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REVIEW

Histological Evaluation of Bone Repair with Hydroxyapatite: A Systematic Review

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Abstract The aim of this study was to evaluate the morphological bone response in animal experiments by applying hydroxyapatite grafts in critical and non-critical size bone defects. Current report followed the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Animal experiments were selected by assessing repair of bone defects with hydroxyapatite as bone graft and with blood clot only as control. Eight articles were identified in specialized literature and included in the meta-analysis. Statistical analysis was carried out with a random-effect model ($p = 0.05$). Subgroup analyses were further performed to investigate bone repair in critical and non-critical bone defects. Comprehensive analysis of bone repair outcome showed a statistically significant difference between hydroxyapatite and blood clot control ($p < 0.05$). Subgroup analyses showed statistically significant difference for critical bone defects ($p < 0.05$). No statistically significant difference was reported in non-critical bone defects ($p > 0.05$). Although animal studies revealed a high risk of bias and results should be interpreted with caution, the literature suggests that non-critical bone defects may heal spontaneously and without the need of a bone graft. Conversely, when critical-size defects are present, the use of hydroxyapatite bone graft improves the bone repair process.

Keywords Hydroxyapatite · Bone repair · Bone graft · Critical size defect

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Introduction

Bone is a composite natural tissue susceptible to fracture which may be caused by trauma, pathology and resorption [1]. Defects and functional disorders of the tissue have become a global health care problem and tissue engineering has turned to be an important approach in bone regeneration research [2]. Bone tissue engineering involves the use of suitable materials for temporary tridimensional matrix to guide cell adhesion, differentiation, proliferation and subsequent tissue regeneration [3]. Techniques for bone engineering currently include the replacement of damaged bone with autograft, allograft, xenografts and artificially synthesized bone materials [4]. Autologous grafts, or tissues from the patient, are considered the gold standard since they have all the characteristics necessary for the growth of new bone [5]. In the case of autologous

bone grafting, bone is transplanted from one site of the body, usually the iliac crest, to another site of the same patient [6]. However, autografting has several drawbacks, such as risks and discomfort to the patient due to additional surgery for bone tissue removal, pain at the donor site, limited bone supply at the donor site or even infections in the region [4, 7].

The above difficulties have triggered researchers for solutions, and as result, the development of bone repair materials has become a hotspot in research. The use of natural bone substitutes or synthetic grafts may overcome the disadvantages of using autologous bone grafts [6]. Biocompatibility, non-toxicity, low cost, non-carcinogenicity, with excellent osteoconductive and osteoinductive properties, are among the desirable characteristics of biomaterials for bone replacements [8].

The primary materials that have been used in bone fracture repair include bone, bone cements, metals, ceramics, polymers and composites [9]. Calcium orthophosphate ceramics (CaPs) is one of the most popular bone substitutes since its chemical composition is almost identical to bone mineral [4]. These materials have excellent biological behavior (biocompatibility, bioactivity and osteoconductivity); they are low cost and widely available [10]. CaPs represents a large family of substances with a Calcium/Phosphate molar ratio between 0.2 and 2.0, including tricalcium phosphate (TCP), hydroxyapatite (HA), biphasic calcium phosphates (BCP), monocalcium phosphate monohydrate (MCPM) and unsaturated apatite (AP) [11].

HA is a widely available bioactive and bioresorbable calcium phosphate that constitutes most of the inorganic component of bone tissue [7]. HA $Ca_{10}(PO_4)_6(OH)_2$ may be found in chemically identical natural or synthetic forms, differing only in their physical microstructure, crystal size and porosity [4]. HA directly bonds with live bone after implantation in cases of bone defects [3]. This feature enhances appropriate vascularization and stem cell proliferation, and guides bone regeneration without causing any local or systemic toxicity [11]. Both natural and synthetic HA are available in pastes, putties, solid matrices, and granules [12].

Histological evaluation is the primary means in assessing the effect of bone substitutes in tissues [13]. However, tissue removal is mandatory in this type of analysis, precluding its use in clinical trials. Therefore, most studies on bone repair employ animal experiments which provide relatively reproducible and quantifiable information. The current systematic review evaluates morphological bone response in animal models by applying hydroxyapatite grafts in bone defects characterized as critical and non-critical size. Hypothesis tested whether hydroxyapatite would enhance bone repair in bone defects.

Materials and Methods

Current systematic review followed the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA Statement). The main research question is: do hydroxyapatite grafts improve bone healing in bone defects of critical and non-critical size in animal models?

Systematic Literature Search

Research in specialized literature was conducted by two independent reviewers until October 2016, with no limit in publication year. The following databases were searched: PubMed (MedLine), ISI Web of Science, Lilacs, Ibecs, BBO, Scopus and Scielo. Search strategy developed for PubMed (MedLine) is listed in Table 1, and was adapted for use in other databases. Terms related to hydroxyapatite and animal experiments were crossed to optimize the retrieval of relevant documents and references cited in the papers were also hand-searched to identify other potentially relevant articles. All documents were imported into Endnote X7 software (Thompson Reuters, Philadelphia, PA, USA) to remove duplicates. The reviewers assessed titles and abstracts of all the documents.

Study Selection

Two reviewers independently searched the eligible items according to eligibility criteria (Table 2). As inclusion criteria, it was included animal experiments with hydroxyapatite both natural or synthetic applied in circumscribed bone defects. We included animal experiments once they are the step before clinical evaluation in humans, and their results may provide valuable data for translational research. Moreover, in order to represent the physiological bone healing process it was only included studies in which the defects unfilled (only blood clot) were used as the control group. As exclusion criteria, studies that hydroxyapatite was used in periodontal or alveolar defects, or in tissue engineering approaches (with growth factors or stem cells) were not included, since our purpose was to evaluate the morphological response of hydroxyapatite grafts alone in critical and non-critical size bone defects. Besides, studies investigating only macroscopic, microtomography or x-ray results were not included, once the morphological response with histological analysis could not be evaluated. When relevant information to eligibility criteria was not available in the abstract, the article was selected for full reading. If reviewers disagreed further discussion ensued till consensus. Only articles that fulfilled all eligibility criteria were accepted.

Table 1 Search strategy used in PubMed (MedLine)

	Search terms
#4	Search ((#1) AND #2) AND #3
#3	Search (Animal OR Animal Research OR Research, Animal OR Animal Experimental Use OR Animal Experimental Uses OR Experimental Use, Animal OR Experimental Uses, Animal OR Animal Experiments OR Animal Experiment OR Experiment, Animal OR Experiments, Animal)
#2	Search (Healing* OR Cicatrix OR Cicatrization OR Scar* OR Regeneration OR Repair OR Wound) AND (Bones OR Bone OR Bone Tissue OR Bone Tissues OR bone graft)
#1	Search Hydroxyapatite*

Table 2 Inclusion and exclusion criteria

	Inclusion criteria	Exclusion criteria
Population	Animal experiments	Studies with humans subjects
Intervention	Studies of animals who have undergone the following procedures	Studies of animals who have undergone the following procedures
	Circumscribed bone defects created by researchers filled with hydroxyapatite both natural or synthetic in the form of granules, particles or blocks	Use of hydroxyapatite in tissue engineering approaches (with growth factors or stem cells)
		Guided tissue regeneration
	Control group defects unfilled, only blood clot	Periodontal defects
		Alveolar defects
Outcomes	Studies investigating morphological outcomes and/or bone tissue repair or regeneration	Studies investigating only macroscopic, microtomography or x-ray results
Study design	Animal studies	Clinical trials, in vitro studies, in situ studies, reviews, case reports

Data Extraction

Data were retrieved by standardized form in Microsoft Office Excel 2013 software (Microsoft Corporation, Redmond, WA, USA). If any information was missing, the authors of the included papers were contacted via e-mail to provide the specific data. Reviewers tabulated the following data of all included studies: authors, country, year, type and number of animals, sex, size defect, local defect, type of hydroxyapatite and number of bone defects evaluated (Table 3). Table 4 shows studies included in the meta-analysis, whilst Table 5 describes the main data.

Statistical Analysis

Analyses were performed by Review Manager Software 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). The first global analysis was carried out using a random-effect model and pooled-effect estimates were obtained by comparing standardized mean difference of bone repair in hydroxyapatite group with the control treatments (unfilled bone defects), at $p < 0.05$ significance. Additionally, subgroup analyses were performed for the analysis of bone repair only in critical and non-critical bone defects. Statistical heterogeneity of treatment effect among studies was assessed with Cochran's Q test and inconsistency with I^2 test, in which rates greater than 50% indicated substantial heterogeneity [14].

Quality Assessment

The methodological quality of each included study was independently assessed by the two reviewers, based on the SYRCLE's risk of bias tool for animal studies [15]. The studies were evaluated to provide a framework for judging the methodological quality of animal experiments according to the following information: random sequence generation (selection bias), baseline characteristics (selection bias), allocation concealment (selection bias), random housing (performance bias), blinding of caregivers and/or investigators (performance bias), random outcome assessment (detection bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other biases. Each component was graded as low, unclear or high risk of bias in software RevMan 5.2 (The Cochrane Collaboration, Denmark).

Table 3 Demographic data of the included studies

Study	Country	Year	Number of animals (defect per animal)	Number of defect or animal per group	Animal	Sex	Size defect	Local defect	Type of hydroxyapatite (HA)
Andrade	Brazil	2013	20 (2)	5 animals per group	Albino rats	Male	Non critical	Parietal and tibia	Cylindrical dense HA blocks. (Experimental material)
Appleford	USA	2009	10 (2)	10 defects per group	Foxhound dogs	Male	Non critical	Mandible	Cylindrical porous HA blocks fabricated with micro or nano particles. (Experimental material)
Ashby	USA	1996	24 (1)	4 animals per group	New Zealand rabbits	Female	Critical	Parietal	Coralline-derived porous HA blocks. Average pore diameter 200 μm (Interpore 200®)
Bilkay	Turkey	2004	88 (1)	22 animals per group	New Zealand rabbits	Male	Non critical	Hind limbs	Coralline-derived porous HA blocks. Pore diameter 190–230 μm (experimental material)
Buser	Switzerland	1998	12 (6)	12 defects per group	Miniature pigs	–	Non critical	Mandible	Coral derived hydroxyapatite granules. Particle size 0.425–1.0 mm (Interpore 200®)
Calasans-Maia	Brazil	2009	15 (6)	5 animals per group	New Zealand rabbits	–	Non critical	Tibia	Bovine derived HA granules (A-Osseus® and Bio-Oss®)
Carvalho	Brazil	2007	8 (6)	9 defects per group	Mongrel dogs	Male	–	Humerus	Bovine derived HA particles. Granule size 150 and 300 μm (experimental material)
Doll	USA	1990	60 (1)	15 animals per group	Long-evans rats	–	Critical	Calvaria	Bovine derived porous HA particles. Pore diameter 190–230 μm (experimental material)
Eftekhari	Iran	2015	27 (1)	9 animals per group	Wistar rats	Male	Non critical	Femurs	Synthetic HA granules (experimental material)
Franco	Brazil	2001	40 (1)	10 animals per group	Mongrel dogs	–	Non critical	Tibia	Synthetic HA granules (experimental material HAP-91)
de Girolamo	Italy	2011	12 (2)	6 defects per group	New Zealand rabbits	Female	Critical	Tibia	Cylindrical HA scaffolds. Pore size 150 μm (provided by Finceramica®, Co.)
Hammerschmidt	Brazil	2011	12 (2)	12 defects per group	Wistar-Furth rats	–	Non critical	Mastoid	Synthetic HA granules. Granule size 0.5–0.75 mm (Genius Gen-phos®)
Houshmand	Iran	2007	8 (3)	8 animals and defects per group	Sheep	–	Non critical	mandible	Bovine derived HA particles. Particle size 250–1000 μm (BioOss®)
Klinge	Sweden	1992	13(4)	13 animals and defects per group	New zealand rabbits	Male and female	Non critical	Frontal and parietal	Synthetic dense HA granules. Granule size 0.3–0.6 and 0.6–1 mm (Apaceram®) Bovine derived HA porous particles. Particle size 250–1000 μm (BioOss®)

Table 3 continued

Study	Country	Year	Number of animals (defect per animal)	Number of defect or animal per group	Animal	Sex	Size defect	Local defect	Type of hydroxyapatite (HA)
Kucukkolbasi	Turkey	2009	18 (8)	18 defects and animals per group	New Zealand rabbits	Male and female	Non critical	Tibia	Bovine derived porous HA particles. Pore size of 200 and 500 μm (Ostegraf N®)
Kuhne	Germany	1994	16 (2)	8 defects per group	Chinchilla rabbits	–	Non critical	Femur	Coral-derived porous HA blocks. Pore size 200 and 500 μm (Interpore 200® and Interpore 500®)
Lee	South Korea	2012	16 (4)	10 animals and defects per group	New Zealand rabbits	–	Non critical	Parietal	Synthetic and eggshells-derived HA granules. Pore size <1 μm (Sigma®) and >1 μm (Hungarian Academy of Science) respectively
Lemperle	USA	1998	18 (2)	6 or 12 animals per group	Mongrel dogs	–	Critical	Cranium	Coralline-derived porous HA blocks. Pore size 200 and 500 μm (Interpore 200® and Interpore 500®)
Lindholm	Finland	1994	10 (2)	3 or 7 defects per group	New Zealand rabbits	–	Non critical	temporal	Coralline-derived porous HA granules. Pore size 200 μm (Interpore 200®)
Moreira	Brazil	2003	55 (2)	15 animals per group +10 control	Wistar rats	Male	Non critical	Femur	Synthetic HA granules. Granule size 212, 500 and 1000 μm (GEN-PHOS®)
Nandi	India	2008	12 (1)	6 animals per group	Bengal goat	Male and female	Non critical	Radius	Synthetic porous HA blocks. Pore size 10–20 μm (experimental material)
Notodihardjo	Japan	2012	20 (1)	5 animals per group	Wistar rats	male	Critical	Calvaria	Synthetic dense HA particles. Particle size 300–500 μm (Bonetite®)
Park	South Korea	2009	56 (1)	14 animals per group	Sprague-dawley rats	male	Critical	Calvaria	Eggshell-derived particles. Particle size 300 μm (experimental material). Bovine-derived HA particles (Bio-Oss®)
Razak	Malaysia	2004	8 (2)	8 defects and animals per group	New Zealand rabbits	–	Non critical	Mandible	Synthetic HA blocks (experimental material)
Reedy	USA	1999	7 (4)	7 defects and animals per group	Yorkshire swine	–	Non critical	Calvaria	Coralline-derived porous HA discs. Pore size 500 μm (Interpore 500®)
Rojbani	Japan	2011	72 (2)	12 defects per group	Wistar rats	Male	Critical	Calvaria	HA particles. Particle size 500–750 μm (provided by Advance®, Co.)
Sawada	Japan	2011	–	–	New Zealand rabbits	Male	Non critical	Calvaria	Bovine-derived HA particles (Bio-Oss®)
Soccol	Brazil	2006	24(2)	12 animals per group	Wistar-furth rats	–	Non critical	Mandible	Synthetic HA particles
Sotto-Maior	Brazil	2011	25 (2)	25 defects and animals per group	Wistar rats	Male	Non critical	Femur	Bovine-derived HA particles. Size 0.5–1 mm (Genox®)

Table 3 continued

Study	Country	Year	Number of animals (defect per animal)	Number of defect or animal per group	Animal	Sex	Size defect	Local defect	Type of hydroxyapatite (HA)
Thorwarth	Germany	2007	12 (9)	–	Pigs	Female	Critical	Forehead	Bovine-derived HA particles. Size 0.25–0.42 mm (OsteoGraf®/N-300)
									Algae-derived porous HA particles. Size 0.3–2 mm, pore size 5–10 µm (FRIOS® Algipore®)
Turk	USA	1993	27	5 animals per group + 2	New Zealand rabbits	Female	Critical	Parietal	Coralline-derived porous HA blocks (Interpore 200®)
Zhou	Canada	2013	20 (2)	5 animals per group	New Zealand rabbits	Male	Critical	Calvaria	Algae-derived porous HA. Pore size 10 µm (C-Graft®)

Results

Search Strategy

Initial search in databases identified 4101 potentially relevant records. Figure 1 shows the flowchart that summarizes the article selection process according to the PRISMA Statement. Duplicates were removed and 3414 records were examined by titles and abstracts. Seventy-five articles were focused for full-text reading, after which 41 were excluded because they failed to meet eligibility criteria. Thirty-two studies fulfilled all selection criteria and were included in this review.

Descriptive Analysis

Table 3 shows data on studies in which six different types of animals were evaluated, namely, 13 rabbits [16–28], 10 rats [29–38], 4 dogs [39–42], 3 pigs [43–45], one sheep [46] and one goat [47]. Twelve out of the 33 articles used males [17, 26, 28, 29, 31, 33–37, 39, 40], 4 used females [16, 19, 27, 44], 3 used both sex [20, 21, 47] and 13 failed to report gender [18, 22–25, 30, 32, 38, 41–43, 45, 46]. Regarding to the site for holding the bone defect, the preference sites comprised skull [16, 20, 23, 24, 26–30, 32, 34–36, 42, 44, 45], jaws [25, 38, 39, 43, 46], tibia [29], femur [22, 31, 33, 37], tibia [18, 19, 21, 41], radius [47], humerus [40] and hind limbs [17]. More than half (21) used size defects informed by the author as non-critical, whereas only 10 articles qualified the defects as critical [16, 19, 27, 28, 30, 34–36, 42, 44]. There was doubt whether the defect really exhibited any critical size occurred only in a single article [40]. Although there were great variations in the hydroxyapatite used, coral-derived hydroxyapatite was predominant [16, 17, 22, 27, 30, 38, 42, 43, 45, 48].

Meta-Analysis

A meta-analysis was performed with 8 animal studies [21, 23, 24, 29, 35, 40, 42, 43]. The global analysis of bone repair with hydroxyapatite (Fig. 2) showed a statistically significant difference when compared with control ($p < 0.05$). Although the rate of I^2 test was 75%, in the subgroup analysis with only non-critical bone defects, 5 studies could be included (Fig. 3a), with no statistically significant difference of hydroxyapatite when compared to control ($p = 0.05$; $I^2 = 85\%$). Further, there were statistically significant differences between treatments ($p = 0.03$; $I^2 = 60\%$) in the use of hydroxyapatite in critical bone defects (Fig. 3b).

Table 4 Bone repair outcomes of studies included in the meta-analysis

Author	Animal	Location	Shape	Dimensions	Critical size	Specifications	Hydroxyapatite			Control		
							% New bone	SD	*N*	% New bone	SD	*N*
Andrade	Rat	Parietal tibia	Circular circular	5 mm diameter 4 mm diameter	No	10 weeks	50.8	3.3	5	45	3	5
Buser	Pig	Mandible	Trapezoidal	Base ~12 mm	No							
				Top ~10 mm		4 weeks	20.7	7.9	4	33.8	7	4
				Height ~12 mm		12 weeks	42.8	11.7	4	62.2	5.3	4
				Depth ~5–6 mm		24 weeks	49	2.8	4	55.3	5.3	4
Carvalho	Dog	Humers	Circular	5 mm wide	Yes	12 weeks						
				4 mm long		Bovine HA small	50.59	8.42	4	28.34	2.95	4
						Synthetic HA small	37.5	5.25	4	28.34	2.95	4
						Bovine HA large	35.71	5.05	4	28.34	2.95	4
						Synthetic HA large	25.89	5.16	4	28.34	2.95	4
Kucukkolbasi	Rabbits	Femur	Circular	3 mm diameter	No	1 month	28.2	2.1	6	11.3	1.12	6
						3 months	44.60	3.12	6	31.78	2.90	6
						6 months	64.67	2.97	6	33.43	2.02	6
Lee	Rabbit	Parietal	Circular	8 mm diameter	No	4 weeks						
						Synthetic HA	28.81	12.63	5	17.11	10.24	5
						Natural HA	25.68	10.89	5	17.11	10.24	5
						8 weeks						
						Synthetic HA	38.62	17.42	5	27.5	10.89	5
						Natural HA	41.99	8.44	5	27.5	10.89	5
Lemperle	Dog	Cranium	Retangular	20 × 15 mm	Yes	2 months	12.1	3.8	4	23.3	3	4
						4 months	18.2	1.9	8	18.2	1.4	8
Lindholm	Rabbit	Temporal	Circular	11 mm diameter	No	12 weeks	34.4	3.9	3	47.7	4.4	7
Park	Rats	Calvarium	Circular	8 mm diameter	Yes	6 weeks						
						Bio-Oss®	6.4	4.3	7	3.9	2.1	7
						N HA	11.2	3.3	7	3.9	2.1	7
						12 weeks						
						Bio-Oss®	8.2	3.9	7	6.4	4.8	7
						N HA	19.2	6.1	7	6.4	4.8	7

Quality Assessment

With regard to assessment of risk of bias, Fig. 4 summarizes the information used to assess the studieś methodological quality. Studies scored particularly poorly on the following items: random sequence generation (selection bias), random housing (performance bias), blinding of caregivers and/or investigators (performance bias), random outcome assessment (detection bias), blinding of outcome assessment (detection bias) and selective reporting (reporting bias).

Discussion

The systematic review of animal experiments revealed improvement of bone repair by hydroxyapatite. However, in non-critical bone defects, the use of hydroxyapatite failed to improve the bone repair process when the latter was compared to blood clot control. Consequently, the hypothesis above was only partially proved. Several studies could not be included in the meta-analysis because the quantitative results were incomplete or their results were only qualitative. Further, deep heterogeneity was detected

Table 5 Main findings from included studies

Author	Year	Main findings
Andrade	2013	Chronic inflammatory response or fibrous tissue formation in the neoformed bone tissue/graft implant interface was observed. Also, it was evident a bone neoformation in direct contact with the HA graft
Appleford	2009	3 weeks: Trabecular bone could be observed forming inside the control defect. Control and Nano-Hydroxyapatite scaffolds (N-HA) demonstrated no morphological differences compared to Micro-Hydroxyapatite (M-HA) scaffolds 12 weeks: Tissue infiltration progressed throughout both scaffold designs with few pores left unfilled. Collagen patterning has visibly organized into interlaced strands wrapping around the scaffold struts. Significant difference in vessel distribution and diameter were observed between HA scaffolds and control bone
Ashby	1996	The unreconstructed control group exhibited no healing, except for 2–4 mm of bone ingrowth from defect edges, spanned by fibrovascular bridge. Hydroxyapatite groups exhibited mean volume percent bone ingrowth of 10 mm^2
Bilkay	2004	New bone formation of various degrees was observed in all groups at the second week. By the second week, hydroxyapatite group displayed remarkably significant new bone formation. Excellent bone formation and remodeling were observed in all the specimens treated with hydroxyapatite
Buser	1998	Blood clot demonstrated a reduced total volume of the defects created and after 12 weeks such defects were almost completely filled with new bone. HA granules appeared dispersed in the defects sites of experimental groups. Remodeling process was restricted to the bone compartment and did not extend into the graft material
Calasans-Maia	2009	The control group has completely filled with new bone and there was no adverse inflammation. The microscopic analysis showed very similar patterns in both biomaterials (A-osseous and Bio-Oss). Mild inflammatory infiltrates were present at 7 days and absent at 14 days. No multinucleated giant cells were observed. The regeneration process prevailed after 14 days. Osteogenesis started at 7 days, in the injury margins and expanded throughout the trial period, filling the bone defect
Carvalho	2007	HA granules exhibited direct bone contact, regardless of the origin and the size. Control sites had an increased amount of connective tissue infiltration. Bovine-derived HA exhibited better bone formation than synthetic HA. The synthetic HA delivered reduced amounts of bone compared with the control
Doll	1990	There was no detectable new bony trabeculae across the defects when HA was used. Paucity of new bone was observed for the untreated defects
Eftekhari	2015	On day 5, the healing site of control group showed the defect to be filled with inflammatory cells infiltrate and immature granulation tissue consisting vessels and fibroblasts dispersed among the inflammatory cells. The healing site of HA-treated group at this time showed that the repaired construct was filled with immature granulation tissue consisting of large amounts of plump fibroblasts. On day 30, the healing site of control group contain well-matured granulation tissue. The healing site of HA treated group at this time showed the presence of cartilaginous nodules in the repaired construct indicating that chondrogenesis in fibrous tissue was taking. On day 45, the healing site of control group indicated bone deposition. The healing site of HA-treated group at this time was filled with fibrous connective tissue and newly formed trabecular bone
Franco	2001	At 8 days post-operative, defects treated with HA showed the presence of granulation tissue with a greater number of blood vessels than control. It was also observed trabecular bone formation around the implant, with numerous osteoblasts on trabecular surface and osteocytes. 30 days after implantation, the control defects and those treated with HA showed trabecular bone filling whole defect. At 60 days after surgery, both control and HA groups showed early remodeling lamellar bone that filled the defect. At 120 and 180 days after surgery all groups showed mature bone tissue. There was also hydroxyapatite encapsulated in adjacent tissue but without inflammatory reaction which suggest biocompatibility of HA
De Girolamo	2011	No bone resorption, abnormal bone callous formation, fractures, extrusions, infections, or severe inflammatory reactions were observed. The untreated defects showed little or no new bone formation. In the HA scaffold-implanted defects, new bone formation was strongly observed within the scaffold pores as well a small number of multinucleated giant cells. Osteoblasts were observed in conjunction with bone trabeculae located at the periphery of the scaffold pores. In particular, most of the larger pores were filled with adipocytes, suggesting the presence of poorly differentiated bone marrow
Hammerschmidt	2011	Inflammatory process occurs in both groups (HA and control). Neutrophilic infiltration was present in both groups which demonstrates that this process occurs in the normal healing course. There were no cases of incompatibility signs or extrusion. HA defects had higher bone formation
Houshmand	2007	Histologically, the negative-control cavities (empty) were filled with fibrovascular connective tissue surrounded by bone trabeculae. Few scattered inflammatory cells were still visible in the regenerated tissues of this group of defects. The positive-control defects, which were filled with inorganic bovine hydroxyapatite, demonstrated trabeculae formation around implant particles. The formed trabeculae loosely surrounded the matrix particles. Inflammatory cells were not identifiable in the cavity area. The negative-control group had the least amount of bone regeneration

Table 5 continued

Author	Year	Main findings
Klinge	1992	At 4 weeks, the interstitial space between the HA implants was infiltrated by loose, immature fibrous connective tissue. After 14 weeks, the fibrous tissue was less abundant and appeared in slender bands between the bone trabeculae. Moderate marginal bone necrosis and accompanying local bone resorption were observed at 4 weeks in most specimens. Osseous production was most extensive along the surfaces when smaller HA granular size implants
Kucukkolbasi	2009	HA grafts promote better bone repair outcomes in all periods evaluated. No postoperative complications were observed in the healing period. None of the graft materials caused serious and long lasting allergic, toxic or graft rejection reactions
Kuhne	1994	Spontaneous bone repair of the empty cavities took approximately 12 weeks. Osteocytes and osteoblasts appeared normal, osteoclasts were rare, and fibroblasts were no longer detected. No signs of new bone formation were found when 200 μm pore size hydroxyapatite was used. In contrast, there was substantial production of bone within the 500 μm pore size implants at 12 and 26 weeks
Lee	2012	Both types of HA showed higher bone formation than the unfilled control. However, eggshell-derived HA had significantly higher bone formation than the unfilled control at 8 weeks after operation
Lemperle	1998	In mandibular model, control defects exhibited the greatest amount of bone formation after 4 months. In cranial defects no significantly differences were detected
Lindholm	1994	The control defects without implants did not heal during the observation time of 12 weeks. Histologic analysis detected varying amounts of fibrous connective tissue around the HA granules and scanty mature new bone in the fibrous tissue in the HA granule
Moreira	2003	In the control animals whose bone defects were not filled with HA, bone defect remodeling occurred in shorter periods of time. Remodeling in the bone defect occurred in less time in those bone defects filled with smaller HA granules were the reorganization of the bone defect was observed in 100% of cases
Nandi	2008	No marked inflammatory reactions were observed in the control and experimental groups up to the 90th day postoperatively. Tissue sections from control group showed mild inflammatory reactions with moderate fibro-collagenization. The marrow space showed an adequate amount of marrow material, fat cells, and blood vessels. When HA was used as graft, normal ossification with development of Haversian canals and well-defined osteoblasts at the periphery was showed. The blood vessels in the Haversian spaces were well-developed. The marrow space showed development of blood vessels with very little amount of marrow material. Non-absorbed biodegradable material was also noted
Notodihardjo	2012	Control group showed the lowest levels of trabecular bone growth, muscle formation and vascular tissues regeneration, especially in the central area of the defect. The HAP group showed that the mixed cell type was aligned in the surrounding formations around the HAP granules; cells like immature mesenchymal cells could be observed although neither bony nor cartilaginous tissue were identified
Park	2009	Unfilled defects were filled with fibrous connective tissue comprising fibroblasts and blood vessels. For this group, the original thickness of the calvarium was never restored. HA particles except for those located close to the defect margins were encapsulated by fibrous connective tissue
Razak	2004	Bone formation and maturation in the implant site was ahead of the control site at all the time intervals of 12, 20 and 22 weeks. Bone deposition was found at the bone implant interface with the earlier and less mature stages of bone development, being found towards the center of the implant. At 22 weeks, the implanted defect showed mature bone formation filling almost the whole field
Reedy	1999	Bone ingrowth was not statistically different between the control group and HA. Fibrosis was significant less in HA groups and complete osseous union in all HA specimens occurs. There was concurrent bone deposition and significant volumetric bone gain in the HA group
Rojbani	2011	At 6 and 8 weeks postoperative, in control groups the new bone failed to progress toward the center of the defect and only a thin layer of new bone was seen at the defect margins. Regarding HA groups, in 6 weeks, the new bone took place at the margins of the defects, woven bone was filling the center of the defects and spaces between particles and in 8 weeks, the new bone was filling the spaces between particles. HA particles showed little degradation
Sawada	2011	At 4 weeks after implantation, newly generated bone tissue around the bone substitute was observed when HA were implanted. No bone regeneration in the defect was observed in the untreated group. At 12 weeks after implantation, complete bone regeneration without residual bone substitute in the defects implanted with HA was observed. Defects without histological closure were observed in the untreated group. The area of the regenerated bone in defects implanted with HA was significantly increased
Soccol	2006	Control group without grafting had lower bone neoformation and higher porosity than HA treated group. At the end of the evaluation, HA group presented mature bone in all specimens
Sotto-Maior	2011	On fifth day both groups showed a higher deposit of collagen fibers surrounding the HA particles. Also, the initial mineralization process was noticed in both groups with higher intensity of extracellular fibrillar matrix and osteoid production, which originate new immature bone. Osteoclasts were present on the surface of the bone defects walls as well as on adjacent and more distant HA particles granules. The new formed bone area showed no significant statistical difference between groups at the evaluated periods

Table 5 continued

Author	Year	Main findings
Thorwarth	2007	During the postoperative period of 26 weeks there was only incomplete bony regeneration with a residual defect of about a quarter of the area of the original defect in the unfilled control defects. When HA was used, histological examination showed small particles of the material enclosed in new bone at the end of the trial
Turk	1993	The unfilled group showed a minimal amount of immature woven bone around the edges of the defect. HA group showed significantly greater bone ingrowth. Although there were no indication of infections, there was evidence of foreign-body reactions in the control group
Zhou	2013	Unfilled defects were mostly occupied by fibrous tissue, with bony ingrowth only visible at the margins of the defects. HA scaffold showed little sign of degradation or resorption with little or no bone formation

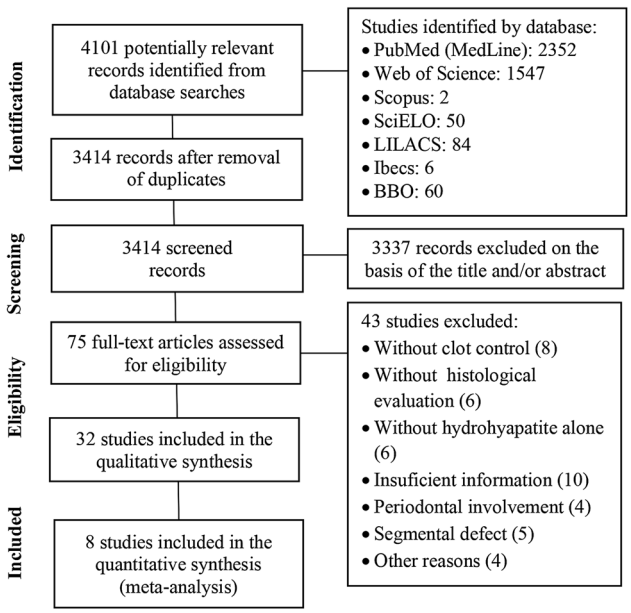

Fig. 1 Search flow (as described in the PRISMA statement)

in the meta-analysis of animal studies, which varied according to the animals evaluated, induced bone defect and different types of hydroxyapatite employed.

By definition, a critical-sized defect (CSD) is the smallest size intraosseous defect in a particular bone that fails to heal completely during the natural lifetime of an animal [49]. In these cases, critical defects are not adequately filled by bone tissue since it lacks spontaneous healing capacity due to its size. Studies analyzed in this review corroborate the above statement. In fact, when a critical size defect is employed, a minimal amount of bone growth has occurred in groups in which no bone graft was used [35, 40, 42]. Results from current meta-analyses suggest that hydroxyapatite may be used successfully as material for bone repair when a CSD was induced.

It has been previously demonstrated that several calcium-phosphate ceramics, including HA, prop new bone formation [50]. These materials, main keys in bone formation, deposit extracellular matrix within the bone defects [11]. Moreover, the presence of Ca^{2+} ions on the material surfaces enhance protein absorption, which facilitates bone-forming cell adhesion and subsequent bone matrix deposition, osteoblast activity and angiogenesis [51–53]. Complex interaction processes between cells and microenvironment, which result in the bone regeneration process, may also be affected by the topography, geometry,

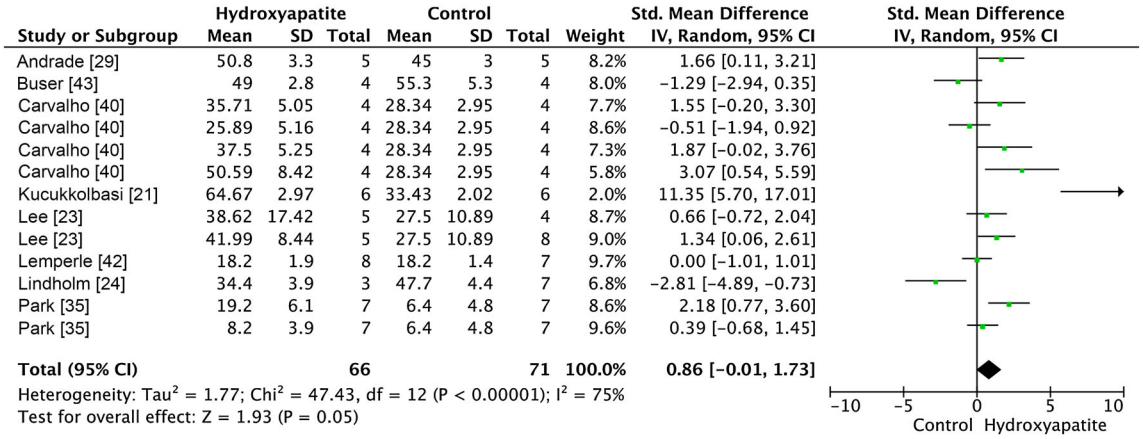

Fig. 2 Results for the global analysis of bone repair with hydroxyapatite compared to control treatments using random-effects models. Statistically significant differences between groups ($p = 0.05$) were observed

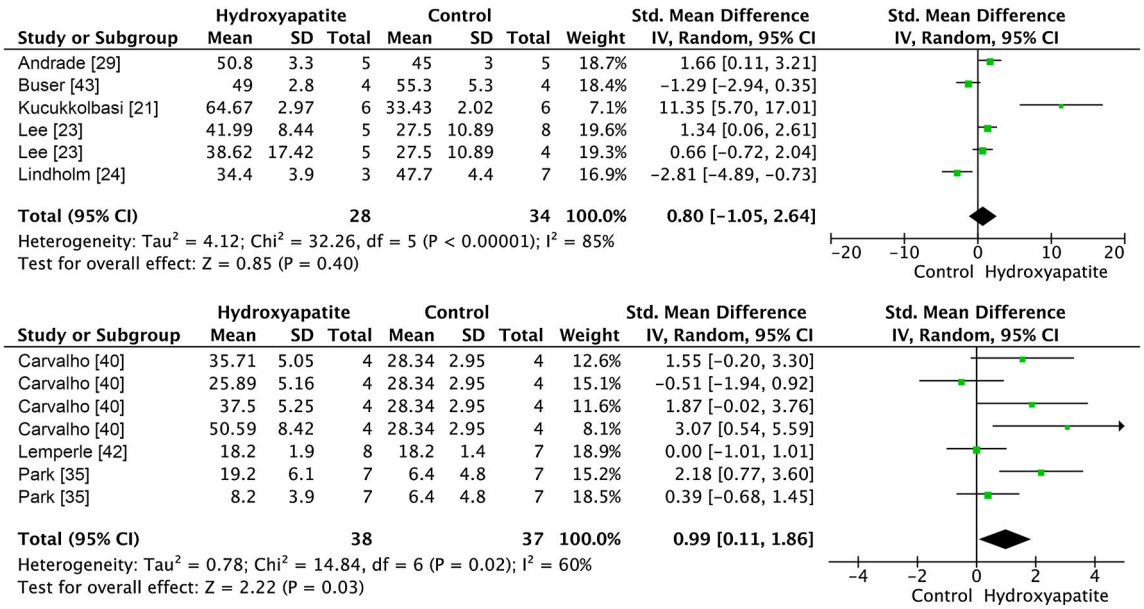

Fig. 3 Results for the subgroup analysis of bone repair with hydroxyapatite in **a** non-critical and **b** critical bone defects compared to control treatments. Statistically significant differences between groups only in critical bone defects analysis ($p = 0.03$)

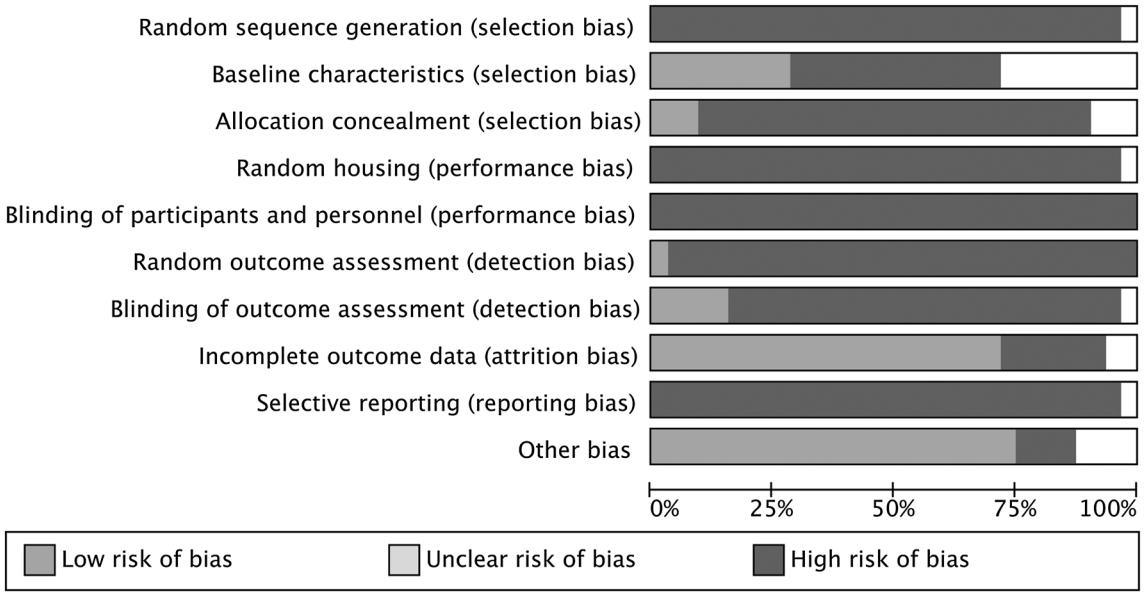

Fig. 4 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

composition, grain size and percent porosity of the scaffolds used [54, 55].

Several researchers discovered that the original definition of CSD is not truly functional and suggested that the critical-size defect in animal research should refer to the size of a defect that will not heal throughout the duration of the study and not to the entire life of the animal [56, 57]. It has also been suggested that critical defects cannot only be defined by their size, but should depend on other factors too, including age, species phylogeny and metabolic and systemic conditions [58, 59]. Five studies were included in the subgroup analysis considering only non-critical bone defects [21, 23, 24, 29, 43]. Meta-analysis of data derived from these studies showed that the use of a bone graft did not improve the healing process, and spontaneous regeneration occurred among the defects evaluated. The latter proves that the injured bone is able to recover itself from small bone defects. The natural healing of bone defects resembles bone formation during organogenesis. After bone injury, an inflammatory response occurs and

extravascular blood cells form a blood clot. This initial response involves the secretion of growth factors which recruit inflammatory cells and promote angiogenesis. After early immune reaction, a soft callus around the injury site is formed leading to a disorganized structure termed woven bone. In later phases of bone regeneration, the woven bone is gradually replaced by a highly organized lamellar bone. Eventually, woven bone and fracture callus are replaced by lamellar or trabecular bone produced by osteoblasts [60, 61].

In general, studies with non-critical size defects show that, during the initial follow-up, the progression of bone growth in the control defects occurs more quickly than in the defects filled with hydroxyapatite. The above corroborates few reports that have described an initial negative cellular response in the presence of calcium orthophosphate ceramics in which low proliferation of osteoblastic cells did not enhance in vitro osteogenesis due to insufficient activation of signaling that forced the cell cycle to progress [62–64]. Since subsequent events leads towards a complete healing, there are indications that the influence of calcium orthophosphate materials (similar to HA) on bone repair may also dependent on other factors, such as animal species, anatomic defect location, fracture stabilization, associated soft tissue and biomechanical conditions, as well as metabolic and systemic conditions, and morbidities affecting defect healing [65].

Since the exact mechanisms of osteoinduction of bone graft materials are still unclear, it is important to mention that the use of small defects in studies with bone substitutes is also extremely relevant when the primary objective refers to the evaluation of tissue response and not merely to the ability to improve regeneration. Other methods are extant to evaluate the growth of bone in bony defects, such as microtomography or x-ray. The histological approach to evaluate the biological performance of bone grafts continues to be very important to report on the qualitative and qualitative fracture repair process [66]. Unfortunately, for ethical reasons, the histological approach is not allowed on humans and different animal models should be used.

Although this is the best currently available evidence demonstrating that hydroxyapatite in critical bone defects is beneficial, only animal studies have been analyzed and the strength of the clinical inference is not strong. Besides, results should be interpreted with caution since animal studies revealed a high risk of bias. The studies also showed heterogeneity concerning the type of hydroxyapatite used and to treatment protocol, which precluded direct comparison. Additionally, the majority of the selected studies investigated calvarial defects, notwithstanding, in humans, bone defects affect more long bones or mandible. It should be noted that clinical application in animal models represents the final step before clinical application in humans, and the results from these studies have provided insights for translational research. Further, the quality of the included studies emphasized the need for further well-designed, randomized and controlled animal studies to highlight the benefits in the employment of hydroxyapatite in bone defects. Factors such as random sequence generation, sample size calculation, blinding outcome assessment, and use of different evaluation methods may improve the quality of more in-depth studies in this research field. Although only a few included studies were published after the publication of Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [67], the compliance to standards in reports on animal studies may improve their quality and facilitate the comparison between different treatments in future systematic reviews.

Evidence found in current systematic review supports the use of pure HA bone grafts, natural or synthetic, to enhance adequate bone healing in critical and non-critical sized defects. Regardless of the form (block, granules or particles), it seems that ceramic calcium orthophosphate ceramic favors bone regeneration in several clinical conditions. In the current review, the analysis of isolated HA without any growth factor or stem cells demonstrated the performance of the substrate as an osteoconductive material which may be an ideal material for scaffolds in future tissue-engineering approaches.

Finally, significant challenges are still extant in bone tissue repair and regeneration. As far as is known, no ideal bone substitute has been developed. The best combination between micro and macro characteristics to achieve adequate osteoconductive properties, coupled to a simple process for manufacturing, are still required. Further, large bone defects still represent a major challenge and future research will have to focus on the development of bone grafts and biomimetics with the appropriate controlled release of osteogenic factors. Although the above are significant challenges, it is becoming evident that the successful development of materials in this research field has long-lasting benefits that surpass potential risks.

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Authors' Contributions HLO, WLOR and EP designed the study. HLO and CECS prepared the first draft of the paper. NLVC, AFS, TNG and OAD contributed to the experimental work. WLOR was responsible for statistical analysis of the data. HLO, CECS, WLOR and EP prepared the final draft of the manuscript. All authors revised the paper critically for intellectual content and approved the final version. All authors agree to be accountable for the work and to ensure that any questions relating to the accuracy and integrity of the paper are investigated and properly resolved.

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Compliance with Ethical Standards

Conflict of interest Authors Héllen L. Oliveira, Wellington L. O. Rosa, Carlos E. Cuevas-Suárez, Neftali L. V. Carreño, Adriana F. Silva, Thomas N. Guim, Odir A. Dellagostin and Evandro Piva declare that they have no conflict of interest.

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