | 2                       | 2                       |
| Hydroxyapatite and scaffold and polysaccharide and doped and cerium | 0                       | 0                       |

Source: Authorship (2017).

Table 1. Number of publications found in the SCOPUS database.
It is possible to note that some of these works shown in Table 3 employ studies of the cerium-doped hydroxyapatite as well as the combination thereof with other metals such as strontium and zirconia and assess their ability to inhibit bacterial. This doping is possible due to the chemical structure of HAp that can accommodate a variety of cationic and anionic substitu-
ents.

Figure 1 shows the projection of the unit cell (hexagonal) of the hydroxyapatite when projected down the c-axis and shows an OH group in the center of the structure and the posi-
tion of the two types of calcium cations: calcium 1 (Ca(1)) and calcium 2 (Ca(2)) for a better understanding of how substitution of HAp ions occurs. Ca(1) atoms are located at the ends of a hexagonal unit cell, while Ca(2) atoms are in a more internal position around the OH group. The phosphate group (P) is the largest ion that constructs the unit cells [15].

The research in the article databases revealed that most of them develop materials for future applications of these in the field of tissue engineering, but there is still a lack of applications of these materials aimed at the field of tissue and biomedical engineering. In other words, the studied materials have the characteristics of inhibition of bacterial growth, but the search showed the nonuse of these biomaterials organized in the form of scaffolds and associated with some polysaccharides.

The four reported articles (Table 3) showed the main objective of doping of HAp and the improvement of its bacteriological growth inhibition properties. Majority of these articles, which aim for this purpose, present the methodologies used for the synthesis and characterization of the materials, and the biological assays are used to investigate the antibacterial properties of these materials. However, they do not elucidate how the mechanism of action of these materials with bacteria works.

| Material                                                                 | Method of synthesis          | Application                               | Author, year of publication |
|-------------------------------------------------------------------------|------------------------------|------------------------------------------|-----------------------------|
| Compound of HAp hydrogel based on xanthan gum                           | Soaking process              | Bone tissue engineering                   | Izawa et al., 2014          |
| Scaffold based on HAp and gum Arabic                                    | C coprecipitation and dissolution | Bone tissue engineering                   | Hadavi et al., 2017         |
| Scaffold nanofibrous cotton based on cellulose and nano-HAp              | Electrospinning              | Bone tissue engineering                   | Ao et al., 2017             |
| Hydroxyapatite co-substituted with strontium and cerium                 | Microwave irradiation        | Inhibition of *Staphylococcus aureus*, *Escherichia coli* | Gopi et al., 2014           |
| Reinforced hydroxyapatite composite with cerium doped glass             | Coprecipitation              | Inhibition of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* | Morais et al., 2015         |
| Hydroxyapatite doped with cerium (IV)                                   | Coprecipitation              | Inhibition of *Staphylococcus aureus*, *Escherichia coli* | Ciobanu et al., 2016        |
| Hydroxyapatite and fluorohydroxyapatite co-substituted with zirconia and cerium | Sol-gel                     | Inhibition of *Staphylococcus aureus*, *Escherichia coli* | Sanyal et al., 2016         |

Table 2. Materials synthesized in the articles and their applications.
One of the causes that leads to failure of conventional implants is the infection caused by bacteria; therefore, the articles aim to improve the biological properties of hydroxyapatite through doping with cerium. Due to this factor, ion has properties that stimulate the formation of new bone tissue and acts as an antibacterial agent. Bacteria, the main cause of infections, are classified as Gram-positive or Gram-negative depending on the difference in...
cell wall architecture. The Gram-positive cell wall consists of a thick layer of peptidoglycan, while the Gram-negative cell wall shows more complex membrane structure and composition. Gram-negative has the finest peptidoglycan layer and the outer surface of the cell has a membrane composed of proteins, lipopolysaccharides, and phospholipids called the outer membrane. The space between the peptidoglycan and the outer membrane is known as the periplasmic space; this space presents in some points enzymes and proteins and performs several physiological functions. Figure 2 shows the comparison between the compositions of the cell walls of Gram-positive and Gram-negative.

Figure 2 shows that the surface of Gram-positive bacteria is mainly covered by neutral and acidic polysaccharides, a large number of different proteins, theichoic acids, whereas the outer membrane of Gram-negative has an irregular distribution of lipids on the external and internal surface, which the outer face contains all lipopolysaccharides, while the inside face contains most of the phospholipids. Gram-positive bacteria are mostly studied bacteria in the articles, especially *Staphylococcus aureus*. *S. aureus* is an exceptionally well-adapted pathogen that can survive under different conditions, without particular nutritional or environmental requirements.

Over the years, infections caused by *S. aureus* have increased as one of the leading causes of bacterial infections in humans worldwide. In the last few decades, treatment of these infections has become more difficult, mainly because *S. aureus* develops mechanisms of resistance to the antibiotics used in the treatments. While for the Gram-negative, the most tested was *E. coli*, which, despite the reduced number in the cause of this type of infection, is a relevant group in clinical practice, presenting a difficulty in its treatment.

Gopi et al. produced nanoparticles of pure hydroxyapatite (n-HAp), hydroxyapatite doped with strontium (Ca/Sr.-HA), hydroxyapatite co-substituted with strontium and cerium (Ca/Sr./Ce-HA) in different concentrations of cerium (0.05, 0.075, and 0.1 mol/L) by using the microwave irradiation method. All the synthesized materials were investigated by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), field emission scanning...
The antibacterial activity of the nanoparticles was evaluated against two prokaryotic strains, *E. coli* and *S. aureus*, by using the disc diffusion method. The results showed that the Ca/Sr./Ce-HAp sample with the 0.1 mol/L concentration of cerium presented higher antibacterial activity in relation to the two strains tested when compared to HA and Ca/Sr./HAp results. According to Gopi et al. [39], Ce$^{3+}$ was important in increasing the antibacterial activity of the synthesized nanoparticle. In order to evaluate the bioactivity of the samples, they tested using simulated body fluid (SBF) for several days and observed that Sr$^{2+}$ and Ce$^{3+}$ ions contributed to the formation of apatite. It can be inferred that the synthesized Ca/Sr./Ce-HAp nanoparticle can be a promising biomaterial for biomedical applications. In the work of Morais et al. [40], a composite of hydroxyapatite reinforced with cerium-doped glass (GR-HAp-Ce) was developed. The phases formed in the synthesized material were identified using SEM techniques coupled with energy dispersed secondary (SEM-EDS) and X-ray diffraction (XRD). In addition to the hydroxyapatite phase, the material presented the β-TCP phases, and the authors concluded that the presence of cerium in the GR-HAp-Ce composite provided an effective antibacterial
effect against bacteria *S. aureus* and *S. epidermidis*, but this effect was not observed for the bacterium *P. aeruginosa*. In addition to investigating the antibacterial activity, the osteoconductive properties of the material were also evaluated, which was performed using human osteoblastic cells and showed that the addition of cerium did not affect the cellular viability of the material and that it showed good osteoconductive capacity.

Ciobanu et al. [41] synthesized nanoparticles of pure hydroxyapatite and cerium-doped hydroxyapatite (HAp-Ce) in different concentrations in the range of 1–25% (with a 5% variation) using the coprecipitation method and studied their antibacterial property. The effects of the replacement of cerium to calcium on the morphology, purity, crystallinity, crystallite size, and antibacterial capacity of cerium HA-substituted powders were investigated using scanning electron microscopy (SEM) coupled with X-ray analysis (XRD), X-ray excited photoelectron spectroscopy (XPS), infrared spectroscopy (FTIR), and Brunauer-Emmett-Teller (BET) surface area analysis confirming the formation of hydroxyapatite and the presence of Ce⁴⁺ and Ce³⁺ ions in its crystal lattice. The doped materials obtained better results of bacterial inhibition indicating that the presence of the ion contributed to the inhibition of bacteria; however, the nanopowders of HAp-Ce were more effective against the *E. coli* bacterium than against *S. aureus*.

Sanyal and Raja [42] studied the effect of the co-substitution of zirconium (Zr) and cerium (Ce) on the structure of hydroxyapatite (HAp) and fluorohydroxyapatite (FHA) gel. The samples were confirmed by the FTIR and XRD spectra; in addition, it was observed that with the increase of the concentration of the Zr⁴⁺ and Ce³⁺ ions, the formation of the HA phase was maintained. Co-substituted materials showed better results of antibacterial activity than pure hydroxyapatite. Materials with higher cerium concentration showed better bacterial inhibition against *E. coli* and *S. aureus* bacteria. All the articles studied describe that the presence of cerium ion in the structure of hydroxyapatite improved the antibacterial activity and also gave the material an improvement in bioactivity and may contribute to the formation of new bone tissues.

### 2.2. Search in the main patents databases

The results of the researches at European Patent Office (EPO), USPTO, and National Institute of Industrial Property (INPI) patents using the separate and combined keywords: hydroxyapatite, scaffolds, polysaccharide, doped, cerium and antibacterial activity are shown in Table 4.

Using the search keywords in English and Portuguese, it was possible to find patent deposits in the main patent databases. According to the data shown in Table 5, INPI found 74 patents deposited with the word hydroxyapatite and 10 patents when the word scaffold was used. For the combination of hydroxyapatite and scaffold, the result of a patent filed with the INPI under number 0905514-2 has been reported. The patent PI 0905514-2 provides a process for the scaffolding of a composite hydrogel biomaterial (CNHAP) based on chitosan (CN) and hydroxyapatite (HA), with potential for application in the medical-dental area, demonstrating the biocompatibility characteristics evidenced by classical in vitro assays. These characteristics are associated to the combination of physical-chemical and biological properties of the materials that compose it. CNHAP was obtained in the form of hydrogel with good mechanical characteristics, easy handling and modeling, high porosity, leading as promising as a bone filling material. The production of the CNHAP composite hydrogel scaffold was performed.
Table 4. Number of patents found in EPO, USPTO, and INPI databases.

| Keywords                                                                 | EPO     | USPTO   | INPI  |
|-------------------------------------------------------------------------|---------|---------|-------|
| Hydroxyapatite                                                         | 6,616   | 579     | 74    |
| Scaffold                                                                | >10,000 | 1870    | 10    |
| Polysaccharide                                                         | >10,000 | 3311    | 5     |
| Doped                                                                  | >10,000 | 24,312  | 22    |
| Cerium                                                                 | >10,000 | 2800    | 60    |
| Antibacterial activity                                                 | >10,000 | 1071    | 55    |
| Hydroxyapatite and scaffold                                            | 192     | 10      | 1     |
| Hydroxyapatite and scaffold and polysaccharide                         | 2       | 0       | 0     |
| Hydroxyapatite and doped                                               | 101     | 4       | 0     |
| Hydroxyapatite and doped and cerium                                     | 1       | 0       | 0     |
| Hydroxyapatite and doped and antibacterial activity                    | 2       | 0       | 0     |
| Hydroxyapatite and scaffold and polysaccharide and doped               | 0       | 0       | 0     |

Source: Authorship (2017).

Table 5. Characteristics of patents found in the EPO.

| Title                                                                 | Classification                  | Country | Abstract                                                                                                                                                                                                 |
|----------------------------------------------------------------------|---------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Polyether ether ketone/nanohydroxyapatite dental implant and         | A61L27/42; A61L27/12; A61L27/18;| China   | Preparation of a biomaterial for dental implant applications based on polyether ether ketone/nano-HAp doped with Ag⁺ and Zn²⁺ presenting antibacterial properties.                                               |
| manufacturing method thereof                                         | A61L27/54                       |         |                                                                                                                                                                                                          |
| Method for preparing antibacterial diamond-like carbon/hydroxyapatite | A61L27/30; A61L27/32; A61L27/54; | China   | Method for the preparation of a carbon/HAp base composite having antibacterial properties to be used as coating materials in the biomedical areas.                                                              |
| gradient multielement nanocoating                                    | C23C14/06; C23C14/35            |         |                                                                                                                                                                                                          |
| Porous polysaccharide scaffold comprising nanohydroxyapatite and     | A61L27/12; A61L27/20; A61L27/56 | France  | Method for the preparation of a scaffold composed of polysaccharide and hydroxyapatite used as support for tissue mineralization.                                                                         |
| use for bone formation                                                |                                 |         |                                                                                                                                                                                                          |
| Continuous gradient composite scaffold and                          | A61L27/26; A61L27/46            | China   | Scaffold composed of magnetic nanoparticles of hydroxyapatite/iron with high cellular biocompatibility and high mechanical resistance after the addition of the natural polysaccharide.                 |
| preparation method thereof                                           |                                 |         |                                                                                                                                                                                                          |

Source: Authorship (2017).
by an *in situ* mineralization procedure of the polymeric hydrogel of CN, by HA. This in situ mineralization method promoted mechanical and bioactivity characteristics to the CNHAP, which is suitable for the medical-dental application.

In addition to biocompatibility, tissue fillers should be able to promote cell adhesion, proliferation, and differentiation, essential requirements for tissue bioengineering, which have been increasingly explored within clinical practice. For the keywords such as *hydroxyapatite* and *scaffolds* and *polysaccharide* and *doped* and *cerium* and *antibacterial activity*, no deposited patents were found. Evaluating the results found, it can be understood that the results show the lack of patent filing implying that this area of research is promising.

In patent searches in the EPO database (*Table 5*), two patent records were found using the expression: *hydroxyapatite* and *doped* and *antibacterial activity*. Using the expression *hydroxyapatite* and *scaffold* and *polysaccharide*, also in the EPO, two patent records were found. *Table 6* shows the information about these patents found.

It is important to note that the polysaccharides cited in the patents (*Table 6*) were defined as a molecule composed of two or more molecules of monosaccharide units. Patents report the use of chitosan, hyaluronic acid, chondroitin sulfate, alginate, chitin, dextran, and other natural polysaccharides, which are the ideal extracellular matrix materials for the composition of scaffolds applied in the areas of tissue and biomedical engineering.

| Company                        | Trademarks          | Characteristics | Applicability       | Country      |
|--------------------------------|---------------------|-----------------|---------------------|--------------|
| JHS Biomateriais               | HAP-91              | Powder          | Bone graft          | Brazil       |
| JHS Biomateriais               | COL.HAP-91          | Scaffold        | Bone graft          | Brazil       |
| Bionnovation®                  | HAP –Bionnovation®  | Powder          | Bone graft          | Brazil       |
| Baumer                         | GenPhos HA TCP      | Powder          | Bone graft          | Brazil       |
| Oral science                   | Remix®              | Toothpaste      | Dental Products     | France       |
| Clarion Pharmaceutical Co.     | MCHC                | Tablets or capsules | Osteoporosis treatment | India       |
| SofSera                        | SHAp                | Powder          | Enxerto ósseo       | Japan        |
| SANGI CO. LTD.                 | Medical nano-hydroxyapatite | Toothpaste | Dental Products     | Japan        |
| Sewon Cellontech Co., Ltd.     | OssFill             | Gel             | Bone graft          | Korea        |
| GranuLab                       | GranuMas®           | Granules        | Bone graft          | Malaysia     |
| Fluidinova                     | nanoMIX®            | Powder          | Biomedical/Cosmetic | Portugal     |
| Berkeley Advanced Biomaterials Inc. | BABI-HAP-G2       | Granules        | Bone graft/Orthopedic Surgery | USA         |
| Berkeley Advanced Biomaterials Inc. | BABI-HAP-N100     | Powder          | Composites, for DNA and protein purification, or as a reference material. | USA         |

Source: Authorship (2017).

*Table 6.* Hydroxyapatite-based biomaterials available on the market.
The data showed that there is a small amount of number of patents related to tissue engineering; in other words, inventions associated with HAp with polysaccharides for the composition of scaffolds, which is the main theme of this work. In particular, there is a deficiency of biomaterials with antibacterial activity properties associated with HAp, polysaccharides, and scaffolds composition for bone tissue regeneration applications. Some of problems of implant may cause to the recipient organism have been addressed throughout this work, for instance, infectious problems originating from bacteria.

For better consistency to the results obtained, the product present in the market based on tuned hydroxyapatite and polysaccharide was examined. The research by companies specialized in the development and commercialization of hydroxyapatite-based biomaterials with applications in tissue engineering was conducted through search engines.

The searched keywords showed information about companies, products, and formulations. For example, the Brazilian company JHS Biomateriais develops a composite named HAP-91 constituted of porous hydroxyapatite, crystalline, biocompatible, pure, and widely tested as bone graft material and with excellent biocompatibility in living organisms. Besides, it is hydrophilic, and the powder can be used directly as a bone graft or it can be added to the patient’s own blood drops.

Another biomaterial developed by JHS Biomateriais is COL.HAP-91. The COL.HAP-91 is a collagen-hydroxyapatite composite spongy (25% collagen and 75% HAP-91), with the hemostatic properties of natural collagen fiber network purified, biocompatible, easy to handle, absorbable, and osteoinductive. Both products are registered with the Ministry of Health from Brazil and have a protected trademark at INPI. Its average market price for these materials ranges from €22.04 to 23.60 per 1000 g.

The Brazilian company Bionnovation®, in its product catalog, sells hydroxyapatite bone graft for applications in orthopedic, maxillofacial, and dental surgeries. The hydroxyapatite is synthesized from calcium hydroxide and phosphoric acid, and the product has radiopaque particles of varied sizes that support in the development of bone cells and assists the osteoconduction of bone-forming cells.

The US Company Berkeley Advanced Biomaterials Inc. develops hydroxyapatite, tricalcium phosphate, and other calcium-based products. The company’s business focuses on applications in orthopedic surgeries and bone graft. The European company Fluidinova synthesizes nanohydroxyapatite and markets through the nanoMIX® product. The biomaterial company supplies companies that manufacture medical devices, cosmetics, toothpastes, and other applications.

No material was found available for commercialization when the research was carried out with the words hydroxyapatite, tissue engineering, polysaccharides, and scaffold, even with the two patents deposited in the EPO (Table 4). However, when it uses the keywords such as scaffold and tissue engineering, companies and products from several countries were found, for example, Atex Technologies Inc. (China), Electrospinning Company (England), Bio-Scaffold International Pte Ltd. (Singapore), GeSiM (Germany), Matricel (Germany), Silkbiomaterial (Italy), ExCel Matrix Biological Devices (P) Ltd. (USA), and Nanofiber solutions (USA).
Table 6 shows some materials available on the market, that is, hydroxyapatite-based materials, used in the field of tissue engineering. Also present in the table are the data referring to companies, headquarters, characteristics, application and trademarks of some products on the market.

3. Conclusion

Analyzing the results, it can verify the use of hydroxyapatite in the areas of tissue engineering and bone regeneration. Many papers and technological innovation patents were found by searching only the keywords such as hydroxyapatite and scaffold. However, the combinations of the keywords mentioned in the experimental session showed that the number of articles and technology innovation patents was reduced. Synthesis of scaffolds is associated with natural polysaccharides or biopolymers due to their high biocompatibility, but the number of articles and patents decreases when it uses the hydroxyapatite and polysaccharides in the scaffold composition. Cerium-doped hydroxyapatite and its association with polysaccharides and biopolymers is an area that is still poorly studied and quite promising. This conclusion is supported by the small number of publications and patents. Therefore, the data presented for patent deposits and published articles show that there are no papers that contain the chosen and all combined keywords. For the use of these types of biomaterials with antibacterial properties, the research studies showed that the bacteria *Escherichia coli* and *Staphylococcus aureus* were the most investigated. This is explained considering that the bacteria are more accessible for research; in addition, *Staphylococcus aureus* is one of the most common agents present in bone infections. In the search for products, corporate brands, and companies in the areas of tissue engineering and bone regeneration, biomaterials that were found in the market used a hydroxyapatite in biomedical applications, bone graft, and composition of cosmetics. As for the association of hydroxyapatite and polysaccharides, no materials were found on the market when using the keywords such as hydroxyapatite, tissue engineering, polysaccharides, and scaffold.

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References

[1] Oliveira LSAF, Oliveira CS, Machado APL, Rosa FP. Biomateriais com aplicação na regeneração óssea – método de análise e perspectivas futuras. Revista de Ciência Médicas e Biológicas. 2010;9(1):37-44

[2] Tabata Y. Biomaterial technology for tissue engineering applications. Journal of the Royal Society Interface. 2009;6:S311-S324

[3] Kalambettu A, Dharmalingam S. Fabrication and in vitro evaluation of sulphonated poly-ether ether ketone/nano hydroxyapatite composites as bone graft materials. Material Chemistry and Physics. 2014;147:168-177

[4] Best SM, Porter AE, Thian ES, Huang J. Bioceramics: Past, present and for the future. Journal of the European Ceramic Society. 2008;28:1319-1327

[5] Dorozhkin SV. Bioceramics of calcium orthophosphates. Biomaterials. 2010;31:1465-1485

[6] Guillaume O, Geven MA, Sprecher CM, Stadelmann VA, Grijpma DW, Tang TT, Qin L, Lai Y, Alini, M, de Bruijn JD, Yuan H, Richards RG, Eglin D. Surface-enrichment with hydroxyapatite nanoparticles in stereolithography-fabricated composite polymer scaffolds promotes bone repair. Acta Biomaterialia. 2017;54:386-398

[7] Kawabata K, Yamamoto T, Kitada A. Substitution mechanism of Zn ions in β-tricalcium phosphate. Physica B: Condensed Matter. 2011;406:890-894

[8] Ryabenkova Y, Pinnock A, Quadros PA, Goodchild RL, Möbus G, Crawford A, Hatton PV, Miller CA. The relationship between particle morphology and rheological properties in injectable nano-hydroxyapatite bone graft substitutes. Materials Science and Engineering C. 2017;75:1083-1090

[9] Kawachi YE, Bertran CA, dos Reis RR, Alves OL. Biocerâmicas: Tendências e Perspectivas de uma Área Interdisciplinar. Química Nova [online]. 2000;4(3):518-522.

[10] Lin K, Wu C, Chang J. Advances in synthesis of calcium phosphate crystals with controlled size and shape. Acta Biomaterialia. 2014;10:4071-4102

[11] Sadat-Shojai M, Khorasani M, Dinpanah-Khoshdargi E, Jamshidi A. Synthesis methods for nanosized hydroxyapatite with diverse structures. Acta Biomaterialia. 2013;9:7591-7621

[12] Supová M. Substituted hydroxyapatites for biomedical applications: A review. Ceramics International. 2015;41:9203-9231

[13] An S, Matsumoto T, Miyajima H, Nakahira A, Kimc K, Imazato S. Porous zirconia/hydroxyapatite scaffolds for bone reconstruction. Dental Materials. 2012;28(12):1221-1231

[14] Oyefusi A, Olanipekun O, Neelgund GM, Peterson D, Stone JM, Williams E, Carson L, Regisford G, Oki A. Hydroxyapatite grafted carbon nanotubes and graphene nanosheets: Promising bone implant materials. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;132:410-416
[15] Gayathri B, Muthukumarasamy N, Velauthapillai D, Santhosh SB, Asokan V. Magnesium incorporated hydroxyapatite nanoparticles: Preparation, characterization, antibacterial and larvicidal activity. Arabian Journal of Chemistry (2017), In Press, http://dx.doi.org/10.1016/j.arabjc.2016.05.010

[16] Fook ACBM, Aparecida AH, Fook MVL. Desenvolvimento de biocerâmicas porosas de hidroxiapatita para utilização como scaffolds para regeneração óssea. Revista Matéria. 2010;3(15):392-399

[17] Ao C, Niu Y, Zhang X, He X, Zhang W, Lu C. Fabrication and characterization of electros-puncecellulose/nano-hydroxyapatite nanofibers for bone tissue engineering. International Journal of Biological Macromolecules. 2017;97:568-573

[18] Kikuchi M, Ikoma T, Itoh S, Matsumoto HN, Koyama Y, Takakuda K, Shinomiya K, Tanaka J. Biomimetic synthesis of bone-like nanocomposites using the self-organization mechanism of hydroxyapatite and collagen. Composites Science and Technology. 2004;64:819-825

[19] Swetha M, Sahithi K, Moorthi A, Srinivasan N, Ramasamy K, Selvamurugan N. Bio-composites containing natural polymers and hydroxyapatite for bone tissue engineering. International Journal of Biological Macromolecules. 2010;47:1-4

[20] Izawa H, Nishino S, Maeda H, Morita K, Ifuku S, Morimoto M, Saimoto H, Kadokawa J. Mineralization of hydroxyapatite upon a unique xanthan gum hydrogel by an alternate soaking process. Carbohydrate Polymers. 2014;102:846-851

[21] Hadavi M, Hasannia S, Faghihi S, Mashayekhi F, Zadeh HH, Mostofi SB. Novel calcified gum Arabic porous nano-composite scaffold for bone tissue regeneration. Biochemical and Biophysical Research Communications. 2017;448:671-678

[22] Nagano S, Yokouchi M, Setoguchi T, Sasaki H, Shimada H, Kawamura I, Ishidou Y, Kamizono J, Yamamoto T, Kawamura H, Komiya S. Analysis of surgical site infection after musculoskeletal tumor surgery: Risk assessment using a new scoring system. Sarcoma. 2014;2014:1-9

[23] Namba RS, Inacio MC, Paxton EW. Risk factors associated with deep surgical site infections after primary total knee arthroplasty: An analysis of 56,216 knees. The Journal of Bone & Joint Surgery. 2013;95:775-782

[24] Radovanovic Z, Jokic B, Velijovic D, Dimitrijevic VK, Petrovic R, Janackovic D. Antimicrobial activity and biocompatibility of Ag⁺- and Cu²⁺-doped biphasic hydroxyapatite/α-tricalcium phosphate obtained from hydrothermally synthesized Ag⁺ and Cu²⁺-doped hydroxyapatite. Applied Surface Science. 2014;307:513-519

[25] Ferraris S, Venturello A, Miola M, Cochis A, Rimondini L, Spriano S. Antibacterial and bioactive nanostructured titanium surfaces for bone integration. Applied Surface Science. 2014;311:279-291

[26] Kolmas J, Groszyk E, Kwiatkowska-Różyczka D. Substituted hydroxyapatites with antibacterial properties. BioMed Research International. 2014;2014:1-15
[27] Morais DS, Coelho J, Ferraz MP, Gomes PS, Fernandes MH, Hussain NS, Santos JD, Lopes MA. Samarium doped glass-reinforced hydroxyapatite with enhanced osteoblastic performance and antibacterial properties for bone tissue regeneration. Journal of Materials Chemistry B. 2014;2:5872-5881

[28] Mishra VK, Bhattacharjee BN, Parkash O, Kumar D, Rai SB. Mg-doped hydroxyapatite nanoplates for biomedical applications: A surfactant assisted microwave synthesis and spectroscopic investigations. Journal of Alloys and Compounds. 2014;614:283-288

[29] Aina V, Lusvardi G, Annaz B, Gibson IR, Imrie FE, Malavasi G, Menabue L, Cerrato G, Martra G. Magnesium- and strontium-co-substituted hydroxyapatite: The effect of doped ions on the structure and chemico-physical properties. Journal of Materials Science: Materials in Medicine. 2012;23:2867-2879

[30] Dorozhkin SV. Calcium orthophosphates in nature, biology and medicine. Materials. 2009;2:399-498

[31] Farzadi A, Bakshi F, Solati-Hashjin M, Asadi-Eydivand M, Osman NAA. Magnesium incorporated hydroxyapatite: Synthesis and structural properties characterization. Ceramics International. 2014;40(4):6021-6029

[32] Kannan S, Goetz-Neunhoeffer F, Neubauer J, Ferreira JMF. Ionic substitutions in biphasic hydroxyapatite and β-tricalcium phosphate mixtures: Structural analysis by Rietveld refinement. Journal of the American Ceramic Society. 2008;91:1-12

[33] Shepherd JH, Shepherd DV, Best SM. Substituted hydroxyapatites for bone repair. Journal of Materials Science: Materials in Medicine. 2012;23:2335-2340

[34] Roane TM, Reynolds KA, Maier RM, Pepper IL. Chapter 2. In: Pepper IL, Gerba CP, Gentry T, Maier RM, editors. Environmental Microbiology. Academic Press, Elsevier, 2nd ed. 2009. pp. 9-36

[35] Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harbor Perspectives em Biologia. 2010;2(5):1-17

[36] Baptista I, Rocha SM, Cunha A, Saraiva JA, Almeida A. Inactivation of Staphylococcus Aureus by high pressure processing: An overview. Innovative Food Science & Emerging Technologies. 2016;36:128-149

[37] Stefani S, Campanile F, Santagati M, Mezzatesta ML, Cafiso V, Pacini G. Insights and clinical perspectives of daptomycin resistance in Staphylococcus Aureus: A review of the available evidence. International Journal of Antimicrobial Agents. 2015;46(3):278-289

[38] Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: Risk factors and outcome of treatment. Clinical Infectious Diseases. 2009;49:1036-1043

[39] Gopi D, Ramya S, Rajeswari D, Karthikeyan P, Kavitha L. Strontium, cerium co-substituted hydroxyapatite nanoparticles: Synthesis, characterization, antibacterial activity towards
prokaryotic strains and in vitro studies. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2014;451:172-180

[40] Morais DS, Fernandes S, Gomes PS, Fernandes MH, Sampaio P, Ferraz MP, Santos JD, Lopes MA, Sooraj HN. Novel cerium doped glass-reinforced hydroxyapatite with antibacterial and osteoconductutive properties for bone tissue regeneration. Biomedical Materials. 2015;10(4):1-15

[41] Ciobanu G, Bargan AM, Luca C. New cerium (IV)-substituted hydroxyapatite nanoparticles: Preparation and characterization. Ceramics International. 2015;41:12192-12201

[42] Sanyal V, Raja CR. Structural and antibacterial activity of hydroxyapatite and fluoro-hydroxyapatite co-substituted with zirconium-cerium ions. Applied Physics A. 2016;122(132):2016