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Hydroxyapatite, alginate and gelatin composites used for bone regeneration: a systematic review

Compósitos de hidroxiapatita, alginato e gelatina utilizados para regeneração óssea: uma revisão sistemática

Compuestos de hidroxiapatita, alginato y gelatina utilizados para la regeneración ósea: una revisión sistemática

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Anderson Cunha dos Santos
ORCID: https://orcid.org/0009-0003-9107-475X
Universidade Federal da Bahia, Brazil
E-mail: cunhaanderson84@gmail.com
George Gonçalves dos Santos
ORCID: https://orcid.org/0000-0001-8601-5825
Universidade Federal da Bahia, Brazil
E-mail: geogoncalves@yahoo.com
Fúlvio Borges Miguel
ORCID: https://orcid.org/0000-0002-0607-0208
Universidade Federal da Bahia, Brazil
E-mail: fulviomiguel@yahoo.com.br
Joni Augusto Cirelli
ORCID: https://orcid.org/0000-0002-7082-9290
Universidade Estadual Paulista Júlio de Mesquita Filho, Brazil
E-mail: joni.cirelli@unesp.br
Fabiana Paim Rosa
ORCID: https://orcid.org/0000-0002-3731-2177
Universidade Federal da Bahia, Brazil
E-mail: fprosa@ufba.br

Abstract
Aim: to investigate and describe, through a systematic review, the biological behavior and osteogenic potential of composite biomaterials containing hydroxyapatite (HA), alginate and gelatin, in different associations, after in vivo implantation. Materials and Methods: for the search and selection of articles, the Medical Literature Analysis and Retrieval System Online (PubMed/MEDLINE) and Scientific Electronic Library Online (SciELO) databases were used, published between 2012 and 2022, using the descriptors: “bone regeneration”; “biocompatible material”; “durapatite”; “alginate”; “gelatin”. Initially, an association was made with the Boolean operator "OR" between the descriptors and their respective entry terms, considering that the MeSH and DeCS platforms use different terms to refer to the same keywords. Subsequently, the "AND" operator was used in nine associations between the descriptors. Results: during the searches, 1939 articles were located. After using the inclusion and exclusion criteria, 16 studies were included in the review. The main themes found in the searches were: HA and Alginate; HA and Gelatin; HA, Alginate and Zinc; HA, Gelatin and mesenchymal cells; HA, Alginate and Chitosan; HA, Alginate and Silk Fibrin; HA, Gelatin and titanium dioxide; HA, Alginate and Gelatin. It was observed that HA, when associated with alginate or gelatin, has improved its osteogenic properties. Final Considerations: HA composites associated with alginate and gelatin provide a range of applications and promising strategies applied to bone repair. Studies have shown that these composites have great potential for application in Bone Tissue Bioengineering.
Keywords: Bone regeneration; Biocompatible material; Durapatite; Alginate; Gelatin.

Resumo
Objetivo: investigar e descrever, por meio de revisão sistemática, o comportamento biológico e o potencial osteogênico de biomateriais compósitos contendo hidroxiapatita (HA), alginato e gelatina, em diferentes associações, após implantação in vivo. Materiais e Métodos: para a busca e seleção dos artigos utilizou-se as bases de dados Medical Literature Analysis and Retrieval System Online (PubMed/MEDLINE) e Scientific Electronic Library Online (SciELO), publicados entre 2012 e 2022, empregando os descritores: “bone regeneration”; “biocompatible material”; “durapatite”; “alginate”; “gelatin”. Inicialmente, foi feita uma associação com o operador booleano “OR” entre os descritores e seus respectivos entry terms, tendo em vista que as plataformas MeSH e DeCS utilizam diferentes termos para referir às mesmas palavras-chave. Posteriormente, empregou-se o operador “AND” em nove associações

entre os descritores. Resultados: durante as buscas localizou-se 1939 artigos. Após o emprego dos critérios de inclusão e exclusão, foram incluídos 16 estudos na revisão. Os principais temas encontrados nas buscas foram: HA e Alginato; HA e Gelatina; HA, Alginato e Zinco; HA, Gelatina e células mesenquimais; HA, Alginato e Quitosana; HA, Alginato e Fibrina de seda; HA, Gelatina e dióxido de titânio; HA, Alginato e Gelatina. Observou-se que a HA, quando associada ao alginato ou a gelatina, tem suas propriedades osteogênicas aperfeiçoadas. Considerações Finais: compósitos de HA associados ao alginato e à gelatina proporcionam uma gama de aplicações e estratégias promissoras aplicadas ao reparo ósseo. Os estudos mostraram que estes compósitos apresentaram grande potencial para aplicação na Bioengenharia Tecidual Óssea.
Palavras-chave: Regeneração óssea; Material biocompatível; Durapatite; Alginato; gelatina.

Resumen
Objetivo: investigar y describir, a través de una revisión sistemática, el comportamiento biológico y el potencial osteogénico de biomateriales compuestos que contienen hidroxiapatita (HA), alginato y gelatina, en diferentes asociaciones, después de la implantación in vivo. Materiales y Métodos: para la búsqueda y selección de artículos se utilizaron las bases de datos Medical Literature Analysis and Retrieval System Online (PubMed/MEDLINE) y Scientific Electronic Library Online (SciELO), publicados entre 2012 y 2022, utilizando los descriptores: “regeneración ósea”; “material biocompatible”; “durapatita”; “alginato”; "gelatina". Inicialmente, foi feita uma associação com o operador booleano “OR” entre os descritos y seus respectivos entry terms, tendo em vista que as plataformas MeSH y DeCS utilizan diferentes termos para referir às mesmas palavras-chave. Posteriormente, empregou-se o operador “AND” em nove associações entre os descritores. Resultados: durante las búsquedas se encontraron 1939 artículos. Después de utilizar los criterios de inclusión y exclusión, se incluyeron 16 estudios en la revisión. Los principales temas encontrados en las búsquedas fueron: HA y Alginato; HA y gelatina; HA, Alginato y Zinc; HA, Gelatina y células mesenquimales; HA, Alginato y Quitosano; HA, alginato y fibrina de seda; HA, Gelatina y dióxido de titanio; HA, alginato y gelatina. Se observó que el HA, asociado a alginato o gelatina, ha mejorado sus propiedades osteogénicas. Consideraciones finales: Los compuestos de HA asociados con alginato y gelatina brindan una gama de aplicaciones y estrategias prometedoras aplicadas a la reparación ósea. Los estudios han demostrado que estos compuestos tienen un gran potencial para su aplicación en la bioingeniería del tejido óseo.
Palabras clave: Regeneración ósea; Material biocompatible; Durapatita; Alginato; Gelatina.

1. Introduction

Bone loss displaying critical morphology and dimensions can be a result of different inhospitable situations, such as extensive surgical resections, trauma, and severe fractures, degenerative diseases, and congenital anomalies, among others (Oryan et al., 2016; Soundarya et al., 2018). In experimental surgery, in order to simulate extensive bone loss, critical bone defects are used in different animal models. Conceptually, they are defined as those in which there is no spontaneous regeneration of the surgical site, completely throughout the animal's life (Schimidt & Hollinger, 1986), without any intervention. In these cases, tissue filling is consolidated by the deposition of fibrous connective tissue (Spicer et al., 2012; Miguel et al., 2013; Lappalainen et al., 2015; Horváthy et al., 2016; Kheiri et al., 2020). Therefore, the regeneration of these losses and defects, as well as the aesthetic and functional restoration in these conditions, remains a major challenge in clinical approaches. In this context, the use of bone grafts becomes the main form of intervention to enable and promote bone regeneration (Li et al., 2014).

The autograft, considered gold standard in view of its osteogenic properties, is biocompatible and meets the ideal mechanical and biological requirements for the induction, viability and maintenance of the physiological events involved in bone regeneration (Amini, Laurencin & Nukavarapu, 2012; Fernandez de Grado et al., 2018). However, this type of graft is associated with the risk of surgical complications at both sites, donor and recipient, due to susceptibility to infections and risk of morbidity and deformity; and limited tissue availability from the donor site (He et al., 2018). Faced with these limitations and in the search for alternatives, bone tissue engineering researchers, an interdisciplinary and multidisciplinary area, have proposed the elaboration and improvement of bone substitutes that mimic natural bone tissue, without the disadvantages presented by autografts (Moshaverinia et al., 2013; Jin et al., 2021). Thus, synthetic biomaterials, also known as alloplastic grafts, are emerging as promising alternatives today (Santana et al., 2016; Haugen et al., 2019).

These materials can be produced from metals, ceramics, polymers, and composites (Jahan &Tabrizian, 2016; Roseti et al., 2017). Among the various possibilities for formatting biomaterials – granules, spheres and microspheres, disks, plates, fibers, membranes –, three-dimensional scaffolds (3D) are the most suitable as bone substitutes, as they provide mechanical support for adhesion, migration and growth of osteogenic lineage cells and, consequently, favor new bone formation within the graft (Ranganathan, Balagangadharan & Selvamurugan, 2019; Mohammadpour, 2021; Santos et al., 2021).

In the context of raw materials, ceramics stand out mainly for their similarity to the inorganic phase of human bone and for their biocompatibility (Ma et al., 2018; Dixon & Gomillion, 2021; Laird et al., 2021; Santos et al. al., 2021). HA, for example, is a ceramic widely used in different bone regenerative techniques, applied individually or in association with other types of biomaterials, mainly due to its bioactivity, osseointegration, osteoconduction and non-toxicity (Jo et al., 2017; Yu et al., 2017; Jyoti et al., 2021). Furthermore, the chemical structure of this ceramic makes it possible to carry out ionic substitutions that alter its physical-chemical, biological and mechanical properties (Szurkowska et al., 2021). However, HA presents slow biodegradation and bioresorption rates after *in vivo* implantation, which occur asynchronously to the mechanism of bone regeneration (Kim et al., 2016; Adamski & Siuta, 2021; Santos et al., 2021). Because it is very rigid and friable, most of the time, this ceramic remains in the implantation site for weeks, months and even years, depending on the synthesis and processing method (Lee et al., 2013; Chao et al., 2015; Kim et al., 2020; Santos et al., 2021).

Faced with these limitations, different researchers have associated HA with other materials and developed composites (Kato et al., 2014) capable of showing improvements in their physicochemical and biological properties – biodegradation, biocompatibility and osteoinduction – compared to their individual forms (Saltz & Kandalam, 2016; He et al., 2018; Akgöl et al., 2021). Thus, considering that bone tissue has polymeric components in its organic portion and calcium phosphates (CaP) of the HA type in its inorganic portion (Perić Kačarević et al., 2020), composites consisting of polymer-ceramic phases have gained notoriety, since they mimic this structure in a peculiar way (Venkatesan et al., 2015). This way, the association of HA with biodegradable polymers (collagen, alginate, gelatin, among others) represents a promising strategy for the development of bone substitutes (Kato et al., 2014). In this context, the use of biomimetic biomaterials, extracted from nature, is a favorable option applied to tissue engineering, since these materials have desirable characteristics such as biocompatibility, hydrophilicity, bioactivity, osteoconductivity and osteoinductivity. In addition, natural biomaterials obtained in a sustainable way can be extracted on a large scale and the generated waste contains less toxicity. (Ma et al., 2018; Haugen et al., 2019; Ranganathan et al., 2019).

Among the natural polymers most used for this purpose, alginate, derived from brown seaweed, has physicochemical characteristics that allow changes in its properties and functions, such as biodegradability, mechanical resistance, gelling property and cell affinity, especially for applications in the administration of drugs, biodegradable dressings and in the area of tissue engineering (Venkatesan et al., 2015; Tong et al., 2017). Consequently, composite biomaterials produced based on alginate are promising in bone tissue applications (Venkatesan et al., 2015). Thus, among the various possible associations of this polymer, the HA-alginate combination results in bioactive and biocompatible biomaterials that may have a porous structure (Rossi, 2012; Jo et al., 2017; Santos et al., 2019), favorable physical-chemical characteristics for bone regeneration.

In addition to alginate, gelatin, obtained by the hydrolysis of animal collagen, has gained prominence for bone tissue applications, as it is a biocompatible, biodegradable, non-toxic, low-cost, widely available natural polymer (Tomas et al., 2019). However, this material does not show bioactivity, which creates the need to associate it with other raw materials, such as inorganic materials, especially CaP or bioactive glasses (Thomas & Bera, 2019; Bello et al., 2020). In this way, HA-gelatin composites have superior mechanical properties than HA and gelatin when used individually, by increasing mechanical strength, due to the molecular bonds between calcium compounds and gelatin (Chiu et al., 2015).

Given the above, this study carried out a systematic review of the literature to describe the biological behavior and osteogenic potential of composite biomaterials containing HA, alginate and gelatin, in different associations, after implantation in vivo.

2. Methodology

Prior to the development of this study, a detailed protocol was drawn up with the aim of clearly and transparently defining the entire process and methods used during this research. For this purpose, the present work was previously registered in the database of protocols of systematic reviews with health outcomes *International Prospective Register of Systematic Reviews* (PROSPERO). This record is intended to avoid the involuntary duplication of publication of systematic reviews that evaluate the same object of study.

The construction of the intervention question was structured in the PICO format, where "P" refers to the population [laboratory animals submitted to the implantation of biomaterials]; "I" for Intervention [implantation of hydroxyapatite composites, associated with alginate and gelatin, in critical bone defects]; "C" for Comparator [without intervention]; and "O" for Outcome [biological behavior and osteogenic potential of biomaterials *in vivo*].

To carry out the search and selection of scientific articles, two main steps were defined: first - selection of works based on titles and abstracts; second - screening by reading the full texts of manuscripts eligible for inclusion in the review. At each stage, two researchers evaluated each article independently. Soon after, disagreements were analyzed, in pairs and independently, through joint discussion.

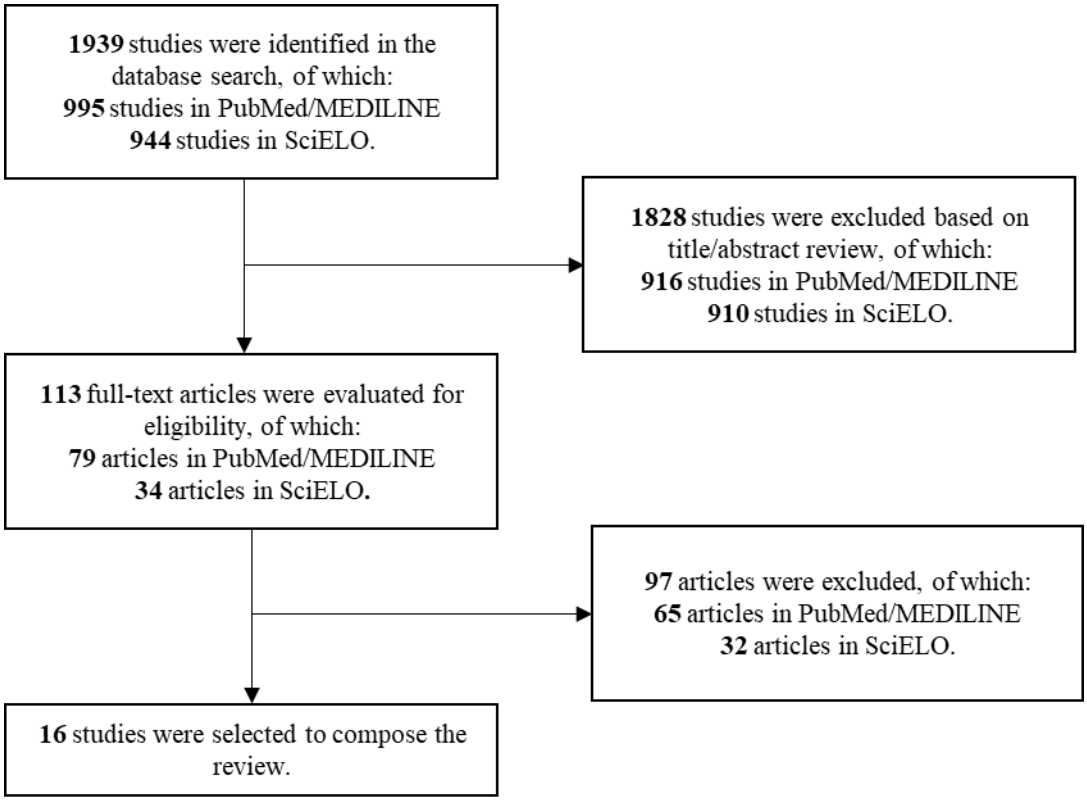
The inclusion criteria were: studies *in vivo* in animal models; interventions with the use of composite biomaterials of hydroxyapatite, alginate and gelatin, where hydroxyapatite was always associated with, at least, alginate or gelatin; written in English and Portuguese; and published in the last ten years (2012 to 2022). Exclusion criteria were: non-original articles; literature reviews; editorials; studies *in vitro*; and the use of other types of association of biomaterials.

The searches were carried out in the databases *Medical Literature Analysis and Retrieval System Online (PubMed/MEDLINE)*; and *Scientific Electronic Library Online (SciELO).* For this purpose, the following keywords available in Health Sciences Descriptors (DeCS) and in the *Medical Subject Headings (MeSH)*: "*bone regeneration*"; "*biocompatible material*"; "*durapatite*"; "*alginate*"; "*gelatin*". Initially, an association was made with the Boolean operator "*OR*" between descriptors "*bone regeneration*", "*biocompatible material*", "*durapatite*" and their respective *entry terms* "*osteoconduction*", "*biomaterials*", "*hydroxyapatite*", given that MeSH and DeCS platforms use different terms to refer to the same keywords. Subsequently, the operator "*AND*" in nine associations between the descriptors: 1) (*bone regeneration OR osteoconduction*) *AND (biocompatible material OR biomaterials) AND (durapatite OR hydroxyapatite) AND alginate; 2) (bone regeneration OR osteoconduction) AND (biocompatible material OR biomaterials) AND (durapatite OR hydroxyapatite) AND gelatin; 3) (bone regeneration OR osteoconduction) AND (biocompatible material OR biomaterials) AND (durapatite OR hydroxyapatite) AND alginate AND gelatin; 4) (bone regeneration OR osteoconduction) AND (durapatite OR hydroxyapatite) AND alginate; 5) (bone regeneration OR osteoconduction) AND (durapatite OR hydroxyapatite) AND gelatin; 6) (bone regeneration OR osteoconduction) AND (durapatite OR hydroxyapatite) AND alginate AND gelatin; 7) (durapatite OR hydroxyapatite) AND alginate; 8) (durapatite OR hydroxyapatite) AND gelatin; 9) (durapatite OR hydroxyapatite) AND alginate AND gelatin*, to obtain the greatest possibility of existing combinations. At the end, duplicate references were excluded from the study.

3. Results

After carrying out searches in the aforementioned databases, 1939 articles were found. After reading the titles and abstracts, 113 papers were selected. Then, after reading the manuscripts in full, 16 articles were included in this review, as shown in the flowchart below (Figure 1).

Figure 1 - Study search and selection flow diagram.



Source: Authors.

The summary of the articles included, as well as the systematization of the main methodological characteristics, are described in the synoptic table of Table 1.

Table 1 - Synoptic table of studies included in the review.

Author	Aim	composites	bone defect (mm)	Animal (n)	follow-up period	Conclusions
Cardoso et al. (2014)	To investigate the gelation mechanism and biocompatibility of alginate-glycerol-CaP composites, which cross-linked by glucono-delta-lactone - induced acidification to monetite and HA little crystalline.	Alginate/CaP	Femur (6 mm x 4 mm)	*New Zealand* white rabbits (6)	6 and 12 weeks	Beneficial bone response was observed in the current *live* render these gels promising for minimally invasive application as bonefilling material.
Santos et al. (2019)	To analyze the influence of the geometry of nanostructured HA (HAn) and alginate composites in the initial phase of bone repair.	HAn/alginate	Skulls (8 mm)	*Wistar* albino rats (5)	15 days	The geometry of the biomaterials influenced the tissue response to implantation of HAn and alginate composites. The most visible histological micro-features are produced by what the spheres present.
Cuozzo et al. (2020)	The objective of this study was to evaluate the *in vitro* and *live* biological response to nanostructured calcium alginate-HA (HA) and zinccontaining HA (ZnHA).	Nanostructured calcium alginate-HA (HA) and zinccontaining HA (ZnHA).	Skulls (8 mm)	*Wistar* albino rats (5)	1, 3, and 6 months	Both of the studied biomaterials were found to be cytocompatible, biocompatible and osteoconductors. The addition of zinc to calcium alginate-hydroxyapatite improved bone repair.
Ferreira et al. (2013)	Generate novel cell aggregate-loaded macroporous scaffolds combining the osteoinductive properties of titanium dioxide (TiO2) with HA-gelatin nanocomposites (HAP) for regeneration of craniofacial defects.	Titanium dioxide (TiO2) with HA-gelatin (HAP)	Skulls (8 mm)	*Sprague-Dawley* rats (5)	4, 8 and 12 weeks	That$_2$-HAP-GEL scaffolds loaded with OD-MAPCs promoted osseointegration, newly formed bone in macroporous areas and significantly improved bone regeneration. Finally, constructs with TiO_2-HAP-GEL scaffolds and ODMPCs had greater bone healing than TiO2-HAP-GEL without cells with closure.
Sarker et al. (2015)	To evaluate bone regeneration in a critical bone defect after the use of oxidized biphasic calcium phosphate alginate-gelatin-phosphate hydrogel (OGB00) and 25 wt% granule-loaded hydrogel (OGB25) and 35 wt% granule-loaded hydrogel biomaterials (OGB35)	HAp granules encapsulated oxidized alginate–gelatin–biphasic calcium phosphate hydrogel	Femur (8 mm x 5mm)	*New Zealand* White rabbits (?)	4, 8 weeks	It was found that the OGB25 sample shows a significantly earlier and higher (***$p < 0.001$) bone formation than OGB00 and negative control after 4 and 8 weeks of implantation.
Wang et al. (2015)	To investigate whether 3D-printed Atsttrin-incorporated alginate Alg)/HA (nHAp) scaffolds can facilitate bone healing through affecting the TNF/TNFR signaling. Followed by evaluation of its effectiveness on bone regeneration both in vitro and *in vivo*.	Atsttrin-incorporated alginate (Alg)/HA (nHAp) *scaffolds*	Skulls (7mm x 5mm)	C57BL/6J Mice (5)	7 days, 8, 16 weeds	A composite of alginate and nHAp forms an appropriate bio-ink for 3D printed scaffolds, and that Atsttrin is a promising bioactive factor that can be incorporated within these 3D printed scaffolds to enhance bone-defect repair with TNF/TNFR signaling involvement.

Zelaya et al. (2019)	To evaluate the osteoconduction behavior of microspheres produced from nanostructured Zn doped carbonated HA (ZncHA), in critical-sized calvarial defects in the rat.	Nanostructured Zn doped carbonated HA (ZncHA)	Skulls (8 mm)	*Wistar* albino rats (5)	1, 3, and 6 months	The nanometric size of cHA and Zn-cHA was a decisive factor in accelerating the in vivo availability of calcium and zinc. The high calcium and zinc accumulation in the defect, which was not cleared by the biological medium, played a critical role in inhibiting osteoconduction and thus impairing bone repair.
He et al. (2014)	To prepared CAH/B2 scaffolds through in situ coprecipitation and freeze drying, and evaluated the efficacy of the porous scaffolds for critical sized calvarial defect repair in rats.	Chitosan/alginate/hydroxyapatite *scaffold*	Skulls (8 mm)	*Sprague-Dawley* rats (6)	12 weeks	On the basis of the data presented here, it appears that CAH scaffolds could be used for the repair of bone defects and functional bone tissue engineering applications. The use of osteogenically differentiated MSCs and a combination of MSCs and BMP-2 may further enhance osteogenesis.
Jo et al. (2017)	To evaluate the bone regeneration efficacy of the AL/HA/SF composite *live* by analyzing TNF-α, FGF-23, OPG, and Runx2 expression levels.	Silk Fibroin-Alginate-Hydroxyapatite Composite	Skulls (8 mm)	*Sprague-Dawley* rats (5)	4 and 8 weeks	The AL/HA/SF scaffolds contributed to new bone regeneration in rat calvarial defects and were stably biodegraded without inducing foreign body inflammatory reactions.
Johari et al. (2016)	To evaluate the effect of co-culturing and transplantation of nanocomposite scaffold/osteoblasts/endothelial cells compared with nanocomposite scaffold/osteoblasts and bare scaffold.	Gelatin/hydroxyapatite *scaffolds*	Calvarias (5 mm)	*Wistar* albino rats (9)	1, 4, and 12 weeks	In this study, nanocomposite Gel/HA scaffold was shown to have the advantages of good cell attachment, lack of cytotoxicity toward cells, and biodegradability within a time longer than 12 weeks for being fully degraded *live*.
Yin et al. (2016)	To evalueted, *in vitro* and *live*, the morphological characteristics, biocompatibility and osteogenesis ability of scaffolds hybrid of collagen-derived gelatin (Gel) and nano-HA (nHA) (Gel:nHA = 1:0, Gel:nHA = 1:1, and Gel:nHA = 1:3), followed by comparison with non-modified porous titanium.	Gel/nHA Micro-*Scaffolds*	Radio (15 mm)	*New Zealand* white rabbits (4)	12 weeks	Gel:nHA showed the best performance. All of these findings indicated that Gel/nHA 3D micro-scaffold modification hybrid scaffold had a good biocompatibility and bone regeneration capability, which may potentially be applied in the clinic.
Rossi et al. (2012)	To investigated the mineralized material surrounding HA-Alg composite and sintered HA (HA-Sint) used to regenerate critical bone defects in rat calvaria by high-resolution electron microscopy (HRTEM)/electron diffraction and Fourier transforminfrared attenuated total reflectance microscopy (FTIR).	Hydroxyapatite/alginate and sintered hydroxyapatite	Skulls (8 mm)	*Wistar* albino rats (6)	3 monthes	The main structural differences were found in the vicinity of the biomaterials (cement layer-like region) where the HA-Alg sample was surrounded in a large extent by a DML, indicating that this material had/induced a more diverse ultrastructure than HA-Sint.

Song et al. (2021)	To evaluated the swelling behavior, mechanical strength and degradation ability of the nanocomposites nHA–Gel. Furthermore, the bone regenerative abilities via the *live* rat critical-size calvarial defect implantation	nHA–Gel nanocomposites	Skulls (5 mm)	*Sprague-Dawley* rats (4)	8 weeks	Although further long-term evaluations are required, we believe that Sr–nHA–Gel composites developed in this research can be potential.
Fayyazbakhsh et al. (2017)	To fabricate a novel BTE composite scaffold and double hydroxides-HA/gelatin to facilitate the bone healing process. Subsequently, in vitro and in vivo studies were run to declare the cellular/tissue responses and biocompatibility of the scaffolds.	Layered double hydroxides-hydroxyapatite/gelatin scaffolds	Radio (15 mm)	*New Zealand* white rabbits (6)	4, 8, and 12 weeks	A composite consisting of LDH (double layered hydroxides) -HA/GEL, was shown to be biocompatible and osteoinductive.
Chao et al. (2015)	To evaluate cell proliferation and viability in osteoblast-like cells (in vitro), and the bioactive, biocompatible capabilities of microspheres composed of gelatin and HA (G HA), fibroin glue (F) and OSTEOSET® (OS), applied to the bone repair *live*.	Gelatin–hydroxyapatite composite, fibroin glue and OSTEOSET	Skulls (4,6 mm)	*Sprague-Dawley* rats (?)	4, 8, and 12 weeks	Composite G-HA microspheres are biocompatible and bioactive. G-HA structures indicated higher osteoconductivity and bioactivity than F and OS.
Hamidabadi et al. (2018)	To evaluate the effectiveness of HA-gelatin seeded with bone marrow stromal cells construct for healing of critical-sized bone defect i*n alive.*	Hydroxyapatite-gelatin *scaffold* and hydroxyapatite-gelatin seeded with BMSCs.	Skulls (7 mm)	*Wistar* albino rats (5)	1, 4 weeks	It seems that hydroxyapatite-gelatin scaffold reinforced with bone marrow mesenchymal stem cells is playing a pivotal role in bone healing and can be used as a useful therapeutic strategy for large bone defects

Source: Authors.

When analyzing the characteristics of the studies included, there was a predominance of studies that evaluated the association of HA-alginate or HA-gelatin, exclusively; followed by studies that evaluated HA-alginate-zinc composite biomaterials; and only one study associated HA-alginate-gelatin (Table 2).

Table 2 - Number of articles according to the type of composite evaluated.

Evaluated composites	**n**
HA and Alginate	4
HA and Gelatin	4
HA, Alginate and Zinc	2
HA, Gelatin and mesenchymal cells	2
HA, Alginate and Chitosan	1
HA, Alginate and Silk fibroin	1
HA, Gelatin and Titanium Dioxide	1
HA, Alginate and Gelatin	1
Total	**16**

Source: Authors.

4. Discussion

Bone tissue is a specialized connective tissue consisting of an inorganic phase, composed essentially of minerals, and an organic phase, predominantly composed of polymers (eg type I collagen). In an attempt to mimic this structure, researchers around the world have developed composite biomaterials that have similar morpho-physical-chemical properties to this tissue (Hamidabadi et al., 2018; Manda et al., 2018). Therefore, among the essential characteristics to be considered during the development of composite biomaterials, the following stand out: chemical composition, physical and mechanical properties, biodegradability, biocompatibility, biofunctionality and bioactivity (Georgopoulou et al., 2017).

In this context, HA has been widely used, as it is biocompatible, osteoconductive and bioactive. However, it is a fragile material with low mechanical strength (Song et al., 2020; Fitzpatrick et al., 2021). To overcome these limitations, this ceramic has been associated with other types of materials (Liao et al., 2018; Dubey et al., 2021; Ait Said et al., 2021), such as collagen, chitosan, silk, fucoidan, elastin, hyaluronic acid, gelatin and alginate (Bharadwaza & Jayasuriya, 2020). The latter two stand out for their biocompatibility, biodegradability, absence of toxicity and immunogenicity (Meimandi-Parizi et al., 2018; Shi et al., 2019). Both, when combined with HA, improve the mechanical properties and the ability to promote bone regeneration, compared to their applications alone (Chiu et al., 2015; Barros et al., 2019; Tomas et al., 2019; Shi et al., 2019). These natural polymers, in general, are used with the aim of providing adequate bioactive support for the cellular events involved in bone repair, as well as serving as a favorable mechanical support for the clinical application of the material (Bharadwaza & Jayasuriya, 2020).

Alginate is a polyanionic copolymer consisting of a sequence of two hexuronic acid residues: an acid unit *a*-L-guluronic and *b*-D-manuronic acids in varying proportions (Venkatesan et al., 2015). The addition of divalent cations, such as calcium to alginate, in aqueous solution, can induce cross-linking between molecules and the formation of hydrogels, with a 3D network that stabilizes the polymers permanently (Saltz & Kandalam, 2016; Ge et al., 2018; Zheng et al., 2018). This

ability supports the versatility of this polymer, which can be widely used in the biomedical area, especially in tissue engineering (Catazano et al., 2015; Rodriguez et al., 2018).

Biomaterials synthesized from polymeric hydrogels, processed with scaffold design, have unfavorable mechanical performance for bone regeneration (Stagnaro et al., 2018). Cardoso et al. (2014) when using alginate hydrogels associated with calcium phosphates (monetite and HA), in various combinations with glucono-deltalactone (CDL) and glycerol, showed mechanical instability of the scaffolds, as a result of premature degradation of the organic phase of the composite. However, it was noticed that the biomaterial was biocompatible and osteoconductive, through the observation of evident bone formation in the entire area previously occupied by the biomaterial, which is found degraded. In addition, new bone formation was visualized around the remaining particles of the composite and absence of fibrous connective tissue.

Sarker's study et al. (2015), using alginate-gelatin-biphasic calcium phosphate hydrogels (OGB00), and Hydroxyapatite granules (HAp), encapsulated in an OGB00 network with 25% and 35% of the weight of HAp, (OGB25) and (OGB35) respectively, showed that HAp granules in the hydrogel matrix are essential to promote structural stability, osteoconductivity and edema control. This hypothesis is reinforced from the best performance, in vivo, of OGB25 for new bone formation, compared to the control group, without biomaterials, explained by a double effect of the osteoconductivity of the HAp granules and the biocompatibility of the OGB polymeric matrix.

The association of alginate with HA improves its performance when applied for bone repair (Mahmoud et al., 2020). A study with HA-alginate-silk fibroin (AL/HA/SF) composites demonstrated biocompatibility, bioactivity, low toxicity rate and potentiated osteogenesis in critical bone defects in rat calvaria. This effect is attributed to the lower expression of TNF-α, higher levels of osteogenic markers and greater size and irregular structure of the AL/HA/SF particles, that is, greater surface area of the biomaterial for adhesion of osteogenic cells. The results demonstrated the superiority of this composite in relation to the association of HA-alginate and alginate evaluated individually (Jo et al., 2017).

Composites of nanohydroxyapatite and alginate (nHAAlg) have been widely applied in bone tissue engineering (Wang et al., 2015; Santos et al., 2019; Zelaya et al., 2019; Cuozzo et al., 2020), considering that the characteristics of nHAAlg can be modified from the association with chemical elements, cells and other biomaterials or raw materials, such as, for example, the use of this nanocomposite containing zinc (Zelaya et al., 2019; Cuozzo et al., 2020). Although, according to Zelaya et al. (2019), with regard to osteoconduction and bone repair, the presence of this metal in the HA structure, when compared to all-ceramic (HA), is still controversial. These authors found a discreet osteoconductive potential of the cHA and Zn-cHA microspheres and bone repair restricted to the edges of the defect. This may have occurred due to two main factors: little fragmentation of the microspheres and high amounts of Ca, P and Zn released from the microspheres, far above the body's ability to reabsorb these elements. On the other hand, Cuozzo et al. (2020), observed the association of Zn to the HA-alginate composite, favored bone neoformation when compared to pure HA. According to the authors, these results were probably due to the presence of zinc, which led to a reduction in the rates of active bioresorption by osteoclasts.

With regard to gelatin – a polymer that can be extracted from various collagen sources, under basic and acidic conditions, at different molecular weights and isoelectric points (Kanda et al., 2015) –, it has been observed that this raw material can be used in bone tissue engineering in scaffold designs; associated with bioceramics; integrated with other natural polymers such as hyaluronic acid, silk, alginate hydrogels, among others); integrated into synthetic polymers such as polylactic acid-co-glycolic acid (PLGA), polycaprolactone, polyhydroxybutyrate, polyvinylpyloridone, among others) and as a vehicle for the controlled release of drugs (Su & Wang, 2015).

Composites that present hydroxyapatite (HA) deposition in natural collagen/gelatin phases provide the biomaterial with biomimetic properties of natural bone tissue, desirable characteristics to stimulate bone regeneration (Bartmański et al., 2022). Chao et al. (2015) when comparing the use of HA-Gelatin microspheres (21%/79%) (HA-Gel); fibrin glue (F); and

Osteoset® (OS), in a critical bone defect in the calvaria of rats, observed better osteoconductivity and more evident bone formation for the HA-Gel composite in relation to the other materials – F and OS. The HA-Gel group filled 90% of the defect area with newly formed bone tissue, which demonstrates the great potential of this biomaterial. According to Hamidabadi et al. (2018), this is due to biocompatibility, absence of cytotoxicity and ability to enable cell growth, differentiation and migration in critical bone defects.

Biocompatibility and biodegradability are properties that can also be optimized during the association of gelatin with other biomaterials, such as chitosan (CS) (Georgopoulou et al., 2017). According to Oryan et al. (2016), this association (CS-Gel), without HA, favored greater bone neoformation in critical bone defects when compared to the use of pure chitosan (CS). However, gelatin (Gel) when used individually showed: greater biodegradability; new bone formation from the edges to the center of the defect; greater number of cartilaginous cells and absence of exacerbated inflammation.

Furthermore, Johari et al. (2016) evaluated scaffolds of HA and gelatin nanocomposites (nHA-Gel) and showed bone neoformation after implantation in bone defects in rat calvaria, after 60 days. These authors attributed this effect to the porosity of the material, good cell adhesion; the absence of cytotoxicity; and biodegradability, characteristics that improved the osteoconduction and osseointegration of the materials.

It was evaluated in the study developed by Yin et al. (2016), inside porous titanium implants, micro-scaffolds 3D hybrids of nHA-Gel, with different proportions (0/1; 1/1; and 3/1) and observed that the composite in the proportion 1/1 presented better performance related to cell adhesion, proliferation and differentiation, in the same way that it promoted greater bone formation when compared to the other proportions studied. The authors justified these results as a function of the average pore size of scaffolds. The group with nHA-Gel (1/1) showed a porous structure in multilayers, with a more adequate size for cell adhesion and growth.

The nHA-Gel composite (0/1), due to the absence of ceramics, when in contact with the body liquid, presented swelling of the gelatin, which reduced the size of the pores and restricted cell growth. As for the nHA-Gel (3/1), the higher proportion of HA resulted in more collapsed areas and more inadequate spaces for cell growth in the scaffold. However, all composites performed well compared to the control group (titanium implant without scaffold). These results suggest that the inclusion of scaffolds of nHA-Gel to the porous titanium implant contributed to better proliferation and adhesion of osteoblasts, osseointegration and mineralization.

Song's study et al. (2021) evaluated scaffolds of HA nanocomposites with gelatin (nHAGel) and the same nanocomposite with strontium (Sr-nHA-Gel), in bone defects in rat calvaria. Both biomaterials showed superior results in terms of bone regeneration capacity, compared to the group with pure Gel (p-Gel). According to the authors, the poor performance of p-Gel was associated with rapid biodegradation, low mechanical strength and, therefore, an early disintegration of biomaterials and inability to support cell attachment and growth, and bone formation. The incorporation of Sr as a bioactive trace element contributed to a better regenerative capacity and osteogenic differentiation, as already reported in studies by Neves et al. (2016) and Ehret et al. (2017).

The nHA-Gel composites can also be added to other substances in order to optimize their osteoinductive properties. In one study, Ferreira et al. (2013) evaluated the following biomaterials: 1) PLGA; 2) collagen; 3) nHA-Gel macroporous composite; 4) macroporous composite of nHA-Gel and titanium dioxide (nHA-Gel-TiO_2) neat; 5) autograft; 6) nHA-Gel-TiO_2 associated with undifferentiated multipotent adult progenitor cells (MAPC); 7) nHA-Gel-TiO_2, associated with osteogenically differentiated adult progenitor cells (OD-MAPC). After 12 weeks, the percentage of new bone formation was significantly higher in the nHA-Gel-TiO_2 pure scaffolds, compared to autograft, other groups of biomaterials.

The association with the cells, MAPC and OD-MAPC group, increased the osteogenic capacity of the materials. The authors correlate the good results of nHA-Gel-TiO_2 to the proposed mechanism of osteoinduction, related to the hydroxyl

groups and calcium ions present on TiO surfaces$_2$. These groups can promote the adsorption of calcium-binding extracellular matrix proteins and specific peptide sequences of RGD (eg fibronectin, bone sialoprotein). The authors stated that the scientific literature is controversial regarding in vivo bone repair, induced by the use of undifferentiated and osteogenically differentiated adult stem cells. Although the study by Hamidabadi et al. (2018) indicates that the association of undifferentiated mesenchymal stem cells to the HA-Gel composite improves the osteogenic properties of the biomaterial.

Based on the works found in this literature review, it could be observed that HA, when associated with alginate or gelatin, has its osteogenic properties improved. However, the performance of these composites can be modified from the association with other components and oligoelements, format of the biomaterial, size and site of the bone defect. As indicated in Sarker et al. (2015), gelatin together with HA-Alginate adds pending mechanical stability in composites formed only from HA-Alginate, described in the works of Stagnaro et al. (2018) and Cardoso et al. (2014). Silk fibroin linked to HA-alginate optimized the bone repair capacity of this biomaterial according to Jo et al. (2017). As for the inclusion of Zn, it is not possible to have a consistent estimate of this effect, as verified in the contradictory results between works by Zelaya et al. (2019) and Cuozzo et al. (2020). With regard to HA-Gel composites, it is noted that when linked to mesenchymal stem cells derived from bone marrow and chitosan the osteogenic potential is optimized, the same happens when adding Sr and TiO_2, described in studies by Hamidabadi et al. (2018), Oryen et al. (2016), Song et al. (2021) and Ferreira et al. (2013), respectively.

Biomaterials in scaffolds design were used in nine of the 16 articles found in the results. Of these, eight were implanted in calvaria, which indicates that the scaffolds, compared to other formats, are promising when applied to this anatomical site, as they provide mechanical support for the migration of osteoprogenitor cells, enabling angiogenesis and diffusion of nutrients and oligoelements inside the implant. Despite this, Santos et al. (2019) obtained good performance regarding bone repair of critical bone defects, using microspheres and granules of the same composite (nHA-Alginate), where a better result was noted with the use of microspheres. This was attributed to the irregular surface of the granules, which interferes with the cellular response observed in the interstitium between the biomaterial particles.

Research in the area of bone tissue engineering has focused on critical bone defects, due to the challenge of regenerating this type of defect, and also due to the formation of fibrotic scars and bone neoformation limited to the margins of the defects (Cardoso et al., 2006; Miguel et al., 2006; Miguel et al., 2013; Ribeiro et al., 2015; Santos et al., 2019; Almeida et al., 2020; Santos et al., 2021; Lappalainen et al., 2015). From chart 1, it is possible to notice the lack of standardization of the size of the defect considered critical in experimental works. Despite this, most of the works found in our study carried out the experiment on rat calvaria and attributed the defect with a diameter of 8 mm as critical, which is generally accepted in the scientific literature (SPICER et al., 2012). However, Wang et al. (2015) e Hamidabadi et al. (2018) consider a diameter of 7 mm as a critical defect and Johari et al. (2017) e Song et al. (2021), 5 mm. This factor is important, since in a bone defect of non-critical size, the capacity for bone regeneration becomes more viable, as a result of the vascular network present at the margins of the defect providing nutritional support for the regenerative process (HE et al., 2020). That said, in addition to the composition of the composites and their components, it is necessary to evaluate the influence of the geometry of the biomaterial and its behavior in a critical defect, suitably standardized in terms of osteogenic potential.

5. Final Considerations

Based on the results presented in the researched literature, it is concluded that HA composites associated with alginate and gelatin provide a range of applications and promising strategies applied to bone repair. Studies have shown that these composites have great potential for application in Bone Tissue Engineering. Therefore, it is necessary to develop new studies involving the analysis of the biological behavior and the osteogenic potential from the use of composites consisting of HA, alginate and gelatin, in various proportions.

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