UNESP - Universidade Estadual Paulista
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Eficácia da hidroxiapatita sintética particulada na formação óssea: revisão sistemática e meta-análise

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RESUMO

Atualmente, a engenharia tecidual óssea tem buscado desenvolver biomateriais que tenham capacidade de promover o reparo tecidual em defeitos ósseos extensos. A hidroxiapatita sintética na forma particulada tem mostrado resultados promissores nesse processo, porém algumas dúvidas ainda surgem quanto a sua eficácia. O objetivo desta revisão sistemática e meta-análise foi avaliar a formação óssea em defeitos preenchidos com hidroxiapatita sintética (HA) na forma particulada em modelos animais. Além disso, investigou-se a influência dos parâmetros físico-químicos da HA, incluindo o tamanho da partícula, porosidade, tamanho dos poros e cristalinidade, na formação óssea e taxa de reabsorção das partículas. Buscas eletrônicas nas bases de dados PubMed/MEDLINE, Embase, Web of Science e Scopus foram realizadas para publicações até maio de 2021. Todos os artigos incluídos eram estudos in vivo que relataram defeitos ósseos em animais preenchidos com HA pura sintética particulada. A formação óssea em defeitos ósseos preenchidos com HA em comparação com coágulos sanguíneos foi avaliada por meio da meta-análise de efeitos aleatórios. Além disso, o efeito do tamanho das partículas na formação óssea foi avaliado por meio da análise de meta-regressão. Cinquenta e seis estudos foram incluídos na análise qualitativa (revisão sistemática) e 2 estudos compuseram a meta-análise/meta-regressão. Os estudos incluídos mostraram formação óssea nos defeitos preenchidos com HA sintética particulada. Em comparação aos defeitos preenchidos com coágulo sanguíneo, a HA apresentou superioridade na formação óssea nas análises qualitativa e quantitativa. Partículas pequenas, principalmente em nanoescala (nano-HA), apresentaram maior taxa de reabsorção óssea e maior formação óssea na análise qualitativa. No entanto, nenhuma influência do tamanho das partículas na formação óssea foi demonstrada na análise de meta-regressão (considerando apenas três observações). Maior porosidade e tamanho dos poros parecem promover maior formação óssea nos defeitos preenchidos com HA. Em conclusão, as partículas de HA são osteocondutoras, proporcionando uma superfície para a formação de novo osso. Além disso, alterações nas propriedades físico-químicas da HA, incluindo tamanho de partícula, porosidade e tamanho dos poros, influenciam diretamente o resultado da formação de osso novo e as taxas de reabsorção.

Palavras chave: Durapatita. Osteogênese. Regeneração óssea.

Lara M. Synthetic hydroxyapatite in particulate forms in bone formation: systematic review and meta-analysis [dissertação de mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2022.

ABSTRACT

Currently, bone tissue engineering has sought to develop biomaterials that are able to promote tissue repair in extensive bone defects. Synthetic hydroxyapatite in particulate form has shown promising results in this process, but some doubts still arise about its effectiveness. The aim of this systematic review and meta-analysis of animal studies was to evaluate the bone formation in defects filled with synthetic hydroxyapatite (HA) in particulate forms. Moreover, we identified the influence of HA particle size, porosity, pore size, and crystallinity in bone formation outcomes and particle resorption. Electronic searches in the PubMed/MEDLINE, Embase, Web of Science, and Scopus databases were conducted for publications up to May 2021. All articles were in vivo studies that reported bone defects filled with synthetic pure HA in particulate form in animals. The bone formation in bone defects filled with synthetic pure HA in comparison to blood clots was calculated using a random-effects model meta-analysis. Moreover, the effect of particle size on bone formation was evaluated with a meta-regression analysis. Fifty-six studies were included in the qualitative analysis (systematic review) and 2 studies composed the meta-analyses/meta-regression. The studies included showed bone formation in defects filled with HA particles. In comparison to defects filled with a blood clot, the HA showed superiority in bone formation outcomes in qualitative and quantitative analysis. Small particles, mainly at the nanoscale (nano-HA), showed a higher rate of bone resorption and greater bone formation in qualitative analysis. However, no influence of particle size in bone formation was shown in meta-regression analysis (considering only three observations). Higher porosity and pore size seem to increase bone formation in defects filled with HA. In conclusion, the HA particles are osteoconductive, providing surfaces for new bone formation. Moreover, alterations in HA physicochemical properties including particle size, porosity, and pore size directly influence the outcome of new bone formation and resorption rates.

Keywords: Durapatite. Osteogenesis. Bone regeneration.

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1 INTRODUÇÃO

A regeneração tecidual óssea é uma área que vem ganhando destaque dentro das pesquisas científicas devido a sua ampla aplicação clínica, uma vez que o tecido ósseo se apresenta susceptível a fraturas, traumas, tumores, infecções ou distúrbios genéticos[1,2]. A formação e reabsorção óssea é realizada por osteoblastos, osteoclastos, osteócidos. Ela é orientada por uma via de sinalização óssea e requer vascularização adequada, estabilidade, presença de fatores de crescimento e uma matriz de crescimento[3].

Em determinadas situações, esse ciclo de reparo ósseo é insuficiente e requer o auxílio de enxertos[3]. Quando idealizamos um enxerto ideal, algumas características são imprescindíveis: ser osteocondutor, ter propriedades osteoindutoras e osteogênicas[3]. A osteoindução consiste no processo pelo qual as células mesenquimais são recrutadas para se diferenciarem em condroblastos e osteoblastos, sendo este processo modulado por fatores de crescimento derivados da matriz do enxerto cuja atividade é desencadeada quando o mineral ósseo é removido. Um material osteocondutor, por meio de uma estrutura tridimensional, possibilita o crescimento interno de vasos sanguíneos e células osteoprogenitoras. A osteogênese remete-se à osteo-diferenciação e posterior formação de novo osso, com o envolvimento de mesenquimais viáveis, osteoblastos e osteócitos[4-8].

Para que o enxerto ósseo seja bem-sucedido, não basta o material apresentar atividade osteogênica e formar osso. A formação óssea deve ser regular e apresentar resistência mecânica adequada para exercer sua função[6].

O enxerto mais eficaz é, indubitavelmente, o enxerto autógeno, considerado o padrão ouro, apresentando histocompatibilidade completa e todas as propriedades necessárias. Sendo assim, é osteocondutor, osteoindutor, apresenta células osteogênicas viáveis, e não promove respostas imunes[2,3,6]. Além disso, algumas formas de enxerto autógeno também podem fornecer suporte estrutural[3]. As áreas doadoras mais comuns são o crânio, costelas ou crista ilíaca[9]. Apesar do enxerto autógeno ser referência, apresenta uma série de desvantagens, incluindo o desconforto para o paciente devido à necessidade de um sítio doador, quantidade óssea limitada, aumento da perda de sangue, potencial de infecção do local doador,

aumento da hospitalização e custos associados, pode apresentar reabsorção imprevisível e pode ser difícil a adaptação ao esqueleto craniofacial[2,6,8,10,11].

Algumas desvantagens encontradas no enxerto autógeno podem ser superadas utilizando os enxertos alógenos. O aloenxerto vem em uma variedade de formas, incluindo derivados ósseos osteocondrais, corticais, esponjosos e altamente processados, como a matriz óssea desmineralizada[2,6]. Geralmente colhido de secções da pelve de cadáver ou de cabeças femorais removidas em artroplastias totais de quadril[2,12]. O aloenxerto apresenta potencial osteocondutor, e possibilidade de capacidade osteoindutora, estando relacionada ao processo de preparação. Normalmente, não possuem células viáveis, não sendo osteogênicos[4,6]. Os maiores problemas associados aos aloenxertos são as transmissões de doenças e infecções, tanto de origem viral como bacteriana[4,6,10].

A matriz óssea desmineralizada (MOD) é uma forma de aloenxerto altamente processado, descalcificado utilizando solução ácida para remover componentes minerais, contendo colágeno, proteínas não-colágenas, proteínas morfogenéticas ósseas (BMPs) e outros fatores de crescimento que lhe conferem propriedades osteocondutoras e também osteoindutoras sendo o potencial osteoindutor maior do que o aloenxerto, porém dependente também da forma de preparação[6,13]. O risco de infecção e reação imunológica é reduzido devido ao processo de desmineralização, assim como destrói as células originais, inviabilizando atividade osteogênica[13,14]. A quantidade de BMPs nas MOD é altamente variável e é menor do que o encontrado no osso humano normal, havendo ampla diferença entre os produtos comercialmente disponíveis[14].

Enxertos ósseos obtidos de animais (comumente bovinos ou suínos), chamados xenógenos, foram desenvolvidos como uma alternativa ao uso do enxerto autógeno. Os enxertos de origem bovina têm demonstrado resultados promissores em diversos estudos[15-17]. Destaca-se propriedades como formação óssea adequada, baixa taxa de reabsorção, características osteocondutoras e compensação da reabsorção óssea natural causada pela remodelação ósseo[18]. Esse tipo de biomaterial é utilizado com frequência na odontologia para elevação do assoalho do seio nasal, preenchimento de alvéolos em exodontias e no tratamento de defeitos periodontais[18].

O biomaterial xenógeno líder no mercado mundial é o Bio-Oss®, produzido pela empresa Geistlich Pharma A, obtido de osso bovino. O Bio-Oss® se apresenta

como um enxerto ideal devido às suas propriedades mecânicas e ao seu comportamento biológico, apresentando características químicas e físicas semelhantes ao osso humano; é osteocondutor, sendo que sua estrutura altamente porosa e uma grande área de superfície de contato promovem o crescimento capilar e migração e proliferação de osteoblastos; estimula adequada formação óssea, apresenta baixa taxa de reabsorção, não estando associado ao aumento das taxas de complicações, além de evitar a necessidade de áreas doadoras[19,11,20]. Devido a estas propriedades, seu uso costuma apresentar resultados comparáveis aos enxertos ósseos autógenos[20].

Para obtenção do Bio-Oss®, o osso bovino é submetido a um tratamento alcalino forte como hidróxido de sódio (NaOH) e, posteriormente com peróxido de hidrogênio (H_2O_2), além de um tratamento térmico (temperatura de cerca de 300°C), para a retirada de matéria orgânica composta basicamente de colágeno. Contudo, mesmo utilizando métodos agressivos para a retirada de matéria orgânica nesse tipo de enxerto, uma parte ainda permanece no material (aproximadamente 1180-1200 ppm (partes por milhão) de matéria orgânica, que correspondem a 620 ppm de proteína, 500 ppm de colágeno e 60 ppm de outros componentes)[20]. Surge, assim, o questionamento de até que ponto enxertos xenógenos podem ser considerados seguros e quais seriam os riscos de transmissão de doenças aos humanos, como a encefalopatia espongiforme bovina[21]. Os antígenos celulares dos enxertos xenógenos demonstraram provocar respostas inflamatórias, estimulando liberação de mediadores inflamatórios do receptor, acarretando falha do enxerto[22]. Além disso, alguns pacientes podem recusar o uso de materiais de origem animal[11].

A engenharia tecidual óssea tem buscado desenvolver incessantemente biomateriais que apresentem biocompatibilidade, osteocondução e osteoindutividade como substitutos para autoenxertos, enxertos alógenos e xenógenos, tendo sido reconhecidos como alternativas promissoras para resolverem problemas de disponibilidade e limitação de volume, reações imunológicas e transferência de patógenos[7,22].

A hidroxiapatita (HA) ($Ca_{10}(PO_4)_6(OH)_2$) é um dos materiais biocerâmicos mais conhecidos com variadas aplicações dependendo de sua morfologia, tendo sido amplamente utilizada para o preenchimento de defeitos ósseos[23].

A HA é uma das biocerâmicas à base de fosfato de cálcio mais estáveis e menos solúveis que possui excelentes propriedades de biocompatibilidade,

bioatividade, bioafinidade e osteocondução, osteointegração, não tendo sido relatado toxicidade local ou sistêmica adversa. Possui boa afinidade biológica com o tecido ósseo devido a sua semelhança química e biológica com o mineral ósseo natural[5,24]. Além disso, a HA tem demonstrado atuar muito bem como um suporte durante o processo de adesão e proliferação de osteoblastos in vitro e possui capacidade de estabelecer uma forte ligação com o fase mineral óssea recém-depositada in vivo[25].

As diferentes formas de HA empregadas para aplicações biomédicas incluem blocos porosos (scaffolds) e densos, grânulos, pasta, cimento e revestimentos[24].

As propriedades mecânicas do HA dependem da porosidade, densidade, sinterabilidade, tamanho do cristal e composição da fase[24]. Pesquisadores têm buscado aprimorar as propriedades desse material alterando sua composição, morfologia e tamanho de partículas para aumentar sua bioatividade, resistência mecânica, solubilidade e sinterabilidade[5]. Alterações nas propriedades físico-químicas da HA como a porosidade, forma e tamanho de partículas, estão mostrando resultados positivos no processo de formação e regeneração óssea, além de promover propriedades osteoindutoras[26].

O aprimoramento dessas características pode ser modulado variando o método e as condições de síntese[26]. A HA pode ser sintetizada por vários métodos, como pirólise por pulverização, síntese por combustão, por hidrólise, método sol-gel, método de emulsão, método mecânico-químico, síntese hidrotermal, método de troca iônica, reação de estado sólido, e métodos de precipitação[27,28]. Um dos métodos mais usados é a precipitação úmida, em que as reações químicas ocorrem entre os íons cálcio e fósforo sob um pH e temperatura controlados da solução[26]. Cada método de síntese requer vários parâmetros de processamento para a produção de uma HA pura, como pH, temperatura, proporção molar de produtos químicos, entre outros, resultando em diferentes tamanhos e morfologias. As características diferentes das HA obtidas afetam significativamente a bioatividade, propriedades mecânicas e biológicas. Assim, o método de síntese está intimamente relacionado ao controle da morfologia, cristalinidade, tamanho e composição química da HA, sendo que estas características determinam a aplicação biomédica da HA[29].

Estudos clínicos e histológicos com partículas sólidas e densas de HA demonstraram boa estabilidade e biocompatibilidade do material, mas a taxa de crescimento ósseo apresentou um aumento mínimo nessas regiões[30,31]. Atualmente,

a HA particulada tem mostrado maior eficácia no processo de reparo ósseo, pela sua maior área de superfície e espaço entre os grânulos, o que beneficia a invasão do tecido nessa região[31].

Ademais, diversos estudos têm mostrado a influência do tamanho da partícula no processo de reparo ósseo com biomateriais[25,32,33]. Essa característica afeta diretamente a taxa de absorção dos materiais utilizados. Resultados promissores foram observados quando a HA é reduzida a uma escala nanométrica. A nanohidroxiapatita (nanoHA) favorece o aumento da área de superfície e, consequentemente, uma maior solubilidade cinética. Dessa forma, o material se integra melhor ao defeito ósseo, proporciona maior biodegradação e promove maior remodelação óssea[25,31,32,34].

A capacidade de um biomaterial de aumentar a expressão osteogênica e fornecer um suporte para a formação de um novo tecido ósseo é diretamente ligada ao tamanho do poro e porosidade[32]. O grau de porosidade regula a bioatividade dos biomateriais para enxerto, sendo que a interconectividade dos poros, geometria, topografia modulam a osteogênese, que promove sinergicamente a osteocondutividade do enxerto ósseo[24]. A porosidade refere-se à porcentagem geral de espaços vazios dentro de um sólido, enquanto o tamanho dos poros reflete o diâmetro individual dos vazios no material[35]. A importância dessas características pode ser atribuída à estrutura nativa do osso, que é um tecido poroso. O osso medular é uma estrutura altamente porosa com valores típicos de porosidade entre 50 e 90%. As regiões porosas do osso cortical permitem a vascularização e a infiltração celular da estrutura. A porosidade e o tamanho dos poros demonstraram crescimento de tecido vascularizado, melhoria na fixação celular e consequentemente, afeta a densidade da semeadura, distribuição e migração celular[35,33].

Em um estudo realizado por De Val et al.[36], foi feita uma comparação das propriedades físico-químicas de HA sintética com materiais comerciais. Uma análise detalhada das curvas de distribuição do tamanho dos poros foi obtida pela intrusão de mercúrio e revelou que os biomateriais à base de HA, puros e bifásicos, são meios eficazes para processos de regeneração óssea; destes, materiais com maior porosimetria inicial e secundária permitem maior colonização celular e, portanto, substituição mais efetiva por um osso novo[35]. O uso de HA in vivo tem sido avaliada há anos com a obtenção de resultados efetivos para regeneração óssea, sendo

utilizada pura ou associada a outros ativos com o objetivo de melhorar suas propriedades mecânicas, químicas e/ou biológicas[37-42,11,43-46].

As evidências científicas mostram que nenhum substituto ósseo ideal foi desenvolvido até o momento[5]. Há a necessidade de uma melhor combinação entre macro e micro características, além da simplificação do processo de fabricação e que tenha um custo acessível. Com isso, essa revisão sistemática traz dados promissores que mostram eficácia do uso HA sintética em estudos in vivo durante o processo de regeneração óssea. Além disso, faz o delineamento e a análise das características físico-químicas da HA e sua comparação com outros enxertos ósseos presentes no mercado atualmente.

2 PROPOSIÇÃO

O objetivo desta revisão sistemática e meta-análise foi avaliar a formação óssea em defeitos preenchidos com HA sintética em forma de partículas em modelos animais. Além disso, buscamos verificar a influência do tamanho das partículas de HA, porosidade, tamanho dos poros e cristalinidade nos resultados de formação óssea e reabsorção de partículas.

3 PUBLICAÇÃO

Synthetic hydroxyapatite in particulate forms in bone formation: a systematic review and meta-analysis*

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ABSTRACT

The aim of this systematic review and meta-analysis of animal studies was to evaluate the bone formation in defects filled with synthetic hydroxyapatite (HA) in particulate forms. Moreover, we identified the influence of HA particle size, porosity, pore size, and crystallinity in bone formation outcomes and particle resorption. Electronic searches in the PubMed/MEDLINE, Embase, Web of Science, and Scopus databases were conducted for publications up to May 2021. All articles were in vivo studies that reported bone defects filled with synthetic pure HA in particulate form in animals. The bone formation in bone defects filled with synthetic pure HA in comparison to blood clots was calculated using a random-effects model meta-analysis. Moreover, the effect of particle size on bone formation was evaluated with a meta-regression analysis. Fifty-six studies were included in the qualitative analysis (systematic review) and 2 studies composed the meta-analyses/meta-regression. The studies included showed bone formation in defects filled with HA particles. In comparison to defects filled with a blood clot, the HA showed superiority in bone formation outcomes in qualitative and quantitative analysis. Small particles, mainly at the nanoscale (nano-HA), showed a higher rate of bone resorption, and a greater bone formation observed in qualitative analysis. However, no influence of particle size in bone formation was shown in meta-regression analysis (considering only three observations). Higher porosity and pore size seem to increase bone formation in defects filled with HA. In conclusion, the HA particles are osteoconductive, providing surfaces for new bone formation. Moreover, alterations in HA physicochemical properties including particle size, porosity, and pore size directly influence the outcome of new bone formation and resorption rates.

Keywords: hydroxyapatite; bone formation, osteoconduction.

1. INTRODUCTION

The bone is a specialized connective tissue formed by organic and inorganic elements, being the major portion of a rigid mineral structure composed of hydroxyapatite (HA) [1]. Using a dynamic and complex process, the bone has the ability to regenerate completely, supplying the mineral needs and providing the mechanical support of the body [2]. However, bone defects caused by trauma, infections, congenital defects, and tumors may be too large for a spontaneous and physiologic repair [3]. In such cases, bone grafts are used as mineral bone substitutes for defect reconstruction [2]. In attempts to achieve rapid and proper bony healing, several forms of autogenic bone transplants have been used. However, the utilization of such bone has some disadvantages including a need for a second surgical site, higher patient morbidity, increased operative time, and limited bone availability in donor area [1]. In this context, several alloplastic materials have been proposed as bone graft substitutes to overcome these complications [4]. In addition to unlimited availability, safety, and lack of immunogenicity and toxicity, the alloplastic materials' properties can be modified during the synthesis process to improve the graft's biological behavior [5].

Among the alloplastic materials, HA synthetic ceramic has been extensively used as bone substitute material in bone defects due to its similarity to the natural bone [6]. The HA synthetic shows excellent biocompatibility, bioactivity, and osteoconductive properties and can be fabricated easily into any size or shape [5,7]. In comparison to other bone graft materials, HA synthetic demonstrated high dimensional stability and very low resorption rates [5]. Moreover, the HA synthetic support osteoblast adhesion and proliferation in vitro [8] and promote bone formation in vivo [9]. On the other hand, despite the HA synthetic showing a good compressive strength is characterized to be weak in tension and shear [6].

Usually, the HA synthetic comes as blocks or particle materials, and the bone formation and graft incorporation vary according to the type [6,10]. The HA in particulate forms is indicated for filling various types of bone defects because are easily applicable. Additionally, interparticle space allows the invasion of osteoblastic cells around the particles which promotes bone tissue invasion [11].

However, the HA effectiveness is controversial once the slow biodegradability of HA has been identified as a disadvantage in relation to other biomaterials [5]. It is

discussed whether more effective reabsorption of the graft material can result in a more gradual replacement of the particles' space by new bone [5]. Moreover, the alterations in the physicochemical properties of HA particles such as porosity, shape, and size, are described as influencing the outcome of new bone formation [12].

The aim of this systematic review and meta-analysis of animal studies was to evaluate the bone formation in defects filled with synthetic HA in particulate forms. Moreover, we indent to identify the influence of HA particle size, porosity, pore size, and crystallinity in bone formation and HA particle resorption results.

2. MATERIALS AND METHODS

2.1 Protocol

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13] and a protocol was registered in PROSPERO (ID: CRD42022307364).

2.2 Focused question

The focus question of the review was “Does particulate synthetic HA effective for bone repair?”.

2.3 Eligibility criteria

The following inclusion criteria were adopted based on the following PICO criteria: (P) Population/Animals: all animals species (all sexes); (I) Intervention: bone defects filled with synthetic pure HA in particulate form; (C) Comparator: bone defects filled with blood coat, deproteinized bovine bone mineral (DBBM) and synthetic HA in the particulate form associated with others biomaterials; (O) Outcomes: bone formation in defects filled with synthetic HA in particulate form; (S) Study design: in vivo studies reporting data about bone defects filled with synthetic HA in the particulate form published in English language.

The following exclusion criteria were excluded: i) articles without a group with pure synthetic HA in particulate form; ii) studies including only animals with co-morbidities, submitted to ovariectomy and ex vivo, in vitro, and in silico models; iii) studies evaluating bone defects filled with synthetic HA in other forms (e.g. cement,

paste and block graft) and interventions in other tissues; and iv) letters to the editor, historical reviews, clinical studies, and unpublished articles.

2.4 Literature search

Electronic searches were conducted in the PubMed/MEDLINE, Embase, Web of Science, and Scopus databases up to May 2021. Publications were found using a combination of terms which are shown in supplementary materials (Search Strategy – Supplementary materials). Filter for animal studies was applied, however, search restrictions for language and publication period were not made. Moreover, unpublished articles and grey literature were not searched. The publications found in all electronic databases were transferred to the EndNote Program™ X7 version (Thomson Reuters, New York, NY, USA) to remove duplicate references.

2.5 Data selection and extraction

Two investigators (ML and LFFS) performed the initial search for assessment of titles and abstracts independently, and the results were checked for agreement. The full text of the articles judged to be relevant based on title and abstract were independently read and assessed based on the selection criteria (ML and LFFS). For conflicting evaluations, an agreement was reached following a discussion including a third investigator (SCP). The same two investigators (ML and LFFS) independently reviewed all studies and extracted the data using a standardized table utilizing Microsoft Excel.

The extracted data were: i) specie of the animal model; ii) study groups (experimental groups, control group(s), and the number of animals per group); iii) type and size of bone defects; iv) obtention method, particle size, porosity, crystallinity, pore size and bioactivity of the synthetic HA; and v) bone formation outcomes.

2.6 Quality assessment

Two authors (CMAM and LFFS) assessed the quality of the included studies separately using the SYRCLE's risk of bias tool for animal studies [14]. Any disagreement was discussed with a third reviewer (SCP).

2.7 Data synthesis - Meta-analysis

A meta-analysis evaluating the percentage of bone formation obtained in bone defects filled with synthetic pure HA (different particle sizes) in comparison to the control group (empty defect) was made. The studies were grouped considering the same animal and bone defect models. Among the outcomes reported, the percentage of new bone formation was used to avoid bias regarding the different bone defect sizes used in the included studies. In addition, HA particles size (millimetric and nanometric sizes) were converted into µm.

Random-effects meta-analysis was performed in RStudio version 4.0.4 using the 'meta-package [15] version 4.18-0 [16]. The effect size is presented as mean difference (MD) with standard deviation (± SD), and 95% (convidense interval) CI for the percentage of bone new formation. A forest plot was used to illustrate the outcomes of the analysis. To assess the heterogeneity among studies, inconsistency statistics (I^2) was calculated, and significant heterogeneity was considered to be present when I^2 was > 50% [17]. A significant heterogeneity would indicate random-effects meta-analysis conduction, in order of a fixed-effect model [18].

In an effort to understand the impact of different HA particle sizes on bone formation, a meta-regression was also performed in RStudio version 4.0.4 using the 'meta-package [15] version 4.18-0 [16]. The particle size of each HA biomaterial tested (30nm, 350µm, 630µm) was considered as a continuous covariate to be correlated to the percentage of new bone formation.

3. RESULTS

In the electronic search, a total of 6294 hits were found, being 5376 unique citations. A total of 177 studies were evaluated as full-text copies and 121 of these studies were excluded based on priority criteria (Figure 1). The remaining 56 studies were included in the qualitative analysis (systematic review) and 2 studies composed the meta-analyses.

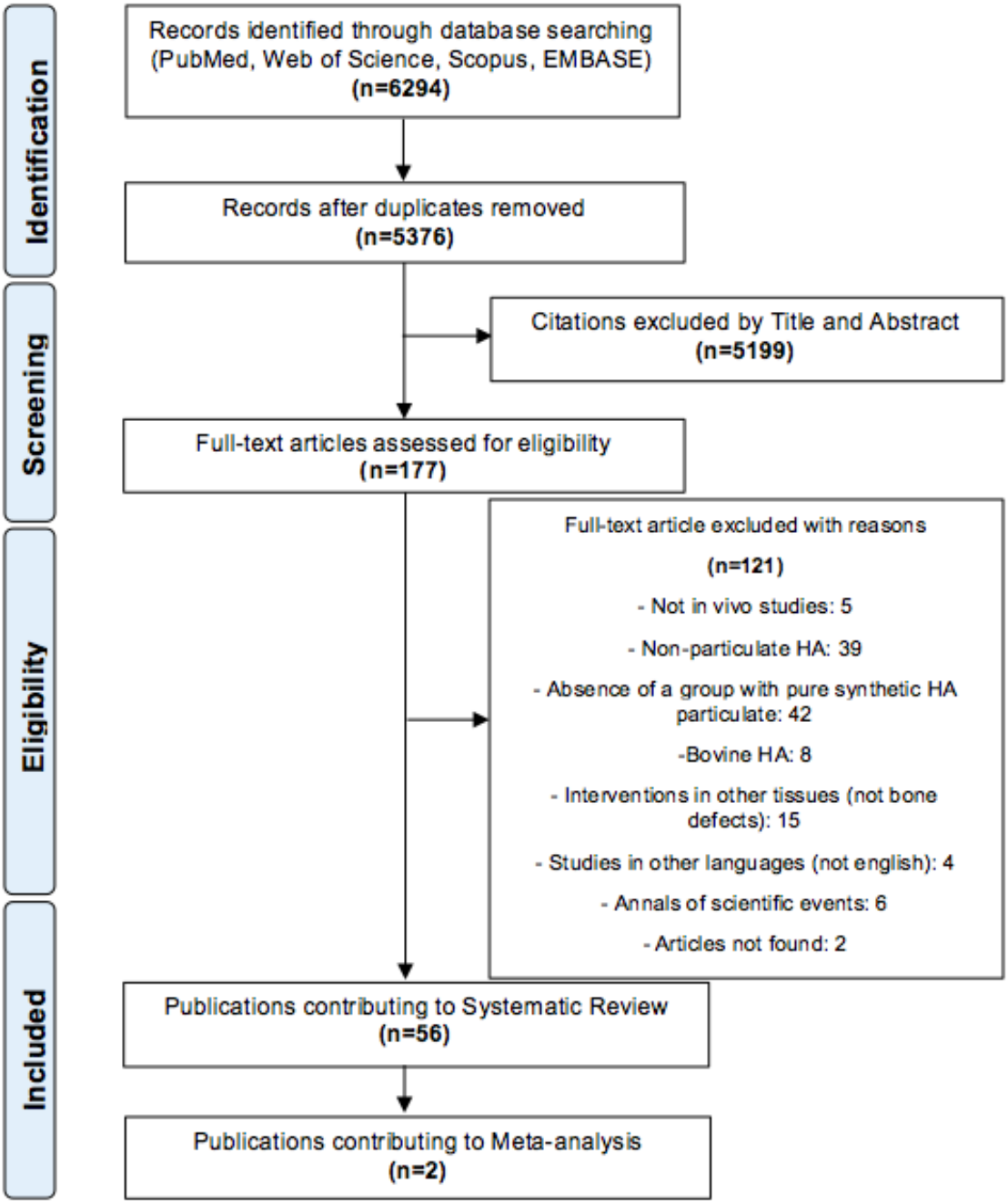

Figure 1: Study search strategy flow-char

3.1 Qualitative analysis (systematic review)

Table 1S (supplementary materials) described the characteristics of studies included in the systematic review according to the PICO´s principles. In addition, the characteristics of synthetic HA including obtention method, particle size, porosity, and pore size were also detailed in table 1S. Considering the 56 animal studies included

in the systematic review that described the bone formation outcomes in defects filled with synthetic pure HA in particulate form, the majority of the studies were performed on rabbits (28 studies) followed by rats (16 studies), dogs (9 studies) and sheep (3 studies) (Table 1S).

Moreover, a wide variety of *types of bone defects* were observed in the animal studies (Table 1S). The most common were defects in the tibia (15 studies), calvaria (13 studies), the mandible (6 studies), femoral condyle (6 studies), and radius (4 studies). Bone defects in femur (3 studies), sinus floor augmentation (3 studies), the distal surface on the third premolar (2 studies), humerus (1 study), iliac (1 study); parietal bone (1 study), periodontal defects (1 study), post-extraction cavity (1 study) and mastoid obliteration (1 study) were also reported. In two articles [19,20], bone defects were performed in more than one region.

Forty-nine studies reported information about *the HA obtention method* (seven studies did not mention this information) (Table 1S). From these, 24 studies reported only the synthetic HA commercial brand. Of the remaining 25 articles, the obtention method was reported in 13 studies the precipitation method (6 studies), followed by the sol-gel method (5 studies), and wet-chemical (2 studies). In addition, HA synthesis temperatures were described in 21 studies and ranged from 500°C to 1225°C. The most used temperatures were 1200°C (5 studies) and 900°C (4 studies), followed by 1100ºC (3 studies), 1000°C (3 studies), 800°C (3 studies), 1150°C (2 studies), 600°C (2 studies), 1225°C (1 study), 850ºC (1 study), 700ºC (1 study) and 500-800°C (1 study).

A high variety of synthetic *HA particle size*s was observed in the 33 studies that reported this information. Particles with 10 nm to 1.5 mm have been reported. Few articles described the data about HA porosity. The most common *degree of porosity* was from 60-85% (9 articles); followed by 35-48% (4 articles), 50-57% (3 articles) and 20-28% (2 articles).

Information about the *HA crystallinity* was described in only two studies [21,22]. Carvalho, et al. [21] comparing synthetic HA with bovine HA (BHA) described that HA samples were highly crystalline, while BHA appeared with a rougher surface at scanning electron microscopy (SEM) analysis. Vieira, et al. [22] produced a HA with good crystallinity, without the presence of intermediate phases using the suspension–precipitation method. Regarding HA bioactivity, only one study reported

this information [23]. Fujishiro, et al. [23] showed that synthetic HA showed lower bioactivity (3.1) in comparison to Bioglass® (12.5) using bioactive bonding indices.

The outcomes of *bone formation* obtained with synthetic HA were described in tables 2S-5S (supplementary materials). The studies were divided according to the comparator group. In 33 studies, an empty group was identified; however, only 23 studies reported the bone formation outcomes obtained in this group in comparison with synthetic HA (Table 2S; supplementary materials). Among these, most studies demonstrated the superiority of synthetic HA in bone formation (21 studies). The empty group showed superior [24] or similar [25] bone formation in comparison to synthetic HA only in two studies (Table 2S in blue lines).

Ten studies compared the bone formation in defects filled with synthetic HA in comparison to DBBM (Table 3S; supplementary materials). The majority of included studies (6 studies) showed no differences in bone formation obtained in the defects treated with both materials (Table 3S in white lines). On the other hand, 4 studies showed the DBBM superiority (Table 3S in grey lines).

In table 4S (supplementary materials) were included the studies that evaluated synthetic HA alone in comparison to HA associated with other biomaterials/treatments or with other biomaterials. Among the 35 included studies, only in 9 studies, the synthetic HA alone show a better [26,27] or similar bone formation compared to the other biomaterials [24,28-33]. In the other studies, synthetic HA performance in bone formation was inferior when used alone in comparison with other biomaterials. Two included studies showed higher bone formation in defects filled with autogenous in comparison to synthetic HA [34,35]. On the contrary, Haas, et al. [29] showed no significant difference between HA and autogenous bone regarding bone-implant contact (Table 4S). Compared with beta tri-calcium phosphate (β-TCP), the synthetic HA showed less bone formation in two studies [24,36]. On the other hand, Okanoue, et al. [37] described that bone ingrowth in the synthetic super porous HA group significantly increased over time, while no significant increase was observed after 8 weeks in the β-TCP group. Lee, et al. [38] not found differences in bone formation between β-TCP and HA groups (Table 4S). Two studies showed superior performance of carbonate apatite in comparison to synthetic HA [39,40] (Table 4S). Finally, three studies showed that synthetic HA particles loaded with recombinant human bone morphogenetic protein-2 (rhBMP-2) promoted more bone formation than pure HA [38,41,42] (Table 4S).

Seventeen studies compared different types of HA regarding bone formation (Table 5S; supplementary materials). Among the four studies that evaluated the effect of different particle sizes on bone formation, two studies showed that nano-HA promoted more bone formation in comparison to micro-HA [3,36] (Table 5S). The other two studies showed that smaller micro-HA particles promoted greater bone formation compared to large micro-HA particles [21,43] (Table 5S). For HA particle shape, Gonda, et al. [11] showed significantly higher newly formed bone in the specimens implanted with rod-shaped HA particles compared to globular-shaped HA particles. Moreover, Kurioka, et al. [44] reported that the compact and multi-nuclear HA particles with continuous pores demonstrated the best osteoconduction 2 weeks after implantation, with little bone resorption in the 6-month specimens, and a stable HA-bone complex in the whole implanted area. Frame, et al. [45] comparing solid and porous HA showed that only porous HA demonstrated a greater amount of bone ingrowth compared with the solid HA. Other studies compared synthetic HA commercial brand [9,43,46-48], changes in chemical composition [49,50] or graft format [26], synthetic versus natural HA [51], and bone defects filled with HA in different period of analysis [52]. The outcomes obtained in these studies were described in table 5S.

The HA reabsorption rate was evaluated in 14 studies (Tables 3S-5S). In comparison to DBBM, two studies [30,53] reported that the amount of residual material was significantly lower in the defects filled with nano-HA particles in comparison to DBBM. Similarly, Orr, et al. [54] study showed a difference between both materials in the percentage of the area occupied by the particles, with the DBBM particles having two-thirds the value of the synthetic HA material. On the other hand, Xu, et al. [32] reported that the amount of residual grafting material was significantly higher in HA in comparison to DBBM. Three studies showed similar residual material amount for synthetic HA and DBBM particles [6,35,55].

Conversely, more gradual resorption of the material was observed for β –TCP in comparison to HA particles [24,37,56]. John, et al. [52] observed that the HA resorption occurs in a slow process, since HA granules still persisted in the defects after 24 weeks. Higher HA particles percentage was also observed in other studies [24,32,48,57]. Mate Sanchez de Val, et al. [58] demonstrated that HA particles of 2000–4000 µm and 1000–2000 µm showed lower rates of resorption in comparison to 600–1000 µm particle size.

3.2 Risk of bias

After analyzing all the included studies using the SYRCLE's risk of bias tool (Figures 2 and 3), it could be noted that for the majority of the manuscripts there were a low risk of bias for random sequence generation, baseline characteristics, incomplete outcome data, selective reporting, and other biases. However, unclear risk of bias was reported for random housing, blinding of participants and personnel, random outcome assessment, and blinding of outcome assessment. A high risk of bias was observed in a few included studies.

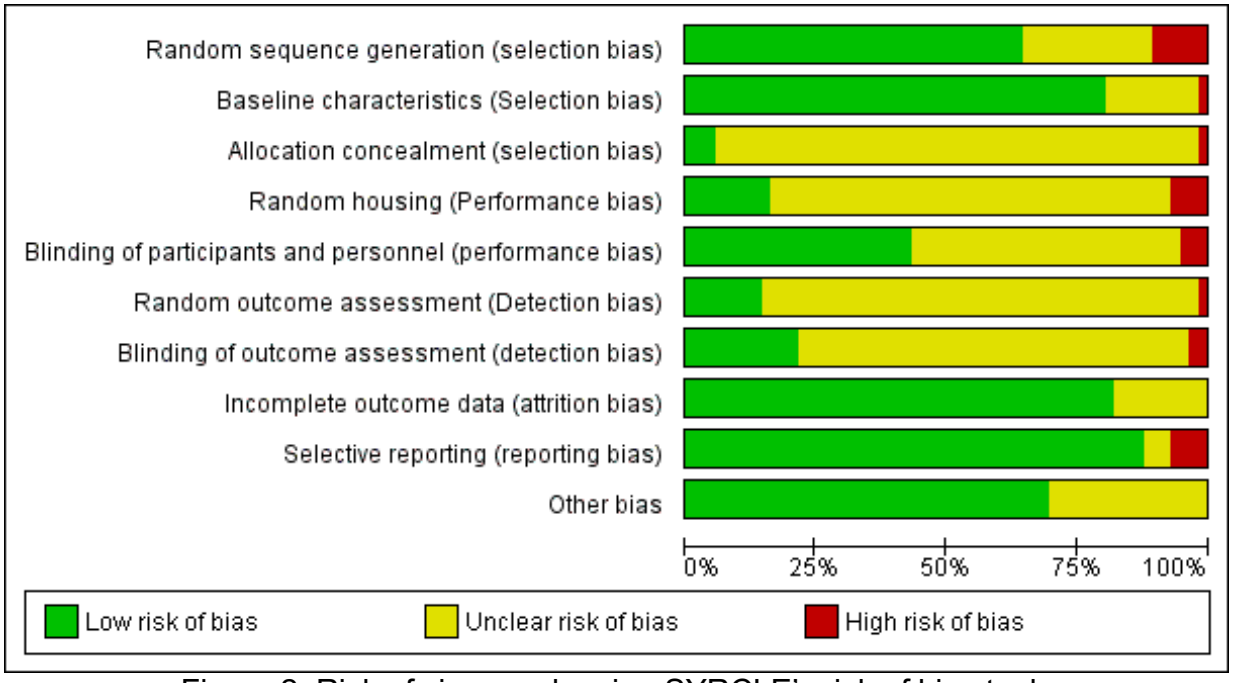

Figure 2: Risk of vies graph using SYRCLE's risk of bias tool

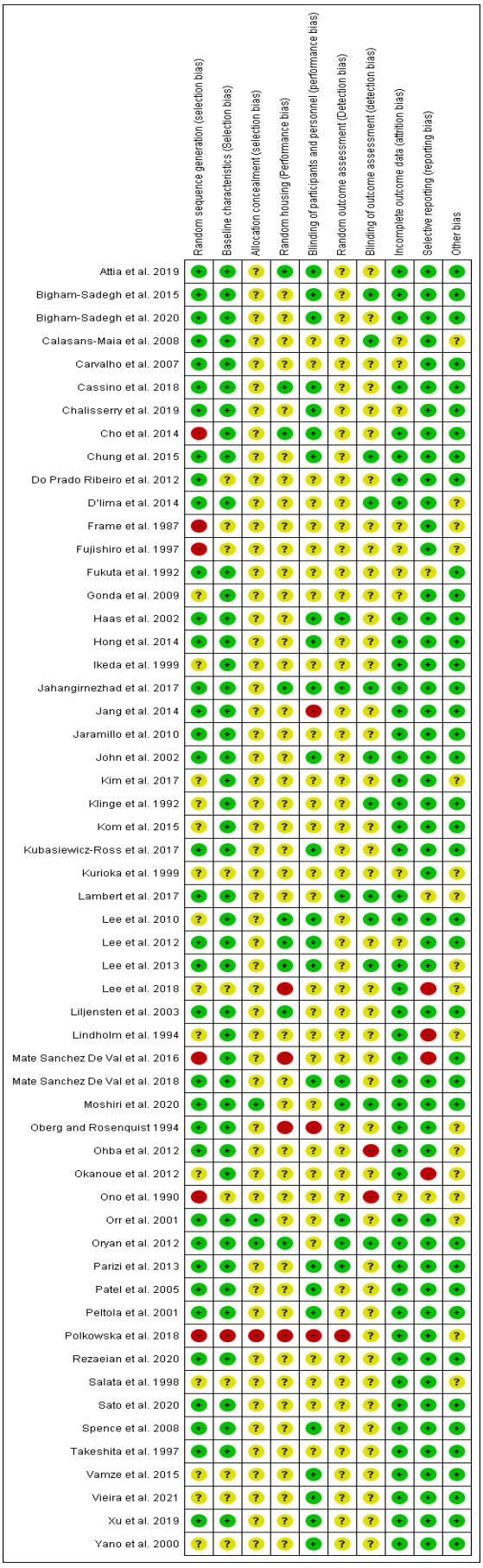

Figure 3: Risk of vies summary using SYRCLE's risk of bias tool

3.3 Meta-analysis and meta-regression

Two studies were included in a total of three comparations for the meta-analysis (k=3). Grafted sites with synthetic HA exhibited a significantly higher *percentage of bone new formation* compared to the control group (MD: 25.6426% [19.7099; 31.5753], p< 0.0001; I²=53%) (Figure 3).

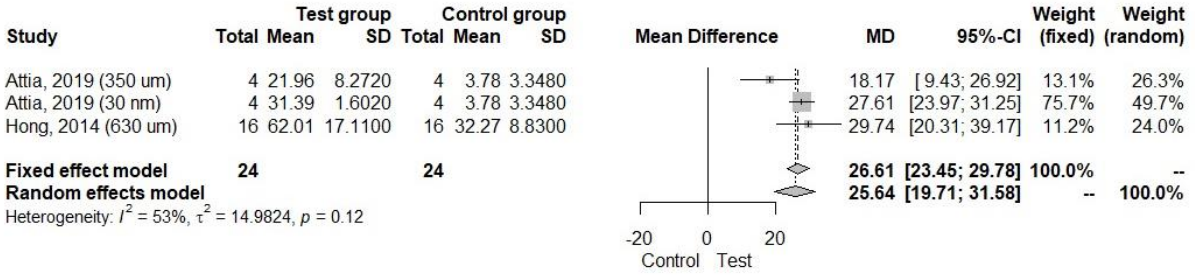

Figure 3: Forest plot.

Meta-regression combined the covariate 'HA particle size' for *the percentage of bone new formation* (k=3). Although a minimum recommend the number of observations (k≥10) was not achieved for this analysis [18], the included HA particle sizes compared did not exhibit statistical significance among them (30nm, 350mm, 630mm; p=0.9677) (Figure 4).

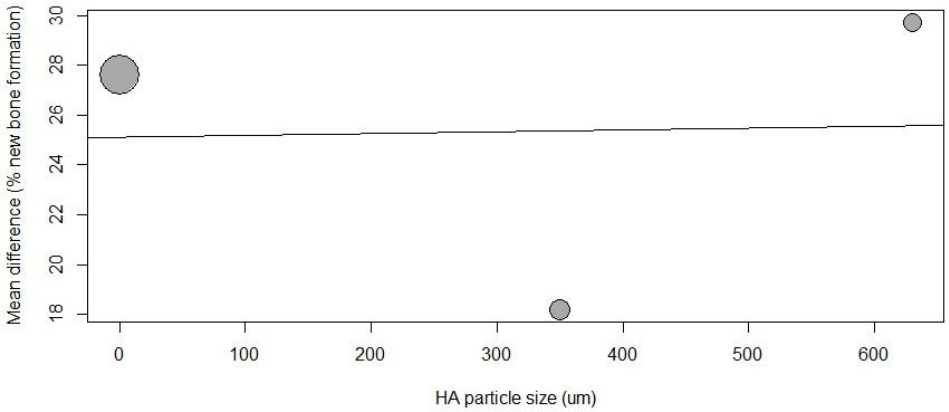

Figure 4: Bubble plot.

4. DISCUSSION

The main aim of bone tissue engineering is to develop materials that mimic the native tissue microenvironment in order to facilitate bone regeneration, and to provide alternative options to classic strategies [59]. The HA is the most common synthetic bone filler used as bone substitute to mimic the inorganic part of the bone microenvironment by virtue of its similarity to the natural bone mineral [6]. However, there remain several questions about the efficacy of HA in bone formation. Therefore, the first purpose of this systematic review with meta-analysis was to evaluate the bone formation in defects filled with synthetic HA in particulate forms in comparison to blood clots (control) and other biomaterials.

In general, it was evident in the included studies that HA particles provided surfaces for new bone formation, thus being osteoconductive. Newly formed bone in direct contact with the particle surface was usually described in the included studies. In comparison to defects filled with blood clots, the HA showed superiority in bone formation outcomes in the majority of included studies in the qualitative analysis. In the meta-analysis, considering the defects filled with synthetic HA of 30nm, 350µm [3], and 630 µm particle size, more bone formation was also obtained in HA than in the empty defects. Evidences suggested that HA itself stimulated and promoted bone formation around HA particles by dissolving Ca [12]; it is probable that high levels of Ca and P stimulate osteogenesis by increasing osteoblast gene expression [60]. Moreover, this ion release can form zones of biological apatite on the agglutinated Ca and P deposits and crystals, which in turn facilitate osteoconduction [5].

Bovine xenografts are natural osteoconductive biomaterials used on a large scale due to their similarity with human bone in terms of chemical composition and structure [61]. In qualitative analysis, the majority of included studies (6 studies) in this review showed no differences in bone formation obtained in the defects treated with HA or DBBM. However, 4 studies showed more bone formation obtained with DBBM particles. According to evidence, the structural similarity of DBBM to cancellous bone act as a template for mineral-collagen interaction and crystallites during the bone formation inside of the defect [1,62]. This structural characteristic of the bone mineral phase is not observed in HA synthetic materials, which are formed without an organic template [62]. Moreover, the DBBM superiority can be also justified by the higher roughness of the DBBM surface in comparison to HA synthetic which seemed to

benefit the osteoconduction process [6]. The increase in surface roughness positively affected osteoblast adhesion which is a mandatory condition for cell proliferation and osteogenesis [63]. It was not possible to perform a meta-analysis comparing HA versus DBBM in this review due to the variability in the study design of the included studies.

The main difference between HA and β-TCP is their slow and fast resorption rates, respectively [64]. It suggested that, although HA has the osteoconductive ability and excellent biocompatibility, it needs a long time to be replaced by natural bone. On the other hand, β-TCP has higher solubility in physiological saline than HA, as well as better bone conductivity and bioresorbability [65]. However, the results from the studies included were contradictory in regard to TCP superiority and a meta-analysis was also not made in this review due to the study design variability. Alternatively, [66] showed that carbonate apatite promoted more bone formation compared to TCP and HA. A skeleton with a porous microstructure morphologically similar to cancellous bone is obtained in the carbonate apatite due to the substitution of the hydroxyl group by the carbonate group [67]. Moreover, carbonate apatite promotes osteoclast recruitment, angiogenesis, and osteoblast differentiation in the early stages of bone repair, probably due to the carbonate and calcium effects [66]. The superiority of carbonate apatite was also observed by Spence, et al. [39]. Furthermore, it was described that HA particles combined with supportive osteoinductive molecules such as BMP showed higher bone formation in comparison to HA alone [38,41,42].

The biological response induced by a biomaterial is affected by its physical and chemical properties [5]. Based on that, this review aimed to identify the influence of HA particle size, porosity, pore size, and crystallinity in bone formation outcomes. In regard to HA particle size, nano-HA showed higher bone formation in comparison to micro-HA in the studies included in the quality analysis [3,36]. The nanostructured HA is obtained by reducing the size of particles at the nanometer scale which increases the surface area and kinetic solubility [68]. These characteristics allowed further grafted material biodegradation and more bone remodeling. Studies reported significant deterioration of the nano-HA particles by solubility and subsequent bone tissue formation [3,36]. Moreover, the nano-HA particles have dimensions similar to the natural crystals (from 1 to 10 nm) and mimic the extracellular matrix of bone in size and structure [69]. In the same way, the other two studies comparing micro-HA

particles showed that smaller particles promoted greater bone formation compared to larger particles [21,43]. Mate Sanchez de Val, et al. [58] demonstrated that HA particles of 2000–4000 µm and 1000–2000 µm showed lower rates of resorption in comparison to 600–1000 µm particle size. However, in our meta-regression comparing three different sizes of particles (30nm, 350µm, and 630 µm), the bone formation was not influenced by the particle size. However, this result needs to be interpreted with caution because only 3 observations were included and a minimum of 10 observations is recommended to make this analysis [18].

Porosity indicates the overall percentage of empty spaces within a particle, while the pore size refers to the diameter of each space [5]. Both parameters affect the ability of the material to promote osteoconduction and vascularization [5]. The majority of included studies reported porosity ranged between 60-85% (9 articles). Maté Sánchez de Val, et al. [5] reported that materials with higher initial and secondary porosimetry allow greater cell colonization resulting in more effective substitution by new bone. Moreover, the high porosity facilitates the resorption process, resulting in an increase in Ca and P ion release. Kurioka, et al. [44] reported that particles with continuous pores ranging from 30 to 200 µm in diameters show good initial osteoconduction and long-term stability. Due to the great variability of porosity and pore size between the studies included, no comparison between these parameters is possible in this review. In addition, only two studies reported information about the crystallinity of the HA particles. Carvalho, et al. [21] described that HA samples evaluated were highly crystalline which characterizes a material as less soluble and more resistant to granular disintegration by extracellular fluids [70,71].

Preferably, the bone substitute should promote bone formation at the same time it is completely resorbed and substituted by new bone tissue. However, in general, a higher HA particles percentage was observed in the included studies even after a long period of analysis, except for nano-HA which showed high rates of resorption [30]. The HA resorption can be mediated by cells (degradation by macrophages and osteoclasts) or by the physicochemical surface dissolution and disintegration of the material into smaller particles by the extracellular fluids (chemical dissolution) [62]. Some authors described that the slow biodegradability of HA particles might not be a disadvantage for clinical application once the release of high concentrations of calcium to the microenvironment results in a change of the pH, and promotes a mild inflammatory response, which favors fibrous tissue formation inside

the bone defect [72,73]. Moreover, materials with a high rate of resorption may degrade faster than the rate of bone formation which affects the bioceramic's physical structure, mechanical stability, and cell attachment [74].

The main limitation of this review study included the great variability among the included reports in regard to the animal specie, type/size of bone defects, euthanasia periods, and HA synthetic properties (obtention methods, particle/pore size, and porosity). Moreover, the studies varied in regard to the analysis used to evaluate bone formation (histological and histomorphometric analyses, microCT, or radiography). Although the a high number of included studies, only two of them were eligible to perform the meta-analysis and meta-regression. Therefore, these results need to be evaluated with caution. Information about the obtention method and HA properties were not usually described in the included studies. Moreover, few studies described and discussed the influence of HA physicochemical properties on bone formation outcomes. In future studies, details about the physical-chemical properties of the evaluated materials need to be described given the importance of these parameters in the biomaterials' efficacy.

In conclusion, the HA particles are osteoconductive, providing surfaces for new bone formation. Moreover, alterations in HA physicochemical properties including particle size, porosity, and pore size directly influence the outcome of new bone formation and resorption rates.

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SUPPLEMENTARY MATERIALS

Search strategy

Publications were found using the combination of the following terms:

Pubmed: (((((("Hydroxyapatite"[All Fields] OR "Hydroxyapatites"[All Fields] OR "Calcium Hydroxyapatite"[All Fields] OR "hydroxyapatite calcium"[All Fields] OR "Durapatite"[All Fields]) AND "Bone graft"[All Fields]) OR "Graft"[All Fields] OR "Grafting"[All Fields]) AND "Bone Formation"[All Fields]) OR "Osteogenesis"[All Fields] OR "Bone Regenerations"[All Fields] OR "Bone Regeneration"[All Fields]) AND "Osteoconduction"[All Fields]) OR "Osteoconductive"[All Fields] OR "Osteoconductivity"[All Fields]) AND (animal[Filter]).

Web of Sience: (Hydroxyapatite OR Hydroxyapatites OR Calcium Hydroxyapatite OR Hydroxyapatite, Calcium OR Durapatite) AND (Bone graft OR Graft OR Grafting) AND (Bone Formation OR Osteogenesis OR Bone Regenerations OR Bone Regeneration OR Regeneration, Bone OR Regenerations, Bone OR Osteoconduction OR Osteoconductive OR Osteoconductivity) AND (Rats OR Rat OR Mouse OR Mouses OR Mice OR Murine OR Murines OR Rabbit OR Rabbits OR Monkey OR Monkeys OR Animal Experimentation OR Animal Experiments OR Animal Research OR Animal Experimental Use OR Animal Experimental Uses).

Embase: ('hydroxyapatite' OR 'hydroxyapatites' OR 'calcium hydroxyapatite' OR 'hydroxyapatite, calcium' OR 'durapatite') AND ('bone graft' OR 'graft' OR 'grafting') AND ('bone formation' OR 'osteogenesis' OR 'bone regenerations' OR 'bone regeneration' OR 'osteoconduction' OR 'osteoconductive' OR 'osteoconductivity') AND ('animal experiment'/de OR 'animal model'/de OR 'animal tissue'/de OR 'canine model'/de OR 'experimental model'/de OR 'experimental study'/de OR 'in vivo study'/de OR 'nonhuman'/de OR 'ovine model'/de OR 'rabbit model'/de).

Scopus: (hydroxyapatite OR hydroxyapatites OR calcium AND hydroxyapatite OR hydroxyapatite, AND calcium OR durapatite) AND (bone AND graft OR graft OR grafting) AND (bone AND formation OR osteogenesis OR bone

AND regenerations OR bone AND regeneration OR regeneration, AND bone OR regenerations, AND bone OR osteoconduction OR osteoconductive OR osteoconductivity) AND (rats OR rat OR mouse OR mouses OR mice OR murine OR murines OR rabbit OR rabbits OR monkey OR monkeys OR animal AND experimentation OR animal AND experiments OR animal AND research OR animal AND experimental AND use OR animal AND experimental AND uses) AND (EXCLUDE (EXACTKEYWORD , "Human") OR EXCLUDE (EXACTKEYWORD , "Humans") OR EXCLUDE (EXACTKEYWORD , "Controlled Study") OR EXCLUDE (EXACTKEYWORD , "Review") OR EXCLUDE (EXACTKEYWORD , "In Vitro Study")).

Table 1S: Characteristics of studies included in the systematic review according to the PICO´s principles.

Author/ Year	Studies characteristics					Hydroxyapatite characteristics			
	Animal model	Experimental groups	Total of animals (animals per group)	Bone defect type	Bone defect syze	Obtention method	Particle size	Porosiy	Pore Size
Attia, et al. [1]	Dog	G1: Clot; G2: Nano-HA; G3: Micro-HA; G4: Nano-HA+Micro-HA	8 (2)	Distal surface of third premolar	5mm x 7 mm	Nano Bone	Nano- HA: 10 a 50nm; Micro-HA: 300 a 400 microns;	NI	NI
Bigham-Sadegh, et al. [2]	Rabbit	G1: HA; G2: HA+DCFGP; G3: DCFGP	15 (5)	Mid-radius bone	10mm	OS Satura	NI	NI	NI
Bigham-Sadegh, et al. [3]	Rabbit	G1: Empty; G2: HA; G3: HA+Royal jelly	15 (5)	Mid-radius bone	10mm	NI	NI	NI	NI

Calasans-Maia, et al. [4]	Rats	G1: Autogenous bone; G2: HA; G3: Zn-HA	45 (15)	Calvaria	8mm	CBPF (Brazilian Center on Physics Research)	NI	NI	NI
Carvalho, et al. [5]	Dogs	G1: Bovine HA (BHA)[Small]; G2: BHA[Large]; G3: HA[Small]; G4: HA[Large]; G5: Empty	8 (4)	Humerus	5mm x 4mm	Hydrolysis and subsequently sintered at 1200ºC for 4 hours. The matter was ground in a ball-grinder and sieved into particles sizes of 150 to 300 µm.	HA small: 150 µm; HA large: 300 µm	NI	NI
Cassino, et al. [6]	Rats	G1: Low purity HA (HAP); G2: Strontium HA (HAPSr); G3: HA doped with gallium (HAPGa); G4: Empty	40 (10)	Tibia	1cm	The synthesized powder was calcined at 700°C. These volumes correspond to a ratio of 5:3 between $Ca(OH)_2$ H_2PO_3	NI	NI	NI
Chalisserry, et al. [7]	Rabbits	G1: NanoHA; G2: NanoHA + Simvastatin (SIM)	12 (12)	Femoral condyle	5mm x 8 mm	Sigma	<200nm	NI	NI
Cho, et al. [8]	Rabbits	G1: HA; G2: Sodium-substituted HA (NaAp)	8(4)	Calvaria	8mm	Calcium hydroxide and phosphoric acid sintered at 1100 ºC for 3 h	G1:0.326 ±0.14 nm; G2: 0.976 ±0.38 nm	NI	NI

Chung, et al. [9]	Rats	G1: HA; G2: ErhBMP-2 loaded HA; G3: Empty	20 (10)	Calvaria	8mm	Manufactured by BioAlpha Inc. (Bongros®, Gyeonggi, South Korea)	0.6 a 1mm	NI	NI
D'Lima, et al. [10]	Dogs	G1: OsteoGenR (HA Resorb)™; G2: Chitra granules	4 (2)	Mandible	2-3 mm x 4.5-5 mm	OsteoGenR (HA Resorb)™; Chitra granules	OsteoGen R: 300 to 400 microns; Chitra granules: 0.5 to 1mm.	NI	Chitra granules: 100-200 microns
Frame, et al. [11]	Dog	G1:Solid HA; G2: Porous HA; G3: Solid HA + autogenous bone; G4: Solid HA + plaster of paris; G5: Porous HA + plaster of paris	6 (6)	Mandible	NI	Interpore 200, Interpore International, Irvine, CA	NI	NI	NI
Fujishiro, et al. [12]	Rabbit	G1: Bioglass®; G2: HA	NI	Femur	NI	NI	100-300µm	NI	NI
Fukuta, et al. [13]	Rabbits	G1: DBBM (Bio-Oss®); G2: Porous HA; G3: Autogenous bone; G4: Empty	20 (5)	Calvaria	8x8mm	Interpore 200	NI	NI	NI
Gonda, et al. [14]	Rats	G1: Rod-shaped HA particles (HHA); G2: Globular-shaped HA particles (SHA)	48 (6)	Tibia	2mm x 3mm	HHA: hydrothermally synthesized HA, composed of rod-shaped particles; SHA: Spherical HA granules were prepared by sintering at 900 ºC for 3 h.	G1: 0.5-0.6mm	G1 and G2: 70%	NI
Haas, et al. [15]	Sheep	G1: Porous HA; G2: Autogenous iliac crest bone; G3: Empty	27 (9)	Sinus floor augmentation	1cm x 1cm	Porous HA: Interpore-200, Interpore International	NI	NI	NI
Hong, et al. [16]	Dog	G1: HA; G2: BCP; G3: β-TCP; G4: Empty	8 (2)	Mandibular/maxillary third premolars	NI	Calcitite® 2040; Calcitek Inc. Zimmer Dental, Carlsbad, CA, USA	420-840µm	NI	NI
Ikeda, et al. [17]	Rats	G1: Apatite- and wollastonite-containing glass –ceramic (A-W GC) dense; G2:A-W GC small porous; G3:A-W GC large porous;	132 (6)	Tibia	3mm x 4mm	The sintering temperatures were 1200 ºC (HA-A), 1150 ºC (HA-S), 900 ºC (HA-M) and 1100 ºC (HA-N)	0.6-1.0 mm	HA-A: 20%; HA-S: 35-48%; HA-M: 60%; HA-N: 35%	HA-A: 100-300 µm, HA-S: 50-300 µm; HA-M: 300 µm; HA-

		G4:HA-A; G5:HA-S; G6:HA-M; G7:HA-N							N: 1-15 µm
Jahangirnezhad, et al. [18]	Rats	G1: Empty; G2: NanoHA; G3: Laser; G4: NanoHA +Laser	56 (14)	Calvaria	NI	Sol-gel combustion method	NI	NI	NI
Jang, et al. [19]	Rats	G1: HA; G2: DBBM	20 (10)	Mastoid obliteration	NI	Aqueous precipitation method with Heat treatment at 1100°C for 1 h.	850 mm to 1 mm	NI	NI
Jaramillo, et al. [20]	Rabbits	G1: OseoU; G2: Osteogen®	70 (10)	Tibia	5mm	OseoU: Hydro-thermal process	300 µm	NI	NI
John, et al. [21]	Rabbits	G1: HA	8 (4)	Tibia	2mm	Wet chemical method.	10-1200µm	200-300µm	0.5mm-1mm
Kim, et al. [22]	Rabbits	G1: Empty; G2: DBBM (Bio-Oss®); G3: HA; G4: HA + rhBMP-2	6 (6)	Calvaria	8mm	Bongros; Daewoong Pharmaceutical Co. Ltd., Seongnam, Korea	NI	80%	300 µm
Klinge, et al. [23]	Rabbits	G1: DBBM; G2: SHA; G3: IHA; G4: Empty	13 (6/7)	Calvaria	5mm	Apaceram, Asahi Optical Co, Ltd, Japan	SHA: 0.3 to 0.6 mm; IHA: 0.6 to 1.0 mm	NI	NI
Kom, et al. [24]	Rabbits	G1, G2 and G3: HA based biografs; G4: HA synthesized; G5: Commercial graft; G6: Empty	24 (4)	Tibia	NI	HA synthesized and HA based biografts were produced by Sol-gel method	NI	NI	NI

Kubasiewicz-Ross, et al. [25]	Rats	G1: DBBM (Bio-Oss®); G2: NanoHA + β-TCP; G3: NanoHA; G4: NanoHA + collagen mebrane; G5: Empty	30 (6)	Calvaria	5mm	Wet precipitation combined with the sol/gel reaction at a relatively low temperature 500-800 ºC	<100 nm	70%	50µm
Kurioka, et al. [26]	Rabbit	AP1: round and compact HA spherical granule without pores; AP2: irregular shaped HA granule with continuous pores; AP3: compact and multi-nuclear HA granule with continuous pores; AP4: multi-nuclear HA granule with a coarse, surface and small continuous pores; AP5: compact and multi-nuclear HA granule; AP6: HA with microspores	NI	Tibia	1.5mm x 7mm	Preciptation method from aqueous solutions and then granulated and synthesized at 1200 ºC	AP1: without pores; AP2: <30 µm; AP3: 30-200µm; AP4: NI; AP5: 30-200µm; AP6: <10µm	NI	NI
Lambert, et al. [27]	Rabbits	G1: DBBM 1 (Bio-Oss®); G2: DBBM 2 (Endobon®); G3: HA	24 (8)	Sinus floor augmentation	6mm x 4mm	Synthetic HA powder and sintered at high temperature	G1 and G3: 250–1000µm G2: 500–1000µm	Synthetic hydroxyapatite displayed a much denser surface and the absence of microporosities	NI
Lee, et al. [28].	Rabbits	G1: HA powder (HAP); G2: HA cylinder (HAC); G3: HA + β-TCP cylinder; G4: Titanium cylinder	10 (10)	Tibia	3.5mm	The HA was fabricated by calcining HA for 1 hour at 900°C, with a resulting particle size between 500 µm to 700 µm.	HA: 500-700µm	NI	NI
Lee, et al. [29]	Rabbits	G1: HA; G2: eggshells HA (eHA); G3: Empty	16 (10)	Parietal bone	8mm	Purchase from Sigma	NI	NI	NI

Lee, et al. [30]	Rats	G1: HA; G2: β-TCP; G3: β-TCP/HA (ratio 80/20); G4: β-TCP + rhBMP-2; G5: β-TCP/HA (ratio 80/20) + rhBMP	130 (13)	Calvaria	8mm	Medical grade of pure HA ceramics (Cerectron Co., South Korea) sintered at 1.225 ºC for 2 h	0.6–1.0 mm	79.8%	NI
Lee, et al. [31]	Rabbits	G1: Empty; G2: HA; G3:TiO_2-coated HA; G4:TiO_2-coated HA + UV	12 (6)	Calvaria	8mm	Bongros; Daewoong Pharmaceutical Co. Ltd., Seongnam, Korea	NI	NI	NI
Liljensten, et al. [32]	Rabbits	G1: Resorbable HA; G2: Nonresorbable HA; G3: Empty	18 (6)	Tibia	5mm	G1: Pentax, Hamburg, Germany; G2: OsteoGen® (HA-resorb, Impladent, Holliswood, NY, USA)	G1: 300-600µm; G2: 300-400µm	G2: 40-100 µm	NI
Lindholm, et al. [33]	Rabbits	G1: Autogeneic bone marrow (BM); G2: Empty; G3: HA; G4: HA+BM	10 (NI)	Calvaria	11mm	NI	NI	NI	NI
Mate Sanchez de Val, et al. [34]	Rabbits	G1: HA (2000–4000 µm); G2: HA (1000–2000 µm); G3: HA (600–1000 µm); G4: Empty	15 (15)	Tibia	6 mm	Sol-gel method with heat treatment at: G3: 600°C during 1 h, G2: 800°C during 1 h, and G1: 1000°C during 1 h.	Grupo 1 (2.000 to 4.000 µm), Grupo 2 (1.000 to 2.000 µm) and Grupo 3 (600 to 1000 µm).	Grupo 1: 71%; Grupo 2: 67%; Grupo 3: 65%	G1: 0.5mm; G2: 0.5mm; G3: 0.6mm

Mate Sanchez de Val, et al. [35]	Rabbits	G1: HA 2000–4000 µm; G2: HA 1000–2000 µm; G3: HA 600–1000 µm; G4: biphasic HA/ß-TCP; G5: biphasic HA/ß-TCP4; G6: Empty	45(NI)	Tibia	6mm	Sol-gel method with heat treatment at: G3: 600°C during 1 h, G2: 800°C during 1 h, and G1: 1000°C during 1 h; G4: Ossceram® nano, Bredent medical GmbH & Co. KG, Senden, Germany; G5 Bone® granules; MIS Implants Technologies Ltd, Shlomi, Israel	G1: 2000–4000 µm; G2: 1000–2000 µm; G3: 600–1000 µm; G4: 800–1200 µm; G5: 600–1000µm	G1: 46.4%; G2: 57.2%; G3: 69.3%; G4: 55.4%; G5: 64.6%	NI
Moshiri, et al. [36]	Rats	G1: Empty; G2: Autogenous graft; G3: Gelatin (Gel); G4: Chitosan (Chi); G5: HA; G6: β-TCP; G7: Nano-HA; G8: Strontium carbonate (Sr)	20 (NI)	Femur	3mm	NI	HA: 5.84±0.79 µm; nanoHA: 76.32±12.95 nm	NI	NI
Oberg and Rosenquist [37]	Rabbits	G1: HA; G2: HA + Fibrin glue; G3: Allogeneic bone (AAA) ; G4: AAA + Fibrin glue; G5: HA + AAA; G6: HA + AAA + Fibrin glue	12 (6)	Mandible (4) and tibia (1) cavities	4mm	Interpore 200 ®, Interpore International, Irvine, CA, USA.	NI	NI	NI
Ohba, et al. [38]	Rabbits	G1: PRP+ electrically polarized HA (pHA); G2: PRP+HA; G3: Hyaluronan (HAN)+pHA; G4: HAN+HA; G5:pHA; G6: HA; G7: PRP; G8:HAN; G9: Empty	18 (NI)	Femoral condyles and tibial tuberosities	3mm x 5mm	Precipitation reaction between calcium nitrate and ammonium dihydrogen phosphate and calcined in air at 850 ºC for 2 h; pHA: electrically polarized in a direct-current field of 4 kV/cm at 400ºC for 1h in air.	30-70µm	NI	NI
Okanoue, et al. [39]	Rabbits	G1: Superporous HA (HAp-S); G2: β-TCP	24 (6)	Lateral femoral	5mm x 8mm	Dropping an aqueous solution of phosphoric acid into a suspension of	NI	85%	50-300µm

				condyle		calcium hydroxide and sintering the substance at 1.200 ºC (Apaceram-ax® (HOYA Co. Ltd, Tokyo, Japan)			
Ono, et al. [40]	Rats	G1: A-W.GC; G2: HAM; G3: Alumina (AL); G4: HAK; G5: Empty	90 (NI)	Tibia	4mm	G2: Mitsubishi Mining &Cement Co. Ltd; G4: Kyocera Co. Ltd. Both HA were produced at a sintering temperature of 900 °C.	200µm	50%	NI
Orr, et al. [41]	Rabbits	G1: DBBM (Bio-Oss®); G2: HA; G3: Empty; G4: Autogenous graft	73 (NI)	Medial femoral condyle	3.5mm x 8mm	Calcitek, Carlsbad, CA	0.5-1.0mm	NI	NI
Oryan, et al. [42]	Rabbits	G1: HA; G2: HA + PRP; G3: Empty	36 (12)	Radius	10mm	OS Satura, Isotis Co, Netherland	NI	NI	NI
Parizi, et al. [43]	Rabbits	G1: Coral G2: HA; G3: Empty	36 (12)	Radius	10mm	OS Satura; Isotis Co., Netherlands	NI	NI	NI
Patel, et al. [44]	Sheeps	G1: HA; G2: 0.8 silicate-substituted HA (SiHA); G3: 1.5 SiHA	NI	Femoral condyle	9mm x 9mm	Conventional aqueous precipitation reaction between calcium hydroxide, $Ca(OH)_2$, and orthophosphoric acid, H_3PO_4, solution. The HA samples were sintered at 1200 ºC using a ramp rate of 2.5 ºC per minute, dwell time of 2 h and a cooling rate of 10 ºC per minute to ambient temperature.	0.99 µm	NI	NI

Peltola, et al. [45]	Rabbits	G1: Bioactive glass (BG); G2: HA	21(6/3)	Sinus floor augmentation	5mm	Calcititet 2040-12, Calkitek Inc., Carlsbad, CA	0.5–0.8 mm	NI	NI
Polkowska, et al. [46]	Dogs	G1: HA granule; G2: HA blocks; G3: Empty G4: Empty	40(10)	G1/G3: Periodontal defect; G2/G4: post extraction cavity	G1/G3: Pockets ≥ 4 mm	Wet chemical method with calcination at 800 ºC.	0.3-0.5 mm	68 vol.%porosity	0.1 µm
Rezaeian, et al. [47]	Rats	G1: Empty; G2: Nano Ostrich Eggshell (N-OES); G3: HA	45(15)	Calvaria	7mm	NI	1-5mm	NI	NI
Do Prado Ribeiro, et al. [48]	Rats	G1: Non-OVX; G2: Bilaterally OVX; G3: Bilaterally OVX + estrogen	18(6)	Femur	GenPhos HA TCP Genius Baumer S.A.	NI	0.5–0.75 mm	NI	NI

Salata, et al. [49]	Rats	G1: Empty; G2: Membrane; G3: HA; G4: Ionomeric graft (IM); G5: HA + membrane; G6: IM + membrane	30(10)	Mandible	3 mm	Calcitite, 20 to 40 mesh size, Calcitek, Carlsbad, CA	NI	NI	NI
Sato, et al. [50]	Dogs	G1: Carbonate apatite(Cytrans®); G2: DBBM (Bio-Oss®); G3: β-TCP (Cerasorb®); G4: HA; G5: Empty	16(8)	Mandibular 3 wall bone defects	4mm x 5mm x 5mm	Neobone®, CoorsTec, Tokyo, Japan	NI	NI	NI
Spence, et al. [51]	Sheeps	G1: Carbonate-substituted hydroxyapatite (CHA); G2: HA	12(6)	Lateral femoral condyles	9 mm x 9 mm	An aqueous precipitation reaction between calcium hydroxide and orthophosphoric acid.	NI	28.5% ±2.4	NI

Takeshita, et al. [52]	Dogs	G1: HA; G2: ePTFE; G3: HA + ePTFE; G4: Empty	8(2)	Mandible	4 mm x 12 mm	NI	NI	NI	NI
Vamze, et al. [53]	Rabbits	G1:HA; G2: α -TCP-1 (sintered at 1.000°C); G3: α -TCP-2 (sintered at 1.150°C); G4: HA/ α-TCP-1; G5: HA/ α -TCP-2; G6: Empty	10(2)	Mandible	NI	Pure hydroxyapatite sintered at 1.000 ºC	NI	NI	NI
Vieira, et al. [54]	Rats	G1: Empty; G2: HA; G3: cerium-containing HA (Ce-HA)	36(9)	Tibia	2mm	Suspension–precipitation method. The product was dried in the oven at 110 ºC for about 12 h.	25.87 nm	NI	NI
Xu, et al. [55]	Rabbits	G1: DBBM (Bio-oss®); G2:HA; G3: Nano-HA with collagen I (nHAC)	12(4)	Calvaria	8mm diameter	Bongros®-HA (Bioalpha, Gyeonggi-do, Korea)	NI	NI	NI
Yano, et al. [56]	Dogs	G1: Empty; G2: Allograft; G3: HA	48(16)	Iliac	6 mm x 12 mm	Sintered temperature, 1.150 °C [Sumitomo Cement Co, Ltd (Osaka, Japan)].	0.9–1.5 mm	35%–48%	50–300 μm

Legend: HA: Synthetic hydroxyapatite; DCFGP: demineralized calf fetal growth plate; Zn: zinc; rhBMP-2: *Escherichia coli*-derived recombinant human bone morphogenetic protein-2; DBBM: deproteinized bovine bone mineral; SEM: scanning electron microscopy; BCP: biphasic calcium phosphate; β-TCP: beta-tricalcium phosphate; DFDBA: demineralized freeze-dried bone allograft; GTAM: polytetrafluoroethylene membrane; UV: ultraviolet irradiation; AAA: autolyzed antigen-extracted allogeneic bone; PRP: platelet-rich plasma gel; A-W.GC: Apatite-wollastonite containing glass ceramic; ePTFE: expanded polytetrafluoroethylene; PLGA: poly(DL-lac-tic-co-glycolic acid), PEG: polyethylene glycol.

TABLE 1S REFERENCES

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Table 2S: Studies included in the systematic review that compared defects filled with synthetic HA in particles forms versus blood clot (empty defects).

Author/ Year	Studies characteristics			Outcomes
	Animal model	Experimental groups	Bone defect type	
Attia, et al. [1]	Dog	G1: Clot; G2: Nano-HA; G3: Micro-HA; G4: Nano-HA+Micro-HA	Distal surface of third premolar	The percentage of new bone formed with **nano-HA bone graft (31.39 ±1.6%)** was highly more significant than that obtained by a **micro- or mixed-composite of hydroxyapatite (G3: 21.95 ±8.27%; G4: 23.90 ±7.08%). The lowest mean value was recorded in G1, where no bone graft was added (3.78 ± 0.62%).**
Bigham-Sadegh, et al. [2]	Rabbit	G1: Empty; G2: HA; G3: HA+Royal jelly	Mid- radius bone	Radiological evaluation showed significant superior bone healing in hydroxyapatite and hydroxyapatite- Royal jelly groups in comparison to control group. **Control group was the inferior group between three groups.** There were not any significant differences between three groups in histopathological analysis.
Carvalho, et al. [3]	Dogs	G1: Bovine HA (BHA)[Small]; G2: BHA[Large]; G3: HA[Small]; G4: HA[Large]; G5: Empty	Humerus	Considering the bone density of the apical third, **the bone amount of control group (28.34 ±2.95) was statistically lower** in relation to BHA(S) (50.59 ±8.42), BHA(L) (35.71 ±5.05) and **HA(S) (37.50 ±5.25)**.
Cassino, et al. [4]	Rats	G1: Low purity HA (HAP); G2: Strontium HA (HAPSr);	Tibia	**Length of the primary bone area (mm) in the empty group was 15.13 mm, in HAP group was 49.55 mm**, in HAPSr group was 36.83 mm and in the HAPGa group was 48.74 mm.

		G3: HA doped with gallium (HAPGa); G4: Empty		
Chung, et al. [5]	Rats	G1: HA; G2: ErhBMP-2 loaded HA; G3: Empty	Calvaria	In both histomorphometric and tomographic analyses, the new bone area for ErhBMP-2/HA group (4.75 ± 0.50; 61.12 ± 1.84) was significantly greater than **HA (2.94 ± 0.28; 56.31 ± 2.62) and control groups (1.25 ± 0.09; 5.78 ± 0.54) at 2 weeks.** However, at 8 weeks, no significant difference in new bone area was observed between the ErhBMP-2/HA (3.67 ± 0.19; 67.55 ± 5.48) and **HA groups (3.68 ± 0.24; 72.66 ± 4.07)**. **Control group also showed inferior new bone area at 8 weeks (1.39 ± 0.13; 12.61 ± 1.16).**
Fukuta, et al. [6]	Rabbits	G1: DBBM (Bio-Oss®); G2: Porous HA; G3: Autogenous bone; G4: Empty	Calvaria	The amount of new bone in the autogenous group (2.447 ±0.084) was found to be significantly more than in the other three groups (G1: 0.661 ±0.081; **G2: 0.604** ±0.102; **G4: 0.555 ±0.126)**.
Haas, et al. [7]	Sheep	G1: Porous HA; G2: Autogenous iliac crest bone; G3: Empty	Sinus-lift	**Both groups [HA: 5.9 + 0.3 mm), autogenous bone (5.7 ±0.3 mm)] showed a significantly greater bone-implant contact than the empty control group (3.9 ±0.3 mm).**
Hong, et al. [8]	Dog	G1: HA; G2: BCP; G3: β-TCP; G4: Empty	Mandibular/maxillary third premolars	After 8 weeks, new bone formation was significantly higher in **control group (62.01% ± 17.11)** compared with the grafted groups at all healing periods (**G1: 32.27 ± 8.83%**; G2: 31.82; G3: 48.55 ± 5.94%).
Jahangirnezhad, et al. [9]	Rats	G1: Empty; G2: NanoHA; G3: Laser; G4: NanoHA +Laser	Calvaria	The highest level of bone formation was observed in nHA+LLLT (49 ±6.79) and after that groups of **nHA (41.6 ±14.95), control (17.2 ±14.45)** and laser (5 ±35.17).
Kim, et al. [10]	Rabbits	G1: Empty; G2: DBBM (Bio-	Calvaria	The average new bone formation percentage of **empty**, DBBM, **HA**, and HA-rhBMP-2 at 4 week after surgery were

		Oss®); G3: HA; G4: HA + rhBMP-2		**12.65±5.89%**, 29.63±6.99%, **28.86±6.17%** and 49.56±8.23%, respectively.
Klinge, et al. [11]	Rabbits	G1: DBBM; G2: SHA; G3: IHA; G4: Empty	Calvaria	All defects healed uneventfully, although **the HA seemed to promote initial bone regeneration (4 weeks)**. After 14 weeks, all materials showed the same bone regeneration
Kom, et al. [12]	Rabbits	G1, G2 and G3: HA based biografs; G4: HA synthesized; G5: Commercial graft; G6: Empty	Tibia	HA synthesis and HA based fabricated grafts accelerated the bone defect recovery at 8th week.
Kubasiewicz-Ross, et al. [13]	Rats	G1: DBBM (Bio-Oss®); G2: NanoHA + β-TCP; G3: NanoHA; G4: NanoHA + collagen mebrane; G5: Empty	Calvaria	Histomorphometric analysis revealed an amount of regenerated bone between **34.2 and 44.4% in treated bone defects, whereas only 13.0% regenerated bone was found in controls**. Micro-CT study confirmed the results of the histological examinations.
Lee, et al. [14]	Rabbits	G1: HA; G2: eggshells HA (eHA); G3: Empty	Parietal bone	Both types of **HA showed higher bone formation (G1: 38.62 ±17.42% and G2: 41.99 ±8.44%) than the unfilled control (27.50 ±10.89%) after 8 weeks**. However, only eHA had significantly higher bone formation than the unfilled control at 8 weeks.
Lee, et al. [15]	Rabbits	G1: Empty; G2: HA; G3:TiO_2-coated HA; G4:TiO_2-coated HA + UV	Calvaria	**The newly formed bone ratio was 13.90 ±3.68% (Empty), 29.42 ±5.81% (HA group),** 39.68±6.70% (TiO2-coated HA group) and 49.46±6.54% (TiO2-coated HA with UV group) at eight weeks postoperatively.
Liljensten, et al. [16]	Rabbits	G1: Resorbable HA; G2: Nonresorbable HA; G3: Empty	Tibia	After 3 months histomorphometry analysis revealed significantly **more newly formed bone in the two HA augmented groups compared with that in the control**.

Oryan, et al. [17]	Rabbits	G1: HA; G2: HA + PRP; G3: Empty	Radius	In radiological evaluation, by day 56, there was **75–100% bone formation in the animals of the HA and HA+PRP group** compared to those of **the control group that** showed **50–75% bone formation**.
Parizi, et al. [18]	Rabbits	G1: Coral G2: HA; G3: Empty	Radius	In radiological evaluation, by day 42 and 56, there was 50–75% bone formation in the coral group, **75–100% bone formation in HA group, and 25–50% bone formation in empty group**. In biomechanical evaluation, **the empty group showed weakness of biomechanical properties in comparison with the coral and HA groups.**
Polkowska, et al. [19]	Dogs	G1: HA granule; G2: HA blocks; G3: Empty G4: Empty	G1/G3: Periodontal defect; G2/G4: Post extraction cavity	The mean depth of pockets was significantly reduced in defects treated with both types of HA and considerably better reconstruction of periodontal tissues was observed when compared to the empty group.
Rezaeian, et al. [20]	Rats	G1: Empty; G2: Nano Ostrich Eggshell (N-OES); G3: HA	Calvaria	On day 42, no significant difference in the edges of the defect was observed between all groups. **However, there was a statistically significant difference in the center of the defect between the N-OES group (8.4 ±0.89) with HA (5.4 ± 3.13) and empty groups (6 ±0.7).** Results of the serological assessment indicated that the applied treatments increased the serum levels of alkaline phosphatase.
Salata, et al. [21]	Rats	G1: Empty; G2: Membrane; G3: HA; G4: Ionomeric graft (IM); G5: HA + membrane; G6: IM + membrane	Mandible	Defects implanted with IM exhibited more bone formation (4 weeks = 3.19 ± 0.38mm^2, 10 weeks = 5.35 ± 0.26mm^2) than both defects that received **no treatment (4 weeks = 0.88 ± 0.35mm^2, 10 weeks = 2.1 ± 0.49mm^2**), membrane alone (4 weeks = 1.21 ± 0.05mm^2) or **HA (4 weeks = 1.41 ± 0.46mm^2, 10 weeks = 3.34 ± 0.41mm^2**) at 4 weeks and at 10 weeks.
Takeshita, et al. [22]	Dogs	G1: HA; G2: ePTFE; G3: HA + ePTFE; G4: Empty	Mandible	Histomorphometric data disclosed that the presence of HA granules in the bone defects significantly arrested bone formation. Bone defects were completely filled with connective tissue in the control group.

Yano, et al. [23]	Dogs	G1: Empty; G2: Allograft; G3: HA	Iliac	The volume fraction of newly formed bone was significantly greater in the allograft (93.0% ±6.1%) and **HA groups (25.7% ±3.3%) than in the empty group**.

Legend: HA: Synthetic hydroxyapatite; rhBMP-2: *Escherichia coli*-derived recombinant human bone morphogenetic protein-2; DBBM: deproteinized bovine bone mineral; BCP: biphasic calcium phosphate; β-TCP: beta-tricalcium phosphate; PRP: platelet-rich plasma gel; ePTFE: expanded polytetrafluoroethylene; painted in gray: empty group superior bone formation; bold: results from control group versus HA.

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Table 3S: Studies included in the systematic review that compared defects filled with synthetic HA in particles forms versus filled with deproteinized bovine bone mineral (DBBM).

Author/ Year	Studies characteristics			Outcomes
	Animal model	Experimental groups	Bone defect type	
Bigham-Sadegh, et al. (1)	Rabbit	G1: HA; G2: HA+DCFGP; G3: DCFGP	Mid-radius bone	In the 8th postoperative week, the radiographs showed that HA+DCFGP group was significantly superior to the HA and DCFGP groups. **There were no significant differences between the DCFGP and HA groups on any postoperative day**. On the contrary, macroscopical and histopathological evaluation did not revealed significant differences between the three groups.
Carvalho, et al. (2)	Dogs	G1: Bovine HA (BHA)[Small]; G2: BHA[Large]; G3: HA[Small]; G4: HA[Large]; G5: Empty	Humerus	Considering the bone density of the apical third, the bone amount of control group (28.34 ±2.95) was statistically lower in relation **to BHA(S) (50.59 ±8.42), BHA(L) (35.71 ±5.05) and HA(S) (37.50 ±5.25)**. **BHA(S) was higher than the 2 synthetic ones and BHA(L) was higher than HA(L) (25.89 ±5.16).**
Fukuta, et al. (3)	Rabbits	G1: DBBM (Bio-Oss®); G2: Porous HA; G3: Autogenous bone; G4: Empty	Calvaria	The bone ingrowth in porous HA and DBBM was not significantly different. The surface area occupied by implant material was shown to be fairly consistent over the follow-up period for both HA (1.460 ±0.141) and DBBM (1.263 ±0.388).
Jang, et al. (4)	Rats	G1: HA; G2: DBBM	Tympanic bulla / mastoid obliteration	Enhanced osteoconduction was observed in the DBBM group whereas limited osteoconduction was seen in the HA group. Neither DBBM nor HA exhibited signs of resorption at 12 weeks of obliteration.
Kim, et al. (5)	Rabbits	G1: Empty; G2: DBBM (Bio-Oss®);	Calvaria	**The average new bone formation percentage of** empty, **DBBM, HA**, and HA-rhBMP-2 at 4 week after surgery were 12.65±5.89%, **29.63±6.99%, 28.86±6.17%** and 49.56±8.23%,

		G3: HA; G4: HA + rhBMP-2		respectively. **However, there was no statistical difference in the bone formation between HA and DBBM groups.**
Klinge, et al. [6]	Rabbits	G1: DBBM; G2: SHA; G3: IHA; G4: Empty	Calvaria	All defects healed uneventfully, although the HA seemed to promote initial bone regeneration (4 weeks). **After 14 weeks, all materials showed the same bone regeneration**.
Kubasiewicz-Ross, et al. [7]	Rats	G1: DBBM (Bio-Oss®); G2: NanoHA + β-TCP; G3: NanoHA; G4: NanoHA + collagen mebrane; G5: Empty	Calvaria	**Histomorphometric analysis revealed an amount of regenerated bone between 34.2 and 44.4% in treated bone defects,** whereas only 13.0% regenerated bone was found in controls. Interestingly, in group 3, no significant particles of the nano-HA material were found. In contrast, residual bone substitute material could be detected in all other test groups. Micro-CT study confirmed the results of the histological examinations
Lambert, et al. [8]	Rabbits	G1: DBBM 1 (Bio-Oss®); G2: DBBM 2 (Endobon®); G3: HA	Sinus floor augmentation	**More bone was found with natural HA (G1:19.28 ±2.58%; G2: 17.27 ±2.52%) compared to the synthetic one (G3: 11.93 ±3.37%). Significantly higher bone-to-material contacts were found with G1 (G1: 65.51 ±29.32%; G2: 44.01 ±15.12%; G3: 37.51 ±8.44%).** The percentage of remaining biomaterial were 40.27 ±82.76% for G1, 39.44 ±2.70% for G2 and 38.50 ±3.90% for G3.
Orr, et al. [9]	Rabbits	G1: DBBM (Bio-Oss®); G2: HA; G3: Empty; G4: Autogenous graft	Medial femoral condyle	**After 26 weeks, an equivalent amount of host bone was found in the G1 and G2 sites.** There was a significant difference, however, in the percentage of the area occupied by the particles, with the DBBM particles having two-thirds the value of the synthetic HA material. While the compressive strength of the anorganic bone particles was less than that of normal bone, the site implanted with the bone mineral particles achieved compressive strength greater than normal after 6 weeks.

Xu, et al. [10]	Rabbits	G1: DBBM (Bio-oss®); G2:HA; G3: Nano-HA with collagen I (nHAC)	Calvaria	At 12 weeks, the amount of the **newly formed bone was significantly higher for DBBM (29.57 ± 9.24%) than nHAC group (19.73 ± 6.37%)** and there was no differences between HA (21.20 ± 6.74%) and nHAC. Amount of residual grafting material was significantly higher in HA (34.48 ± 5.96%) in comparison to other groups (G1:16.64 ± 3.78%; G3: 14.54 ± 6.45%).

Legend: HA: Synthetic hydroxyapatite; DCFGP: demineralized calf fetal growth plate; DBBM: deproteinized bovine bone mineral; rhBMP-2: *Escherichia coli*-derived recombinant human bone morphogenetic protein-2; β-TCP: beta-tricalcium phosphate; highlighted in gray: reabsortion rate of materials; painted in gray: DBBM group superior bone formation; bold: results from DBBM group versus HA.

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T**able 4S:** Studies included in the systematic review that compared defects filled with synthetic HA in particles forms versus filled with HA associated with other biomaterials/treatments or with other biomaterials.

Author/ Year	Studies characteristics			Outcomes
	Animal model	Experimental groups	Bone defect type	
Bigham-Sadegh, et al. [1]	Rabbit	G1: Empty; G2: HA; G3: HA+Royal jelly	Mid- radius bone	Radiological evaluation showed significant superior bone healing in HA and HA-Royal jelly groups in comparison to control group. Control group was the inferior group between three groups. There were not any significant differences between three groups in histopathological analysis.
Calasans-Maia, et al. [2]	Rats	G1: Autogenous bone; G2: HA; G3: Zn-HA	Calvarial	In the Zn-HA group the area of neoformed bone was larger than in the HA group, but smaller than in the autograft. A fibrous connective tissue was more evident around HA granules
Chalisserry, et al. [3]	Rabbits	G1: NanoHA; G2: NanoHA + Simvastatin (SIM)	Femoral condyle	The SIM loaded site showed statistically higher bone mineral density (0.9±0.1) and bone volume (58.5±4.5) compared to the nanoHA (0.8± 0.1; 52.9±4.6) by microCT. Higher bone volume was also observed in SIM + nanoHA (57.2±4.8) compared to nanoHA (50.1±5.5) using histological method.
Chung, et al. [4]	Rats	G1: HA; G2: rhBMP-2 loaded HA; G3: Empty	Calvaria	In both histomorphometric and tomographic analyses, the new bone area for ErhBMP-2/HA group (4.75 ± 0.50; 61.12 ± 1.84) was significantly greater than HA (2.94 ± 0.28; 56.31 ± 2.62) and control groups (1.25 ± 0.09; 5.78 ± 0.54) at 2 weeks. However, at 8 weeks, no significant difference in new bone area was observed between the ErhBMP-2/HA (3.67 ± 0.19; 67.55 ± 5.48) and HA groups (3.68 ± 0.24; 72.66 ± 4.07). Control group also showed inferior new bone area at 8 weeks (1.39 ± 0.13; 12.61 ± 1.16).

Fujishiro, et al. [5]	Rabbit	G1: Bioglass®; G2: HA	Femur	For Bioglass, trabecular bone was generated by 1 week, with about 80% interparticle spaces being filled with new bone at the periphery of the implantation site in less than 6 week. The rate of bone growth for HA particles was relatively slow; the percentage of new bone growth is about 30% at 6 week. For the remaining particles, Bioglass® showed 51.6% and HA showed 49.4%
Fukuta, et al. [6]	Rabbits	G1: DBBM (Bio-Oss®); G2: Porous HA; G3: Autogenous bone; G4: Empty	Calvaria	The amount of new bone in the autogenous group (2.447 ±0.084) was found to be significantly more than in the other three groups (G1: 0.661 ±0.081; G2: 0.604 ±0.102; G4: 0.555 ±0.126).
Haas, et al. [7]	Sheep	G1: Porous HA; G2: Autogenous iliac crest bone; G3: Empty	Sinus-lift procedure	There was no significant difference between HA (5.9 + 0.3 mm) and autogenous bone (5.7 ±0.3 mm) regarding bone-implant contact. Both groups showed a significantly greater bone-implant contact than the empty control group (3.9 ±0.3 mm).
Hong, et al. [8]	Dog	G1: HA; G2: BCP; G3: β-TCP;	Mandibular/maxillary third premolars	After 8 weeks, new bone formation was significantly higher in control group (62.01% ± 17.11) compared with the grafted groups at all healing periods (G1: 32.27 ± 8.83%; G2: 31.82;

		G4: Empty		G3: 48.55 ± 5.94%). For the residual particles were observed 48.11 ± 7.35% for HA, 32.39 ± 4.15% for BCP and 10.28 ± 4.14% for β-TCP.
Ikeda, et al. [9]	Rats	G1:Apatite- and wollastonite-containing glass – ceramic (A-W GC) dense; G2:A-W GC small porous; G3:A-W GC large porous; G4:HA-A; G5:HA-S; G6:HA-M; G7:HA-N	Tibia	There were no significant diferences among the osteoconductive potentials of the different sizes of each form of A-W GC granule. The osteoconductive potentials of small porous A-W GC granules were significantly superior to those of commercially available HA granules.
Jahangirnezhad, et al. [10]	Rats	G1: Empty; G2: NanoHA; G3: Laser; G4: NanoHA +Laser	Calvaria	The highest level of bone formation was observed in nHA+LLLT (49 ±6.79) and after that groups of nHA (41.6 ±14.95), control (17.2 ±14.45) and laser (5 ±35.17).
Kim, et al. [11]	Rabbits	G1: Empty; G2: DBBM (Bio-Oss®); G3: HA; G4: HA + rhBMP-2	Calvaria	HA-rhBMP-2 showed significantly higher degree of bone formation compared with DBBM and HA group. The average new bone formation percentage of empty, DBBM, HA, and HA-rhBMP-2 at 4 week after surgery were 12.65±5.89%, 29.63±6.99%, 28.86±6.17% and 49.56±8.23%, respectively.
Kubasiewicz-Ross, et al. [12]	Rats	G1: DBBM (Bio-Oss®); G2: NanoHA + β-TCP; G3: NanoHA; G4: NanoHA + collagen mebrane; G5: Empty	Calvaria	Histomorphometric analysis revealed an amount of regenerated bone between 34.2 and 44.4% in treated bone defects, whereas only 13.0% regenerated bone was found in controls. Interestingly, in group 3, no significant particles of the nano-HA material were found. In contrast, residual bone substitute material could be detected in all other test groups. Micro-CT study confirmed the results of the histological

				examinations
Lee, et al. [13].	Rabbits	G1: HA powder (HAP); G2: HA cylinder (HAC); G3: HA + β-TCP cylinder; G4: Titanium cylinder	Tibia	The HAP group showed the most pronounced effect on the bone ingrowth surface area, which seen at 4, 6, and 8 weeks after graft by radiologic measurement. The earliest difference was seen at 4 weeks after grafting when the HAP group had an average volume of 118.97 ± 7.25 mm^3, whereas the HAC group (86.52 ± 4.92 mm^3), HA/TCP group (78.12 ± 4.91 mm^3), and titanium group (73.07 ± 6.76 mm^3) were relatively poorly healed. In contrast to HAP, which showed better results for the bone ingrowth surface, the HAC showed better results for the sustained morphology.
Lee, et al. [14]	Rats	G1: HA; G2: β-TCP; G3: β-TCP/HA (ratio 80/20); G4: β-TCP + rhBMP-2; G5: β-TCP/HA (ratio 80/20) + rhBMP	Calvaria	In microCT analysis, four weeks after implantation, new bone formation was evidently shown in groups 4 (42.0 ±11.8) and 5 (48.9 ±16.0) showing that the most affected area was filled with newly-formed bone. In the animals of group I (25.7 ±6.87), group II (26.0 ±5.34), and group III (20.3 ±5.84), minimal new bone formation was observed.
Lee, et al. [15]	Rabbits	G1: Empty; G2: HA; G3:TiO_2-coated HA; G4:TiO_2-coated HA + UV	Calvaria	The newly formed bone ratio was 13.90±3.68% (Empty), 29.42±5.81% (HA group), 39.68±6.70% (TiO2-coated HA group) and 49.46±6.54% (TiO2-coated HA with UV group) at eight weeks postoperatively, and the TiO2-coated HA with UV group had a significantly higher new bone percentage than the three other groups. The remaining graft material ratios at 8 weeks were 12.14 ± 5.22% (HA group), 14.64 ± 2.82% (TiO2-coated HA group), and 13.38 ± 3.44% (TiO2-coated HA with UV group), and no difference between the groups was observed.
Lindholm, et al. [16]	Rabbits	G1: Autogeneic bone marrow (BM); G2: Empty;	Calvaria	The area of new bone ingrowth in BM (70.3 ±8.4%) was significantly larger than in HA (34.4 ±3.9%) and in HAg/BM (24.0 ±5.1%).

		G3: HA; G4: HA+BM		
Mate Sanchez de Val, et al. [17]	Rabbits	G1: HA 2000–4000 µm; G2: HA 1000–2000 µm; G3: HA 600–1000 µm; G4: biphasic HA/ß-TCP; G5: biphasic HA/ß-TCP4; G6: Empty	Tibia	The analysis of the SEM images shown as biphasic groups has presented a more gradual resorption of the material. In them can be see areas of new bone formation around stable biomaterial particles. The stable particles must correspond to HA. More bone formation were observed in biphsic groups in comparison to the pure materials.
Moshiri, et al. [18]	Rats	G1: Empty; G2: Autogenous graft; G3: Gelatin (Gel); G4: Chitosan (Chi); G5: HA; G6: β-TCP; G7: Nano-HA; G8: Strontium carbonate (Sr)	Femurs	Both the Gel and Chi had no role on osteoconduction and osteoinduction. Compared to HA, nanoHA showed superior role in increasing new bone formation, mineral density and ash. In contrast to HA and nanoHA, both the TCP and Sr showed superior morphological, radiographical and biochemical properties on bone healing. β-TCP and Sr showed the most effective osteoconduction and osteoinduction, respectively. In the Sr group, the most mature type of osteons formed.
Oberg and Rosenquist [19]	Rabbits	G1: HA; G2: HA + Fibrin glue; G3: Allogeneic bone (AAA) ; G4: AAA + Fibrin glue; G5: HA + AAA; G6: HA + AAA + Fibrin glue	Mandible (4) and tibia (1) cavities	Bone formation was not affected by the addition of fibrin glue.The combination of AAA bone and HA stimulated bone formation more than any other combination (66.4±12.5%). AAA bone alone showed a better bone regeneration (62.7±14.6%) than HA alone (60.3±10.9%).
Ohba, et al. [20]	Rabbits	G1: PRP+ electrically polarized HA (pHA); G2: PRP+HA; G3: Hyaluronan (HAN)+pHA; G4:	Femoral condyles and tibial tuberosities	Although PRP alone could not induce bone formation, PRP+pHA and PRP+HA composites, especially the former, activated osteogenic cells and enhanced bone formation more than in the others groups. This effect was not prominent in the HAN+pHA and HAN+HA composites. When HA microgranules

		HAN+HA; G5:pHA; G6: HA; G7: PRP; G8:HAN; G9: Empty		were incorporated into HAN composites, the osteogenic capacity of the composite was not larger than that of the HA microgranules alone, with or without the electrical polarization treatment.
Okanoue, et al. [21]	Rabbits	G1: Superporous HA (HAp-S); G2: β-TCP	Lateral femoral condyle	Micro-CT showed that bone ingrowth in the HAp-S group significantly increased over time, while no significant increase was observed after 8 weeks in the β-TCP group. Although both materials showed gradual material resorption, β-TCP resorption was significantly greater than HAp-S. The ultimate compressive strength in the HAp-S group significantly increased over time up to six times its original value, while there was no significant increase in the β-TCP group.
Ono, et al. [22]	Rats	G1: A-W.GC; G2: HAM; G3: Alumina (AL); G4: HAK; G5: Empty	Tibia	In the A-W.GC group (apatite-wollastonite containing glass ceramic), the amount of bone at the interface was significantly greater 11 days post-operatively than in the other three groups. In addition, it increased rapidly and was stabilized at about 89% 4 weeks post-operatively. There were no significant differences between the two HA groups (G2 and G4). In these HA groups, the value became significantly greater than that in the AL group 6 weeks post-operatively, and reached aplateau 8 weeks post-operatively. In regard to percentages of the area occupied by granules G1 showed 53.4 ±5.5%, G2: 53.8 ±6.3%, G3: 54.2 ±4.8% and G4: 53.6 ±3.1%.
Oryan, et al. [23]	Rabbits	G1: HA; G2: HA + PRP; G3: Empty	Radius	In radiological evaluation, by day 56, there was 75–100% bone formation in the animals of the HA and HA+PRP group compared to those of the control group that showed 50–75% bone formation. Remodeling was superior in HA+PRP group in comparison with the HA and control groups on 42th and 56th days post-surgery. However, the animals of the HA group showed better remodeling criteria on day 56 than those of the control group.

Parizi, et al. [24]	Rabbits	G1: Coral G2: HA; G3: Empty	Radius	In radiological evaluation, by day 42 and 56, there was 50–75% bone formation in the coral group, 75–100% bone formation in HA group, and 25–50% bone formation in empty group. In biomechanical evaluation, the empty group showed weakness of biomechanical properties in comparison with the coral and HA groups.
Patel, et al. [25]	Sheeps	G1: HA; G2: 0.8 silicate-substituted HA (SiHA); G3: 1.5 SiHA	Femoral condyle of the hind legs	A significant increase in the percentage of bone coverage for 0.8 SiHA and 1.5 SiHA implants was significantly greater compared to HA implants at 6 and 12 weeks. No significant differences in bone in-growth and coverage were observed between 0.8 and 1.5 SiHA implants.
Peltola, et al. [26]	Rabbits	G1: Bioactive glass (BG); G2: HA	Frontal sinuses	The mean new bone formation was higher in defects filled with BG (34.0 ±14.0) than in corresponding HA-filled defects (25.1 ±7.0) after 12 months. The mean values of the remaining filling material were higher with HA (33.7 ±8.5) than with BG (20.6 ±8.0).
Rezaeian, et al. [27]	Rats	G1: Empty; G2: Nano Ostrich Eggshell (N-OES); G3: HA	Calvaria	On day 42, no significant difference in the edges of the defect was observed between all groups. However, there was a statistically significant difference in the center of the defect between the N-OES group (8.4 ±0.89) with HA (5.4 ± 3.13) and empty groups (6 ±0.7). Results of the serological assessment indicated that the applied treatments increased the serum levels of alkaline phosphatase.
Salata, et al. [28]	Rats	G1: Empty; G2: Membrane; G3: HA; G4: Ionomeric graft (IM); G5: HA + membrane; G6: IM + membrane	Mandible	Defects implanted with IM exhibited more bone formation (4 weeks = 3.19 ± 0.38mm^2, 10 weeks = 5.35 ± 0.26mm^2) than both defects that received no treatment (4 weeks = 0.88 ± 0.35mm^2, 10 weeks = 2.1 ± 0.49mm^2), membrane alone (4 weeks = 1.21 ± 0.05mm^2) or HA (4 weeks = 1.41 ± 0.46mm^2, 10 weeks = 3.34 ± 0.41mm^2) at 4 weeks and at 10 weeks. The use of HA was associated with a greater inflammatory reaction than was ionomer in this model.

Sato, et al. [29]	Dogs	G1: Carbonate apatite (Cytrans®); G2: DBBM (Bio-Oss®); G3: β-TCP (Cerasorb®); G4: HA; G5: Empty	Mandibular 3 wall bone defects	All of these bone substitutes showed a similar healing ability to periodontal ligament and cementum. However, the carbonate apatite group showed the fastest bone healing ability compared with the other materials at 4 weeks post-transplantation. At 8 weeks, the carbonate apatite group demonstrated a 20% larger new bone area than the empty group, which was significant, but no differences compared to the other groups. In addition, the recruitment of osteoclasts and endothelial cells was observed in carbonate apatite grafts at 4 weeks, but only detected at 8 weeks in the other materials.
Spence, et al. [30]	Sheeps	G1: Carbonate-substituted HA (CHA); G2: HA	Lateral femoral condyles	At six weeks more bone had grown into the CHA implants than into the HA specimens.
Takeshita, et al. [31]	Dogs	G1: HA; G2: ePTFE; G3: HA + ePTFE; G4: Empty	Mandible	Histomorphometric data disclosed that the presence of HA granules in the bone defects significantly arrested bone formation. Fibrous encapsulation of HA granules was observed in the defects of the HA group. In the group with grafting of HA granules and covering with ePTFE membranes, small amounts of bone tissue were observed among HA granules, but most HA granules were surrounded with fibrous tissue. Bone defects were completely filled with connective tissue in the control group. The ePTFE alone group showed the greatest bone height and bone area among the four groups.
Vamze, et al. [32]	Rabbits	G1:HA; G2: α -TCP-1 (sintered at 1.000°C); G3: α -TCP-2 (sintered at 1.150°C); G4: HA/ α-TCP-1; G5: HA/ α -TCP-2; G6: Empty	Mandible	Pure and mixed HAP and α-TCP sintered at different temperatures do not affect the production of cytokines and bone-specific proteins.
Vieira, et al. [33]	Rats	G1: Empty;	Tibia	The results showed that the animals that had the induced bone

		G2: HA; G3 cerium-containing HA (Ce-HA)		defects filled with the Ce-HA materials had more expressive bone neoformation than the others groups.
Xu, et al. [34]	Rabbits	G1: DBBM (Bio-oss®); G2:HA; G3: Nano-HA with collagen I (nHAC)		At 12 weeks, the amount of the newly formed bone was significantly higher for DBBM (29.57 ± 9.24%) than nHAC group (19.73 ± 6.37%) and there was no differences between HA (21.20 ± 6.74%) and nHAC. Amount of residual grafting material was significantly higher in HA (34.48 ± 5.96%) in comparison to other groups (G1:16.64 ± 3.78%; G3: 14.54 ± 6.45%).
Yano, et al. [35]	Dogs	G1: Empty; G2: Allograft; G3: HA	Iliac	The volume fraction of newly formed bone was significantly greater in the allograft (93.0% ±6.1%) and HA groups (25.7% ±3.3%) than in the empty group. For mechanical test, in the allograft group, the yield strength and stiffness were within normal control levels at 3 weeks and were maintained through 12 weeks. The yield strength and stiffness in the HA group were significantly higher than those in the allograft group at 3 and 12 weeks but decreased to normal control levels at 8 weeks

Legend: HA: Synthetic hydroxyapatite; Zn: zinc; rhBMP-2: *Escherichia coli*-derived recombinant human bone morphogenetic protein-2; DBBM: deproteinized bovine bone mineral; BCP: biphasic calcium phosphate; β-TCP: beta-tricalcium phosphate; A-W.GC: Apatite-wollastonite containing glass ceramic; PRP: platelet-rich plasma gel; painted of gray: no difference between study groups; painted of blue: more bone formation in HA group.

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Table 5S: Studies included in the systematic review that compared defects filled with different types of synthetic HA in particles forms.

Author/ Year	Studies characteristics			Outcomes
	Animal model	Experimental groups	Bone defect type	
Attia, et al. [1]	Dog	G1: Clot; G2: Nano-HA; G3: Micro-HA; G4: Nano-HA+Micro-HA	Distal surface of third premolar	The percentage of new bone formed with nano-HA bone graft (31.39 ±1.6%) was highly more significant than that obtained by a micro- or mixed-composite of HA (G3: 21.95 ±8.27%; G4: 23.90 ±7.08%).
Carvalho, et al. [2]	Dogs	G1: Bovine HA (BHA)[Small]; G2: BHA[Large]; G3: HA[Small]; G4: HA[Large]; G5: Empty	Humerus	Considering the bone density of the apical third, the bone amount of HA(S) with particle size of 150 µm (37.50 ±5.25) was higher than HA(L) with particle size of 300 µm (25.89 ±5.16).
Cho, et al. [3]	Rabbits	G1: HA; G2: Sodium-substituted HA (NaAp)	Calvaria	The areas occupied by new bone were significantly different between the two groups (G1: 5.8 ±1.8%; G2: 51.2 ±5.3%). The occupied portion by material for G1 was 48.3±4.5% and for G2 was 46.1±3.9%.
D'Lima, et al. [4]	Dogs	G1: OsteoGenR (HA Resorb)™; G2: Chitra granules	Lower mandible	The amount of new bone formed with OsteoGenR (HA Resorb) (particle size: 300 to 400 µm) was slightly more than that obtained by Chitra granules (0.5 to 1mm) in histometric evaluation (6493.5 ± 2519.42 microns versus 6087.5 ± 1325.83) at 6 months.
Frame, et al. [5]	Dog	G1:Solid HA; G2: Porous HA; G3: Solid HA + autogenous bone; G4: Solid HA + plaster	Mandible	The ridges augmented with only porous HA demonstrated a greater amount of bone ingrowth compared with the solid HA. Number of particles having surface deposition of bone was counted and presented as a ratio of the total number of particles on each section: G1: 0.03 ± 0.05; G2: 0.28 ± 0.2; G3:

		of paris; G5: Porous HA + plaster of paris		0.09 ± 0.11; G4:0.10 ± 0.06; G5: 0.21 ± 0.23.
Gonda, et al. [6]	Rats	G1: Rod-shaped HA particles (HHA); G2: Globular-shaped HA particles (SHA)	Tibia	Histomorphometric analyses showed that the number of osteoclasts and the amount of newly formed bone were significantly increased in the specimens implanted with HHA compared to SHA.
Jaramillo, et al. [7]	Rabbits	G1: OseoU; G2: Osteogen®	Tibia	The results of this research indicate that 50% repair was reached at 14 and 21 days, evident by new bone in both groups.
John, et al. [8]	Rabbits	G1: HA	Tibia	Good osteointegration of the granules with 54.47% ±6.83 of new bone area after 6 months. There was neither inflammation nor fibrous tissue interposition while resorption of the material was in effect a slow process, since HA granules still persisted after 24 weeks
Klinge, et al. [9]	Rabbits	G1: DBBM; G2: SHA; G3: IHA; G4: Empty	Calvaria	All defects healed uneventfully, although the HA seemed to promote initial bone regeneration (4 weeks). After 14 weeks, all materials showed the same bone regeneration
Kom, et al. [10]	Rabbits	G1, G2 and G3: HA based biografs; G4: HA synthesized; G5: Commercial graft; G6: Empty	Tibia	HA synthesis and HA based fabricated grafts accelerated the bone defect recovery at 8th week.
Kurioka, et al. [11]	Rabbit	AP1: round and compact HA spherical granule without pores; AP2: irregular shaped HA granule with continuous pores; AP3: compact and	Tibia	The quantitative analysis of the bone tissue in the central part of the implanted area indicated that AP3 demonstrated the best osteoconduction 2 weeks after implantation with little bone resorption in the 6-month specimens and maintained a stable HA-bone complex in the whole implanted area. AP2, AP4 and AP5 which exhibited less bone formation in the central area 2 weeks after implantation, showed little bone resorption as

		multi-nuclear HA granule with continuous pores; AP4: multi-nuclear HA granule with a coarse, surface and small continuous pores; AP5: compact and multi-nuclear HA granule; AP6: HA with microspores		observed in AP3 after 6 months. Bone resorption ocorred most aggressively in AP6 among the 6 samples in the central area 6 months.
Lee, et al. [12]	Rabbits	G1: HA powder (HAP); G2: HA cylinder (HAC); G3: HA + β-TCP cylinder; G4: Titanium cylinder	Tibia	The HAP group showed the most pronounced effect on the bone ingrowth surface area, which seen at 4, 6, and 8 weeks after graft by radiologic measurement. The earliest difference was seen at 4 weeks after grafting when the HAP group had an average volume of 118.97 ± 7.25 mm^3, whereas the HAC group (86.52 ± 4.92 mm^3), HA/TCP group (78.12 ± 4.91 mm^3), and titanium group (73.07 ± 6.76 mm^3) were relatively poorly healed. In contrast to HAP, which showed better results for the bone ingrowth surface, the HAC showed better results for the sustained morphology.
Lee, et al. [13]	Rabbits	G1: HA; G2: eggshells HA (eHA); G3: Empty	Parietal bone	Both types of HA showed higher bone formation (G1: 38.62 ±17.42% and G2: 41.99 ±8.44%) than the unfilled control (27.50 ±10.89%) after 8 weeks. However, only eHA had significantly higher bone formation than the unfilled control at 8 weeks. Residual graft was 28.12 ±9.58% in the HA group and 9.60 ±4.95% in the eHA group at 8 weeks after the operation
Liljensten, et al. [14]	Rabbits	G1: Resorbable HA; G2: Nonresorbable HA;	Tibia	After 3 months histomorphometry analysis revealed significantly more newly formed bone in the two HA augmented groups compared with that in the control. A relatively slow

		G3: Empty		degradation process was indicated by the small reduction of the total granule area in the cortical defects. However, a change of granule form was observed.
Mate Sanchez de Val, et al. [15]	Rabbits	G1: HA (2000–4000 µm); G2: HA (1000–2000 µm); G3: HA (600–1000 µm); G4: Empty	Tibia	By increasing the sintering temperature, the HA graft exhibited an increase in crystallinity and crystallite size. The fastest resorption rate of the material was in Group 3 > Group 2 > Group 1.
Moshiri, et al. [16]	Rats	G1: Empty; G2: Autogenous graft; G3: Gelatin (Gel); G4: Chitosan (Chi); G5: HA; G6: β-TCP; G7: Nano-HA; G8: Strontium carbonate (Sr)	Femurs	Compared to HA, nanoHA showed superior role in increasing new bone formation, mineral density and ash.
Ono, et al. [17]	Rats	G1: A-W.GC; G2: HAM; G3: Alumina (AL); G4: HAK; G5: Empty	Tibia	There were no significant differences between the two HA groups (G2 and G4). In regard to percentages of the area occupied by granules G2 showed 53.8 ±6.3% and G4: 53.6 ±3.1%.

Legend: HA: Synthetic hydroxyapatite; DBBM: deproteinized bovine bone mineral; β-TCP: beta-tricalcium phosphate; A-W.GC: Apatite-wollastonite containing glass ceramic; Highlighted in gray: informations about resorption rate.

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4 CONCLUSÃO

Conclui-se que as partículas de HA são osteocondutoras, proporcionando uma superfície para a formação de novo osso. Além disso, alterações nas propriedades físico-químicas da HA, incluindo tamanho de partícula, porosidade e tamanho dos poros, influenciam diretamente o resultado da formação de osso novo e as taxas de reabsorção.

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APÊNDICE A – METODOLOGIA DO ESTUDO

Protocolo

Esta revisão sistemática foi conduzida de acordo com as diretrizes Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[1] e um protocolo foi registrado no PROSPERO (ID: CRD42022307364).

Pergunta principal

A pergunta principal da revisão foi “A hidroxiapatita sintética particulada é eficaz para o reparo ósseo?”.

Critérios de elegibilidade

Os seguintes critérios de inclusão foram adotados com base nos seguintes critérios PICO: (P) População/Animais: todos os modelos animais (todas as espécies, todos os sexos); (I) Intervenção: defeitos ósseos preenchidos com HA pura, sintética, na forma particulada; (C) Comparador: defeitos ósseos preenchidos com sangue, mineral ósseo bovino desproteinizado (DBBM) e HA sintética na forma particulada associada a outros biomateriais; (O) Desfechos: formação óssea em defeitos preenchidos com HA sintética na forma particulada; (S) Desenho do estudo: estudos in vivo relatando dados sobre defeitos ósseos preenchidos com HA sintética na forma de partículas publicadas no idioma inglês.

Seguindo os critérios de exclusão foram excluídos: i) artigos sem grupo com HA sintética pura na forma particulada; ii) estudos incluindo apenas animais com comorbidades, submetidos à ovariectomia e modelos ex vivo, in vitro e in silico; iii) estudos avaliando defeitos ósseos preenchidos com HA sintética em outras formas (por exemplo, cimento, pasta e enxerto em bloco) e intervenções em outros tecidos; e iv) cartas ao editor, revisões históricas, estudos clínicos e artigos inéditos.

Buscas eletrônicas

As buscas eletrônicas nas bases de dados PubMed/MEDLINE, Embase, Web of Science e Scopus foram realizadas para publicações até maio de 2021. As publicações foram encontradas usando uma combinação de termos mostrados no quadro 1. Foi aplicado filtro para estudos em animais, porém, não foram feitas

restrições de busca por idioma e período de publicação. Além disso, não foram pesquisados artigos inéditos e literatura cinzenta. As publicações encontradas em todas as bases de dados eletrônicas foram transferidas para a versão EndNote Program™ X7 (Thomson Reuters, Nova York, NY, EUA) para remover referências duplicadas.

Quadro 1 - Estratégia de busca nas bases de dados

ESTRATÉGIA DE BUSCA PUBMED	(((((("Hydroxyapatite"[All Fields] OR "Hydroxyapatites"[All Fields] OR "Calcium Hydroxyapatite"[All Fields] OR "hydroxyapatite calcium"[All Fields] OR "Durapatite"[All Fields]) AND "Bone graft"[All Fields]) OR "Graft"[All Fields] OR "Grafting"[All Fields]) AND "Bone Formation"[All Fields]) OR "Osteogenesis"[All Fields] OR "Bone Regenerations"[All Fields] OR "Bone Regeneration"[All Fields]) AND "Osteoconduction"[All Fields]) OR "Osteoconductive"[All Fields] OR "Osteoconductivity"[All Fields]) AND (animal[Filter])
ESTRATÉGIA DE BUSCA WEB OF SCIENCE:	(Hydroxyapatite OR Hydroxyapatites OR Calcium Hydroxyapatite OR Hydroxyapatite, Calcium OR Durapatite) AND (Bone graft OR Graft OR Grafting) AND (Bone Formation OR Osteogenesis OR Bone Regenerations OR Bone Regeneration OR Regeneration, Bone OR Regenerations, Bone OR Osteoconduction OR Osteoconductive OR Osteoconductivity) AND (Rats OR Rat OR Mouse OR Mouses OR Mice OR Murine OR Murines OR Rabbit OR Rabbits OR Monkey OR Monkeys OR Animal Experimentation OR Animal Experiments OR Animal Research OR Animal Experimental Use OR Animal Experimental Uses)

Continua

	Continuação
ESTRATÉGIA DE BUSCA EMBASE	('hydroxyapatite' OR 'hydroxyapatites' OR 'calcium hydroxyapatite' OR 'hydroxyapatite, calcium' OR 'durapatite') AND ('bone graft' OR 'graft' OR 'grafting') AND ('bone formation' OR 'osteogenesis' OR 'bone regenerations' OR 'bone regeneration' OR 'osteoconduction' OR 'osteoconductive' OR 'osteoconductivity') AND ('animal experiment'/de OR 'animal model'/de OR 'animal tissue'/de OR 'canine model'/de OR 'experimental model'/de OR 'experimental study'/de OR 'in vivo study'/de OR 'nonhuman'/de OR 'ovine model'/de OR 'rabbit model'/de)
ESTRATÉGIA DE BUSCA SCOPUS	(hydroxyapatite OR hydroxyapatites OR calcium AND hydroxyapatite OR hydroxyapatite, AND calcium OR durapatite) AND (bone AND graft OR graft OR grafting) AND (bone AND formation OR osteogenesis OR bone AND regenerations OR bone AND regeneration OR regeneration, AND bone OR regenerations, AND bone OR osteoconduction OR osteoconductive OR osteoconductivity) AND (rats OR rat OR mouse OR mouses OR mice OR murine OR murines OR rabbit OR rabbits OR monkey OR monkeys OR animal AND experimentation OR animal AND experiments OR animal AND research OR animal AND experimental AND use OR animal AND experimental AND uses) AND (EXCLUDE (EXACTKEYWORD , "Human") OR EXCLUDE (EXACTKEYWORD , "Humans") OR EXCLUDE (EXACTKEYWORD , "Controlled Study") OR EXCLUDE (EXACTKEYWORD , "Review") OR EXCLUDE (EXACTKEYWORD , "In Vitro Study"))

Fonte: Elaboração própria.

Processo de extração e seleção de dados

Dois pesquisadores (ML e LFFS) realizaram a busca inicial para avaliação de títulos e resumos de forma independente, e os resultados foram verificados quanto à concordância. O texto completo dos artigos julgados relevantes com base no título e resumo foram lidos de forma independente e avaliados com base nos critérios de

seleção (ML e LFFS). Para avaliações conflitantes, um acordo foi alcançado após uma discussão incluindo um terceiro investigador (SCP). Os mesmos dois investigadores (ML e LFFS) revisaram independentemente todos os estudos e extraíram os dados usando uma tabela padronizada feita no Microsoft Excel.

Os dados extraídos foram: i) tipo de animal (espécie); ii) grupos de estudo (grupos experimentais, grupo(s) controle e número de animais por grupo); iii) tipo e tamanho dos defeitos ósseos; iv) método de obtenção, tamanho de partícula, porosidade, cristalinidade, tamanho de poro e bioatividade do HA sintético; ev) resultados da formação óssea.

Avaliação de qualidade

Dois autores (CMAM e LFFS) avaliaram a qualidade dos estudos incluídos separadamente usando a ferramenta de risco de viés do SYRCLE para estudos em animais[2]. Qualquer discordância foi discutida com um terceiro revisor (SCP).

Síntese de dados - Meta-análise

Foi feita uma meta-análise avaliando a porcentagem de formação óssea obtida em defeitos ósseos preenchidos com HA pura sintética (diferentes tamanhos de partículas) em comparação ao grupo controle (defeito vazio). Os estudos foram agrupados considerando os mesmos modelos animais e de defeitos ósseos. Entre os desfechos relatados, a porcentagem de neoformação óssea foi usada para evitar viés em relação aos diferentes tamanhos de defeitos ósseos usados nos estudos incluídos. Além disso, o tamanho das partículas de HA (tamanhos milimétricos e nanométricos) foram convertidos em µm.

A meta-análise de efeitos aleatórios foi realizada no RStudio versão 4.0.4 usando o 'meta-pacote[3] versão 4.18-0[4]. O tamanho do efeito é apresentado como diferença média (MD) com desvio padrão (± SD) e IC 95% para a porcentagem de neoformação óssea. Um gráfico de floresta foi usado para ilustrar os resultados da análise. Para avaliar a heterogeneidade entre os estudos, calculou-se inconsistência estatística (I^2) e considerou-se heterogeneidade significativa quando I^2 foi > 50%[5]. Uma heterogeneidade significativa indicaria a condução de meta-análises de efeitos aleatórios, na ordem de um modelo de efeito fixo[6].

Em um esforço para entender o impacto de diferentes tamanhos de partículas de HA na formação óssea, uma meta-regressão também foi realizada. O tamanho de

partícula de cada biomaterial de AH testado (30nm, 350µm, 630µm) foi considerado como uma covariável contínua a ser correlacionada com a porcentagem de neoformação óssea.

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