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Biomaterials in Apical Microsurgery: Beta-TCP/Hydroxyapatite and Bovine Xenograft

André Filipe da Silva Gonçalves

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

Gandra, 26 de setembro de 2020

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Trabalho realizado sob a Orientação do Prof. Doutor Paulo Miller e Coorientação do Prof. Doutor António Ferraz

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AGRADECIMENTOS

Um enorme agradecimento aos meus pais, por todo o apoio, esforço e sacrifício incondicional que fizeram por mim, desde sempre. Pela minha felicidade e por uma melhor formação. Agradeço, a confiança que depositaram em mim, sem nunca duvidarem das minhas capacidades. O meu profundo obrigado.

À minha Luciana, um agradecimento especial. Obrigado por tudo. Por mais uma caminhada que finalizamos juntos. Por me ter dado a mão, com toda a força, nos momentos em que mais precisei. Obrigado pela demonstração de pura genuinidade e resiliência. Pela paciência, toda a ajuda e motivação. Pelos ensinamentos, experiências e toda a transmissão de confiança. Pelos momentos incríveis e felizes que passamos e por todas as aventuras que aí vêm. O meu profundo obrigado.

Agradeço ao meu irmão, António, pelo seu companheirismo e vontade em aprender e evoluir. Pela vontade em me tornar o seu exemplo. O meu obrigado por todos os momentos tão bem passados.

Agradeço aos meus avós, por todos os ensinamentos, que certamente levarei para toda a vida.

Agradeço ao meu binómio João, leal e companheiro, pela grande amizade desenvolvida ao longo destes 5 anos, que será levada para toda a vida. Por todas as trocas de ideias e entreajuda, ao longo desta jornada.

Agradeço aos meus amigos, por todos os grandes momentos que passamos juntos, pelas gargalhadas, aventuras e grande convivência.

Agradeço ao meu Orientador, Prof. Doutor Paulo Miller, por todo o apoio depositado ao longo de toda minha formação universitária. Pela disponibilidade e paciência, pela vontade em ajudar e pela preocupação. O meu enorme obrigado.

E por fim, agradeço ao meu Coorientador, Prof. Doutor António Ferraz, pela sua forma de ensinar e motivar, pela sua pessoa, sempre com um sorriso, pronto a ajudar.

RESUMO

Introdução: As grandes lesões periapicais constituem um desafio no procedimento cirúrgico devido à extensa reabsorção de tecido ósseo, que prolonga, significativamente, o tempo da recuperação óssea. Assim sendo, para além dos biomateriais de origem autóloga, a utilização de biomateriais de origem sintética e animal na microcirurgia apical, como o Beta-TCP/Hidroxiapatita e o xenoenxerto bovino, pode acelerar o processo de regeneração tecidular.

Objetivo: O objetivo deste estudo foi estabelecer uma comparação entre o Beta-TCP/Hidroxiapatita com o Xenoenxerto Bovino, utilizados em microcirurgia apical, a fim de determinar qual biomaterial apresenta melhores resultados na regeneração tecidular a nível apical.

Método: Uma pesquisa bibliográfica foi realizada nas bases de dados PUBMED e EBSCO usando combinações de elementos de pesquisa, a fim de adquirir artigos adequados para este estudo.

Resultados: Foram analisados 8 estudos humanos a nível apical e 13 de comparação dos biomateriais a nível não-periapical. Ambos os materiais demonstraram ser capazes de proporcionar uma regeneração tecidular completa a nível apical. Os ratios de 30%HA/70%β-TCP e de 10%HA/90%β-TCP apresentaram melhores resultados, comparativamente ao xenoenxerto bovino, na formação óssea.

Conclusões: Tanto o xenoenxerto bovino como o HA/β-TCP são potenciais escolhas para aplicação na área da lesão apical durante o procedimento de microcirurgia apical, dado que promovem uma completa regeneração tecidular. Um ratio de HA/β-TCP com níveis diminuídos de HA e níveis mais elevados de β-TCP parece ser o que apresenta melhores resultados, sendo talvez a melhor opção entre os dois tipos de biomateriais.

Palavras-Chave: *"Regeneração Tecidular"; "Lesão Periapical"; "Microcirurgia Apical"; "Xenoenxerto Bovino"; "Beta-TCP/Hidroxiapatita".*

ABSTRACT

Introduction: The large periapical lesions are a challenge in the surgical procedure due to the extensive resorption of bone tissue, which significantly prolongs the time required for bone recovery. Therefore, in addition to biomaterials of autologous origin, the usage of biomaterials from synthetic and animal sources in apical microsurgery, such as Beta-TCP/Hydroxyapatite and bovine xenograft, can accelerate the tissue regeneration process.

Aim: The aim of this study was to establish a comparison between Beta-TCP/Hydroxyapatite with Bovine Xenograft, used in apical microsurgery, in order to determine which biomaterial presents better outcomes in tissue regeneration at the apical level.

Method: A literature search was performed on the PUBMED's and EBSCO's databases using combinations of search terms in order to acquire suitable articles for this study.

Results: 8 human studies at the apical level and 13 studies comparing the biomaterials at the non-periapical level were analysed. Both biomaterials demonstrated to be capable of providing a complete tissue regeneration at the apical level. The ratios of 30%HA/70%β-TCP and 10%HA/90%β-TCP showed better outcomes, compared to bovine xenograft, in bone formation.

Conclusions: Both bovine xenograft and HA/β-TCP are potential choices for application in the apical lesion area during the apical microsurgery procedure, by advancing both a complete tissue regeneration. An HA/β-TCP ratio with diminished levels of HA and higher levels of β-TCP seems to be the one that advances the best outcomes, being perhaps the best option between the two types of biomaterials.

Keywords: *"Tissue Regeneration"; "Periapical Lesion"; "Apical Microsurgery"; "Bovine Xenograft"; "Beta-TCP/Hydroxyapatite".*

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TABLE OF CONTENTS:

LIST OF ABBREVIATIONS:

GTR: Guided Tissue Regeneration

DBBM: Demineralized Bovine Bone Mineral

Beta-TCP/Hydroxyapatite: Beta Tricalcium Phosphate associated with Hydroxyapatite

HA/β-TCP: Beta Tricalcium Phosphate associated with Hydroxyapatite

β-TCP: Beta Tricalcium Phosphate

HA: Hydroxyapatite

BHA: Bovine Derived-Hydroxyapatite

BCP: Biphasic Calcium Phosphate

TCP: Tricalcium Phosphate

VEGF: Vascular Endothelial Growth Factor

HO-1: Heme Oxygenase-1

1. INTRODUCTION

The surgery procedure might be indicated for insistent lesions when the non-surgical approaches failed (1–4). Apical microsurgery is performed in order to eradicate the causative agents of radicular and periradicular lesions and to achieve a reestablishment of the tissues and, consequently, total healing. After the correct curettage of the apical lesion, the defects can be filled with grafts to improve bone and soft tissue regeneration (tissue regeneration) (1,3). Hence, the introduction of Guided Tissue Regeneration (GTR) aligned with the apical microsurgery became a standard treatment for periapical lesions, where a new treatment choice has appeared for such deformities (4–7): the placement of a mechanical barrier besides the graft placement (5,6). However, the efficacy of the endodontic microsurgery depends on the choice of the graft, the healthy quality of the patients, the previous root canal treatment or retreatment that will influence the overall success of the endodontic microsurgery (5,6).

Bone graft placement is an important procedure in the treatment of bone defects and should provide biological compactness, ensure volume maintenance, preserve the coagulum formation and promote angiogenesis, inciting a high rate of newly formed bone and the remodeling process (8–10). The optimal graft material should demonstrate biocompatibility, therefore not trigger immunogenic responses and shouldn't present any risk of disease transmission (2,11). Furthermore, the hard tissue substitute should be reabsorbed while it is being replaced by new bone, thus it's essential to obtain a balance between the rates of newly formed bone and the biomaterials resorption mechanism (9).

There are four types of bone graft materials as the autogenous bone graft, considered the gold standard of the bone grafts, the allografts, the xenografts (from equine, bovine or porcine origin) and the alloplastic bone grafts (calcium phosphate ceramics, polymers and bioactive glasses) (1,9). Despite the excellent osteoinductive and osteoconductive properties of autografts, being capable to promote osteogenesis (9,12), the usage of this type of graft material is related with the necessity of a secondary surgery (1). In addition, limited graft availability, rapid graft resorption, increasement of morbidity and post-operative complications are also some disadvantages of the autogenous material (1,8,9,12).

In order to overcome these disadvantages, the calcium phosphate materials and the bovine xenografts are being used in the endodontic microsurgery (1,9). The bovine xenografts, as Demineralized Bovine Bone Mineral (DBBM), are commonly used due to its great mechanical and osteoconductive properties, acting as a space maintainer for bone growth, and present low resorption rate (8,9,12,13). The Beta Tricalcium Phosphate associated with Hydroxyapatite (Beta-TCP/Hydroxyapatite or HA/β-TCP) is composed by Beta Tricalcium Phosphate (β-TCP) and Hydroxyapatite (HA) (1,2). This association promotes a better control of the high resorption of the β-TCP component by the presence of HA in the material, maintaining the great properties of both components and expedited new bone formation (1,2). This synthetic biomaterial is a cost-effective biomaterial that doesn't present any risk of diseases transmission and is devoid of local or systemic toxicity, in contrast with the bovine xenograft maybe due to its bovine origin (9,14,15).

Therefore, the main aim of this study was to establish a comparison between Beta-TCP/Hydroxyapatite with Bovine Xenograft, used in apical microsurgery, in order to determine which material presents better outcomes in tissue regeneration at the apical level. It was hypothesized that, in endodontic apical microsurgery, the use of Beta-TCP/Hydroxyapatite promotes better outcomes in tissue regeneration than the use of Bovine Xenograft.

2. METHOD

A literature search was performed on PUBMED (via National Library of Medicine) as well as on EBSCO until 4th February of 2020 using the following combination of search terms: “tissue regeneration” AND “periapical lesion”; “tissue regeneration” AND “endodontic apical microsurgery”; “tissue regeneration” AND “bovine xenograft”; “tissue regeneration” AND “Beta TCP/Hydroxyapatite”; “periapical lesion” AND “endodontic apical microsurgery”; “bovine xenograft” AND “Beta TCP/Hydroxyapatite”; “bovine xenograft” AND “Beta TCP/Hydroxyapatite” AND “tissue regeneration”; “periapical lesion” AND “tissue regeneration” AND “apical microsurgery”; “beta tcp ha” AND “bovine xenograft”; “beta tcp/hydroxyapatite” AND “biomaterial” AND “apical surgery”; “bovine xenograft” AND “biomaterial” AND “apical surgery”; “Beta TCP/Hydroxyapatite” AND “biomaterial” AND “apical surgery”; “beta TCP/Hydroxyapatite) AND endodontics”; “endodontics” AND “bovine xenograft”; “beta TCP/Hydroxyapatite” AND “biomaterial” AND “healing process”; “bovine xenograft” AND “biomaterial” AND “healing process”; “endodontic microsurgery” AND “biomaterials”; “beta TCP/Hydroxyapatite” AND “bone recovery”; “bovine xenograft” AND “bone recovery”, “bovine xenograft” AND “apical surgery”. Also, a manual search was performed considering the references within the selected articles. The inclusion criteria involved articles published in the English language since 2000 up to February 2020; bibliographic reviews; randomized controlled trials, clinical human studies; in vivo studies, referring to Beta-TCP/Hydroxyapatite's and Bovine Xenograft's properties and their potential in the apical tissue regeneration, and articles that compared specifically both biomaterials on the regenerative mechanisms. Articles that the outcomes were supported by experiences in rats were the exclusion criteria. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager.

In a preliminary phase, three of the authors (P.M.; A.F.; A.G.), performed a pre-selection of potentially relevant articles, analysing the titles and abstracts of each article. Of 144 pre-selectionated articles, 24 were selected and individually read and evaluated concerning the purpose of this study. The following factors were retrieved for this review: authors' names, study design, publication year, purpose of the study, study population, Bovine Xenograft and Beta TCP/Hydroxyapatite as a mean to new bone formation and complete healing, biomaterials properties and the ratio of β-TCP/HA.

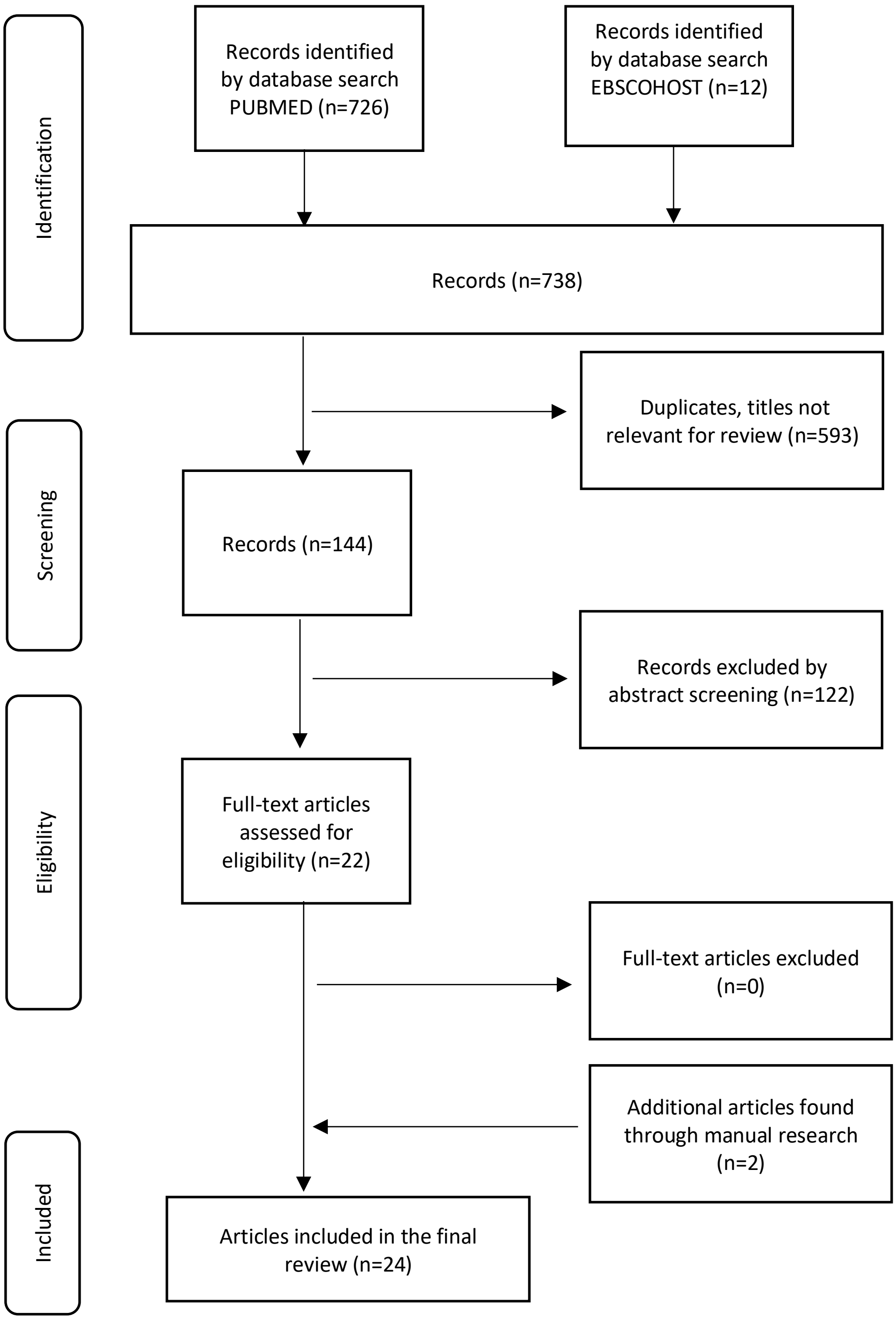

Figure 1 – Flow diagram of the search strategy used in this study

3. RESULTS

In this integrative systematic review, 8 human articles (38,1%) were analysed to establish which discussed biomaterial has the best results at regenerative process level for application in apical sites during endodontic microsurgery, according to the all parameters enumerated below. However, due to the lack of comparison studies with both materials in this referred specific area, it was necessary to evaluate 13 comparison studies at the non-periapical level (61,9%) in order to have a better and accurate final interpretation of the 8 referred human studies.

Out of the studies about the biomaterials at apical surface: two (25%) demonstrated HA/β-TCP as a component to be applied in the required situations (1,2); and six (75%) demonstrated the use of bovine xenograft in this specific area (4–7,16,17). As bovine bone graft material was used: DBBM (4–7,16,17). As alloplastic biomaterial was used a 55%HA/45%β-TCP ratio in one study (1). However, in the other study the percentage of each component was not disclosed (2). All these studies used apical microsurgery as a mean and way to help achieve the success in each case.

In the total investigations at apical surface, three studies (37,5%) had as population more than one person for the specific procedure (1,6,7) and the remaining articles (62,5%) had as population only one patient (2,4,5,16,17). Six studies (75%) were case reports (1,2,4,5,16,17) and the other two (25%) were human clinical trials (6,7). Just one study (12,5%) reffered the realization of a randomized clinical experiment and the presence of a control group to establish comparasion (6).

Among the 8 articles, four (50%) were related to periapical lesions strictly from endodontic provenance (1–3,6) (one was a case of sinus without healing (2)) and in the other four (50%) the procedures were established for endodontic-periodontic lesions (4,5,7,8). In the total of the studies, three (37,5%) demonstrated other materials associated to the evaluated biomaterials (2,5,17) which could indeed influence the finals outcomes.

Overall, 71 patiens encompassed the specific procedures and there was an existence of 90 lesions (100%). From this total, in 51 (56,7%) were lesions where the bovine xenograft material was placed and only in 4 (4,4%) occurred the placement of the alloplast biomaterial, during the microapical surgery procedure (Table 1).

Relatively to the comparison articles at the non-periapical sites: five evaluated the biomaterials approach to the sinus floor augmentation (8,9,14,15,18) and the left investigations encompass themes such as alveolar ridge preservation, wall-bone and intrabony defects, bone level after extractions sockets which the materials were tested, demonstrating their contribution and qualities in the assessed processes (10,12,13,19–23). Two of these studies (15,4% of the 13 articles) compared not the bovine xenograft with the HA/β-TCP, but the xenograft with β-TCP, in order to interpret the efficiency of this last material without association to HA (18,22). Two types of bovine xenograft were used: DDBM(8–10,12–15,19–23) and Bovine Derived-Hydroxyapatite (BHA) (18). There were six *In vivo* studies (10,12,15,19,20,22) and the following seven were humans studies (8,9,13,14,18,21,23). Relatively to the *In vivo* studies: two had mini-pigs as the study population (15,19) and four articles had dogs as its population (10,12,20,22).

	Bovine Xenograft	HA/β-TCP	Control	
Suneelkumar C et al.		1 (+2, from two other patients with only radiographs)		3 lesions (3 patients)
Taschieri S et al (2007)	24 defects		35 defects	59 defects (41 patients)
Dietrich T et al.	23 defects			23 defects (22 patients)
Das AN et al		1 lesion		1 lesion (1 patient)
Komabayashi T et al.	1 lesion			1 lesion (1 patient)
Kourkouta S et al.	1 lesion			1 lesion (1 patient)
Ghezzi C et al.	1 lesion			1 lesion (1 patient)
Taschieri S et al (2012)	1 lesion			1 lesion (1 patient)
	51 lesions	**4 lesions**		**Total of lesions = 90** **Total of patients = 71**

Table 1 – Total of lesions and total of patients of each periapical study.

Author (Year)	Title	Study Design	Aim	Study Population	Bone formation		Properties		Ratio of β-TCP/HA	Healing
					Bovine Xenograft	β-TCP/HA	Bovine Xenograft	β-TCP/HA		
Suneelkumar C et al. (2011)	"Biphasic calcium phosphate in periapical surgery"	Case report	To demonstrate the clinical use of biphasic calcium phosphate as a bone substitute in periapical surgery.	n=1 woman (+2) * *more two cases with just the follow-ups radiographs	—	Increasement and replacement of newly formed bone	—	Decrease of alloplastic material´s density; β-TCP/HA improves cell proliferation, osteogenesis and revascularization.	55%HA/ 45%β-TCP	Complete tissue regeneration; presence of new soft tissue; healing achieved in a large periapical lesion
Das AN et al (2015)	"Interdisciplinary Approach to a Tooth with Open Apex and Persistent Sinus"	Case report	To treat an open apex and persistent sinus with the use of MTA, PRF and β-TCP/HA.	n=20-year-old male	—	—	—	Biocompatibility of the biomaterial.	—	Complete tissue regeneration; presence of new soft tissue; healing achieved at apical site and sinus closure; at four weeks postoperative the regenerative process was complete
Kourkouta S et al. (2014)	"Periradicular Regenerative Surgery in a Maxillary Central Incisor: 7-year Results Including Cone-beam Computed Tomography"	Case report	To treat a patient with persistent symptoms in 21 tooth.	n= 52-year-old female	New bone formation at apically, interproximally and facially sites of the root.	—	Osteoblast activity; DBBM provides stability; osteoconductivity; higher density in new bone than in the adjacent bone	—	—	Presence of new soft tissue; complete tissue regeneration (100% of healing) at apical site; no medical prescription was needed
Taschieri S et al. (2012)	"Treatment of through-and-through bone lesion using autologous growth factors and xenogeneic bone graft: a case report"	Case report	To evaluate the possibility of achieving optimal hard and soft tissue regeneration PRGF and anorganic bovine bone (ABB) for the surgical treatment of a large through-and-through periapical bone lesion.	n=28-year-old man	Positive improvements in new bone gaining	—	—	—	—	Healing of soft tissue was in 1 week; in 1 year it was seen a complete tissue regeneration (100% of healing); absence of any symptoms (pain and swelling) after the endodontic microsurgery and during respectively healing time;
Taschieri S et al. (2007)	"Efficacy of Xenogeneic Bone Grafting With Guided Tissue Regeneration in the Management of Bone Defects After Surgical Endodontics"	Study (human clinical trial)	"The purpose of this prospective clinical trial was to monitor the outcomes of periradicular surgery in large periapical lesions with or without GTR and	n=44 (63 teeth)	—	—	—	—	—	83,3% of the lesions achieved a successfully healing, independently the size of the lesion; there were no significant statistically differences between the bovine xenograft

			anorganic bovine bone.”							group and the empty cavity group;
Dietrich T et al. **(2003)** **(manual research)**	“Periapical and periodontal healing after osseous grafting and guided tissue regeneration treatment of apicomarginal defects in periradicular surgery: Results after 12 months”	Study (human clinical trial)	The aim of the present study was to evaluate the periapical and periodontal healing of apicomarginal defects 12 months after periradicular surgery and guided tissue regeneration in a series of consecutively treated patients.	n= 22 patients (23 defects)	Presence of newly formed bone (median probing depths from 9.0mm to 3.0mm)	—	—	—	—	83% of the apical lesions achieved a successfully healing with the usage of DBBM; presence of new soft tissue (median relative attachment level gain of 2.8 mm); prescription of medication and absence of postoperative symptoms
Komabayashi T et al. (2011)	“Apical infection spreading to adjacent teeth: a case report”	Case report	To treat a patient that described an apical infection that spread to adjacent teeth.	n= 25-year-old female	—	—	DDBM is a space maintainer; promotes osteoconductivity.	—	—	The outcome was excellent, and this material facilitated the healing of the large apical lesion; 100% of healing (complete tissue regeneration); presence of new soft tissue; prescription of medication; absence of postoperative symptoms
Ghezzi C et al. (2012)	“Treatment of Comined Endodontic-Periodontic Lesions Using Guided Tissue Regeneration: Clinical Case and Histology”	Case report	To evaluate at histological level the periapical healing after combined endodontic-periodontic treatment.	n= 38-year-old man	Formation of cementum, ligament, new bone around the apex of the treated tooth.	—	Presence of osteoblast activity	—	—	Gain of new soft tissue (decrease of attachment level); Complete tissue regeneration (100% of healing); prescription of medication after the apical surgery and absence of postoperative symptoms
La Monaca G et al. (2018)	“Comparative Histological and Histomorphometry Results of Six Biomaterials Used in Two-Stage Maxillary Sinus Augmentation Model after 6-Month Healing”	Study	To evaluate the performances of six different bone substitute materials used as graft in maxillary sinus augmentation by means of histological and histomorphometry analysis of bone biopsies retrieved from human subjects after 6 months healing period.	n= 6 (3 males and 3 females, aged 50-72 years)	Newly formed bone: 16.1%; residual bone graft: 37,2% (no significant statistical differences with the HA/β-TCP group);	Newly formed bone: 20,3%; residual bone graft: 37,9% (no significant statistical differences with the DBBM-bovine xenograft group);	Biocompatible; osteoconductive	Biocompatible; osteoconductive; + resorption process	30%HA/ 70%β-TCP	The healing was successfully achieved with both biomaterials and there was an absence of postoperative symptoms for both biomaterials’ groups

Oh J et al. (2018)	"A Comparative Study with Biphasic Calcium Phosphate to Deproteinized Bovine Bone in Maxillary Sinus Augmentation: A Prospective Randomized and Controlled Clinical Trial"	Study	"To evaluate a new graft material, biphasic calcium phosphate, composed of 60% hydroxyapatite and 40% β-Tricalcium phosphate, and deproteinized bovine bone mineral, which is established as a predictable graft material for maxillary sinus augmentation."	n=60 patients (31 with BCP; 29 with DDBM)	Newly formed bone: 25,13% (no significant statistical differences with HA/β-TCP group); new bone volume level: 31,06% (no significant statistical differences between the groups); residual bone graft: 32,19% (no significant statistical differences between the groups)	Newly formed bone: 28,84% (no significant statistical differences with DBBM group); new bone volume level: 35,21% (no significant statistical differences between the groups); residual bone graft: 26,99% (no significant statistical differences between the groups)	Biocompatible; osteoconductive	Biocompatible; osteoconductive; + resorption process	60% HA/40% β-TCP	The healing was successfully achieved with both biomaterials and there was an absence of postoperative symptoms for both biomaterials' groups
Lindgren C et al. (2010)	"Back-scattered electron imaging and elemental analysis of retrieved bone tissue following sinus augmentation with deproteinized bovine bone or biphasic calcium phosphate"	Study	To compare the resorption of a synthetic biphasic calcium phosphate (BCP) bone graft substitute with deproteinized bovine bone (DBB) used for human maxillary sinus augmentation.	n=11 patients	There were no significant statistical differences in new bone formation between both biomaterials´ groups; residual bone graft: DBBM> HA/β-TCP	There were no significant statistical differences in new bone formation between both biomaterials' groups; residual bone graft: HA/β-TCP< DBBM	—	+ resorption process	60% HA/40% β-TCP	The complete tissue regeneration was achieved with both biomaterials
Mardas N et al. (2011)	"Radiographic alveolar bone changes following ridge preservation with two different biomaterials"	Study	To evaluate radiographical bone changes following alveolar ridge preservation with a synthetic bone substitute or a bovine xenograft.	n=27 patients	Similar results in new bone formation between the groups; in the DBBM group, the Mh and Dh showed a mean difference of 0.4 ±1.3 and 0.7 ± 1.3mm, among BL-8M.	Similar results in new bone formation between the groups; in the HA/TCP group, the Mh and Dh showed a mean difference of 0.9± 1.2 and 0.7 ± 1.8mm, respectively, among BL-8M.	—	—	60% HA/40% β-TCP	A complete healing was achieved; Both bovine xenograft and HA/TCP will equally preserve radiographic bone levels up to 8 months following the grafting of the sockets.
Mardas N et al. (2010)	"Alveolar ridge preservation with guided bone regeneration and a synthetic bone substitute or a bovine-derived xenograft: a randomized, controlled clinical trial"	Study	To compare the potential of a synthetic bone substitute or a bovine-derived xenograft combined with a collagen membrane to preserve the alveolar ridge dimensions following tooth extraction.	n=27 patients	The material preserved the mesio-distal bone height of the ridge; no differences in the width of buccal and palatal bone plate between the groups	The material preserved the mesio-distal bone height of the ridge; there were no significant statistical differences between the groups at the new bone formation	Absence of resorption process	Absence of resorption process	60% HA/40% β-TCP	Postoperative symptoms as edema and pain; presence of new soft tissue in both biomaterials' groups; A complete healing was achieved successfully.
Kurkcu M et al. (2012)	"Anorganic Bovine-Derived Hydroxyapatite vs b-Tricalcium Phosphate in Sinus Augmentation: A Comparative	Study	To compare the biological performance of the new BHA graft material and the well-known	n= 23 patients (12 man and 11 woman)	The mean new bone formation was 30.13% in the BHA group. The mean percentage of residual graft particle area was 31.88% in the BHA group.	New bone formation was 21.09% in the β-TCP group; percentage of residual graft particle area was 34.05% in the β-TCP group.	Biocompatible; + osteoconductive	Biocompatible; - osteoconductive	β-TCP alone	Complete healing in both biomaterials´ groups; Higher percentage of new soft tissue in β-TCP group; absence of any

	Histomorphometric Study”		synthetic b-TCP material in the sinus augmentation procedure.		Higher percentage of newly formed bone in BHA group than in β-TCP group. Higher residual bone graft level in BHA group than in β-TCP group	Less percentage of newly formed bone in β-TCP group than in BHA group				postoperative symptoms
Zafiropoulos GK et al. (2007)	“Treatment of Intrabony Defects Using Guided Tissue Regeneration and Autogenous Spongiosa Alone or Combined with Hydroxyapatite/b-Tricalcium Phosphate Bone Substitute or Bovine-Derived Xenograft”	Study	To investigate the clinical regeneration of deep intrabony defects using GTR with autogenous spongiosa (ASB) alone or using GTR with a mixture of ASB with a bovine-derived xenograft (BDX) or a synthetic composite bone substitute (hydroxyapatite/b-tricalcium phosphate [HA/b-TCP]).	n= 64 patients	Similar results at new bone gain level with both biomaterials	Similar results at new bone gain level with both biomaterials	———	———	60% HA/40% β-TCP	Presence of new soft tissue in both biomaterials´ groups (no significant statistical differences between the groups); Complete tissue regeneration was achieved with both materials; absence of postoperative symptoms
Ho K et al. (2016)	“A Novel HA/β-TCP-Collagen Composite Enhanced New Bone Formation for Dental Extraction Socket Preservation in Beagle Dogs”	In vivo Study	To compare the performance of a novel bioresorbable purified fibrillar collagen and hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) versus collagen alone and a bovine xenograft-collagen composite in beagles.	n= 20 male beagle dogs	New bone formation at 8 weeks: 42.68% (36.94±8.68% in the collagen plug group, control group presented 40.43%±3.26%.) Presence of remodelling process	New bone formation at 8 weeks: 56.10% HA/β-TCP > bovine xenograft at new bone formation level Presence of remodelling process	Presence of resorption process; osteoconductive; + cell vitality	Presence of resorption; HA/β-TCP+ purified fibrillar collagen-biomimetic properties; osteoconductive	30% HA/70% β-TCP	The application of HA/β-TCP and DBBM was successfully achieved
Di Raimondo R et al. (2019)	“Alveolar crest contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation”	In vivo Study	To evaluate the changes in alveolar contour after guided bone regeneration (GBR) with two different combinations of biomaterials in dehiscence defects around implants.	n= 8 beagle dogs	No significant statistical differences between the HA/β-TCP and DBBM groups at new bone formation level	No significant statistical differences between the HA/β-TCP and DBBM groups at new bone formation level	———	———	60% HA/ 40% β-TCP	The procedures went well and the application of HA/β-TCP and DBBM was successfully achieved
Vahabi S et al. (2011)	“A Comparison between the Efficacy of Bio-Oss, Hydroxyapatite Tricalcium Phosphate and Combination of Mesenchymal Stem Cells in Inducing Bone Regeneration”	In vivo Study	To compare the quality and quantity of regenerated bone using Bio-Oss, HA/TCP and MSC loaded HA/TCP scaffolds.	n= 5 hybrid dogs	New bone formation: empty cavity- 60.80%; **DBBM-40.60%**; HA/TCP+MSCs-46.38%. No significant statistical differences between HA/TCP and DBBM groups at new bone formation; Residual bone graft: 16,46% (no significant	New bone formation: **HA/TCP-44.93%;** Residual bone graft: 14,87% (no significant statistical differences between the groups)	Osteoblastic activity; presence of giant cells	———	Non-referred	The application of HA/β-TCP and DBBM was successfully achieved; absence of postoperative complications

					statistical differences between the groups)					
Sato N et al. (2020)	"Comparison of the vertical bone defect healing abilities of carbonate apatite, β-tricalcium phosphate, hydroxyapatite and bovine-derived heterogeneous bone"	In Vivo Study	To examine the bone regeneration abilities of various bone substitutes including Cytrans, Cerasorb, Neobone and Bio-Oss in a 3-wall bone defect animal model.	n= 16 healthy adults male beagle dogs	- new bone formation than β-TCP; presence of remodelling process; similarity in bone volume for both biomaterials´ groups; presence of neo-angiogenesis	+ new bone formation than DBBM; presence of remodelling process; similarity in bone volume for both biomaterials´ groups; presence of neo-angiogenesis	- resorption process	-----------	β-TCP alone	Presence of soft tissue (new collagen fibre) in the affected sites at 4 and 8 weeks postoperative for both biomaterials´ groups; the procedures went well and the application of HA/β-TCP and DBBM was successfully achieved; absence of any postoperative omplications
Hung C et al. (2019)	"Bone formation following sinus grafting with an alloplastic biphasic calcium phosphate in Lanyu Taiwanese mini-pigs"	In vivo Study	To evaluate the new bone formation after grafting with a synthetic biphasic calcium phosphate in sinuses with minimal bone height and comparing with xenografts materials into Lanyu Taiwanese mini-pig sinuses via split-mouth design.	n= 6 mini-pigs	New bone formation: at 4 weeks was worst for DBBM than for HA/TCP; at 8 weeks was greater for DBBM than for HA/TCP – Similar performance of both biomaterials in new bone formation; no significant statistical differences between both biomaterials in new bone volume and residual bone graft; presence of remodelling process	New bone formation: at 4 weeks was greater for HA/TCP than for DBBM; at 8 weeks was worst for HA/TCP than for DBBM — Similar performance of both biomaterials in new bone formation; no significant statistical differences between both biomaterials in new bone volume and residual bone graft; presence of remodelling process	Similarity at bone density level	Similarity at bone density level	60% HA/ 40% β-TCP	HA/ β-TCP had similar effectiveness as DBBM for the regenerative process, in both materials´ groups it was achieved a compete healing; presence of new soft tissue in both biomaterials´ groups (no significant statistical differences between the groups)
Dahlin C et al. (2014)	"Bone tissue modelling and remodelling following guided bone regeneration in combination with biphasic calcium phosphate materials presenting different micro porosity"	In Vivo Study	To investigate bone regeneration through the application of a novel biphasic calcium phosphate (90%β-TCP/10%HA) compared to 40% β-TCP/60%HA and to DDBM in the mandible of minipigs with an approach with GBR.	n=16 mini-pigs	DDBM demonstrated more bone formation than 40% β-TCP/60%HA at 8 weeks; DDBM demonstrated less bone formation than 90% β-TCP/10%HA at 8 weeks; DBBM and the other 60%HA/40% β-TCP presented similarity in bone formation no significant statistical differences between both biomaterials in new bone volume; presence of remodelling process	90%β-TCP/10%HA demonstrated more bone formation than 40% β-TCP/60%HA at 8 weeks; 90%β-TCP/10%HA demonstrated more bone formation than DBBM at 8 weeks; DBBM and the other 60%HA/40% β-TCP presented similarity in bone formation; better bone fill with 90%β-TCP/10%HA; presence of remodelling process	- resorption process of DBBM; similarity in bone density level	+ resorption process of 90%β-TCP/10%HA; similarity in bone density level	60%HA/40% β-TCP; 90%β-TCP/10%HA	90%β-TCP/10%HA was the biomaterial that promoted the highest quantity of newly formed bone; in both materials´ groups it was achieved a successfully healing; absence of postoperative complications

3.1- Human studies with bovine xenograft material at apical level:

New bone formation: Four studies (100%) demonstrated that there were positive improvements in bone gaining during the healing period leading to a total bone regeneration at apical sites, after periapical microsurgery (4,5,7,17).

Properties of the material: Two studies mentioned the presence of osteoblast activity (4,17). Two authors referred that the assessed graft material was placed to provide a better stability indeed promoting the osteoconductivity (4,16). One article evaluated the density of the newly formed bone referring a higher density of the new hard tissue rather than the adjacent bone (4).

Healing:

Overall, five studies where the bovine material was placed (80%) referred that healing was successfully achieved when bovine xenograft was used as a complement in periapical surgery indeed leading to an effective regenerative process at apical level, demonstrated during all healing period and respectively follow-ups (4,5,7,16,17) and one study (20%) demonstrated that besides the success of the bovine material as a material to promote the bone healing, there were no significant statistically differences between this material and the empty cavity group (6).

- Two studies didn't reach a 100% of success in all treated lesions, however these articles were the only human clinical trials and indeed there was a minimal percentage of unsuccessful cases (6,7).
- From five authors: all five studies (100%) reported the presence or gain of soft tissue, improving the achievement of total tissue regeneration at apical sites (4,5,7,16,17).
- From five studies: four (80%) indicated complete absence of any symptoms after the procedures and during each follow-up (5,7,16,17). Three studies (60%) mentioned that occurred the prescription of medication after the apical microsurgery procedure (7,16,17); one study (20%) indicated that it wasn't necessary the prescription of medication (4).
- From three studies: relatively to the size of lesions, two studies (66,6%) demonstrated

overall a successfully healing achievement of the defects with the use of bovine xenograft (6,16). In other study (33,3%), the general healing of the defects was achieve and it was revealed that there weren't differences in the final outcome if the defects were involving just one root or those that encompassed more than one root (7).

Both in endodontic-lesion (1,2,6,16) and in endodontic-periodontal lesions (4,5,7,17) the healing was reached.

3.2- Human studies with HA/β-TCP material at periapical level:

New bone formation: One study (100%) mentioned the successfully increasement and replacement of newly formed bone with the HA/β-TCP material placed in the apical cavity (1).

Properties of the biomaterial: One author reported the decrease of the material density during the healing period and mentioned the presence of excellent regenerative properties of HA/β-TCP in promotion of the revascularization, cell proliferation and osteogenesis (1). Another author referred the biocompatibility of the alloplastic material (2).

The influence of the HA/β-TCP ratios: One study reported that the placement of Biphasic Calcium Phosphate (BCP) was with a 55% HA and 45% β-TCP ratio, prepared with the usage of microwave method, and it was shown the replacement of the bone (1).

Healing: From two studies (100%), where occurred the placement of HA/β-TCP material in lesions at apical site (including the healing of sinus (2)), in both (100%), the tissue regenerative process was completely achieved, demonstrated during all healing period and respectively follow-ups, after the periapical microsurgery procedure (1,2).

- Two studies (100%) demonstrated the new soft tissue regeneration when occurred the usage of HA/ β-TCP (1,2). One study reported that at four weeks the tissue regenerative process was complete (2).
- In two articles independently of being a non-sinus healing at the apical site (2) or a large periapical lesion (1) the complete bone regeneration at apical level and soft tissue regeneration was achieved with the use of the alloplastic biomaterial.

Human Studies	Bovine Xenograft	HA/β-TCP	Origin of the lesion	Last Follow-up	% of the healing
Suneelkumar C et al.		✖	Endodontic lesions (Periapical lesion - 15x10mm in the first patient)	1. 2 years 2. 3 months 3. 2 months	100%
Taschieri S et al (2007)	✖		Four-wall defects and through-and-through lesions (Endodontic lesions – at least 10mm of diameter for the defects)	1 year	83,3%
Dietrich T et al.	✖		Endodontic-periodontic lesions (Defects)	1 year	83%
Das AN et al		✖	Endodontic lesion (Non-sinus healing)	1 year (complete healing at 4 weeks postoperative)	100%
Komabayashi T et al.	✖		Endodontic lesion (Periapical lesion - 10x8x4mm)	2 years	100%
Kourkouta S et al.	✖		Endodontic-periodontic lesion	7 years	100%
Ghezzi C et al.	✖		Endodontic-periodontic lesion	2 years	100%
Taschieri S et al (2012)	✖		Endodontic-periodontic lesion (through-and-trhough lesion)	1 year	100%

Table 2 – % of the healing in each periapical study. Reference of the material that was used in each case; origin of the lesion and respectively last follow-up that was clinically performed.

3.3- Comparison studies at the non-periapical level:

3.3.1- Human studies:

New bone formation: Seven studies observed through different assessements the results for newly formed bone (8,9,14,18,21,23)(13). One article (14,3%) had higher percentage of bone regeneration with the alloplastic than with the bovine xenograft (8), but another study (14,3%) demonstrated the opposite (18). In the five other articles (71,4%) there were no significant statistical differences between the groups (9,13,14,21,23).

- One study (100%) referred that there were no significant statistically differences between the materials at bone volume level (9).
- There were four studies that evaluated the residual bone graft (8,9,14,18). Out of these case reports, two (50%) of them had an identical result in this context (8,9). One study (25%) obtained with the xenograft material a higher outcome in residual bone graft compared to HA/ β-TCP (14) and the other study (25%), with the β-TCP alone, occurred the opposite after 6 months and half of healing (18).

Properties of the materials: *Oh et al.* demonstrated even though the similar outcomes of each material, in bovine xenograft group the particles maintained its shape and were installed in the recent hard tissue in spite of the shape and minor size of residual particles in the alloplastic biomaterial (9). In three articles (42,9%) the biphasic calcium phosphate material was referred as a material that mostly develops the resorption process (8,9,14) compared with bovine xenograft material; just one study (14,3%) indicated an absence of resorption in both biomaterials (21). Two studies (28,6%) referred that both materials had similar performances promoting the osteoconductivity and biocompatibility in the sinus floor augmentation procedures (8,9); One study (14,3%) concluded that the bovine material was more efficient in the osteoconductive level when compared with β-TCP alone, and that both biomaterials demonstrated biocompatibility (18).

Ratio of alloplastic material and the different types of bovine xenograft: From the seven studies: one study (14,3%) where the ratio of the alloplastic material was 30% HA and 70% β tricalcium phosphate had better outcomes in new bone formation than DBBM (8); in another study (14,3%), the BHA reached an higher level of new bone than the other biomaterial that was β-TCP alone, without the association to HA (18); The remaining studies (71,4%) had similar outcomes with a ratio of 60% HA and 40% of β-TCP material and with DDBM (9,13,14,21,23).

Healing: In all studies with the tested materials the complete regenerative process was successfully achieved, promoting a complete healing.

- During the healing period there wasn't any postoperative complications for both biomaterials´ groups (8,9,18,21,23), just a presence of slight symptoms as edema or pain (21).
- *Kurkcu et al.* (33,3%) revealed the percentage for the soft tissue and the difference between the groups was statistically significant, with better results with β-TCP (18); one study demonstrated that there were no significant statistically differences between the tested groups relatively to new soft tissue gain (23). Other study (33,3%)

showed the presence of soft tissue in both groups but without any concrete values (21).

3.3.2- In Vivo studies:

3.3.2.1- Dogs Studies:

New bone formation: Two in vivo studies (50%) revealed that alloplastic material in dogs had a better performance compared with bovine xenograft material at new bone formation level (10,22). Two studies (12,20) (50%) demonstrated that there were no significant statistically differences between the xenograft and alloplastic material in relation to the new bone formation.

- One study reported the percentage of the bone volume in the tested materials (22) and it was demonstrated that there were no significant statistically differences between the alloplastic and the bovine xenograft materials (100%).
- Relatively to the residual bone, only one vivo study (100%) mentioned this parameter (20) and the differences between the groups were statistically insignificant.
- Two studies demonstrated the presence of remodelling process in the alloplastic material and in the bovine xenograft (10,22). It was seen in both groups the presence of neo-angiogenesis (22).

Properties of the materials: One article (100%) referred the presence of similar resorption process in alloplastic and bovine xenograft groups (10). One study reported that there was no significant statistically differences between the assessed groups at osteoconductive level; the alloplastic material associated with a purified fibrillar collagen achieved good biomimetic properties (10). *Ho et al*. demonstrated that according to the MTT assay the cell vitality was better in HA/β-TCP group (10). It was shown the osteoblastic activity and the presence of giant cells in the bovine graft group (20). In one study it was referred that bovine xenograft biomaterial didn't present a great resorption process (22).

Ratio of alloplastic material and the different types of bovine xenograft: From the four studies: one (25%) with DBBM as bovine group had worst results in new bone formation than the ratio of 30% HA/70% β-TCP (10); another study (25%) had also worst results for DBBM compared to β-TCP alone (22). The two other studies (50%) performed similar results in new bone formation with DBBM/non-referred alloplastic ratio (20) and with DBBM/a ratio of 60% HA/ 40% β-TCP (12).

Healing: Overall, in each in vivo study the success of the application was achieved well for both groups (100%) (10,12,20,22).

- Just one study referred the presence of soft tissue (22): in DDBM and in HA/β-TCP groups there were a presence of a good quantity of new collagen fibre in the affected sites at four and eight weeks during the healing period (22).
- Two articles referred that during the healing period there wasn't any presence of complications (100%) (20,22) and three studies demonstrated that the procedures and the healing occurred successfully (12,20,22).

3.3.2.2- Pigs Studies:

Dahlin et al. also compared two HA/β-TCP materials with different compositions besides the comparison with the bovine xenograft (19).

New bone formation: One study (50%) demonstrated that the newly formed bone was higher in alloplastic material (10%HA/90%β-TCP) than in the bovine xenograft group (19). However, one study (50%) showed a similar performance of the bovine xenograft group and the alloplastic material group at newly formed bone level (15).

- Two studies (100%) referred that there were no significant statistically differences between the alloplastic and xenograft group in the new bone volume (15,19).
- In one study (100%) there were no significant statistically differences between the groups about the amount of residual bone (15).
- In one study, the bone fill was better in 10%HA/ 90%β-TCP group than in DBBM group, according to the author (19).
- In both groups, the presence of the remodelling process was seen (15,19).

Properties of the materials: One study indicated that the resorption process was present in 10%HA/ 90%β-TCP group in opposite to the bovine group at 8 weeks of follow-up(19). The two articles (100%) affirmed that there were no significant statistically differences between the alloplastic and xenograft group at bone density level (15,19).

Ratio of alloplastic material and the different types of bovine xenograft: One study showed that the highest bone formation was with the 10%HA/ 90%β-TCP; in the same study, DBBM and the other 60%HA/40% β-TCP presented similarity in bone formation at 8 weeks of follow up (19). The other study presented similar outcomes with DBBM and 60% HA/ 40% β-TCP (15).

Healing: The HA/β-TCP and bovine xenograft materials reached complete success of the procedure in each in vivo study (100%) (15,19). It was shown that both presented a similar effectiveness as materials for the regenerative process (15).

- Just one study refereed the presence of soft tissue (15). There was no significant statistically differences between the groups (15).
- One article referred that there weren't any complications during the procedure (19).

4. DISCUSSION

4.1- Endodontic Microsurgery:

In the endodontics' field, the non-surgical treatment is the first choice for a retreatment of previous endodontic treated teeth or in the general cases of periradicular lesions (1,3). However, this approach doesn't present a maximum efficacy in cases of insistent diseases and extensive apical lesions (1,3). Therefore, the surgical procedure is required in case of failure of the non-surgical approaches (1–3). The maintenance of the natural teeth and the success achievement of the case with the apical microsurgery depends on many factors as operator skills, a correct diagnosis and prognosis, techniques application and choice of the grafts that will eventually be placed in the affected site (3,6). Before the placement of biomaterials (bovine xenograft, HA/β-TCP) with the intention to promote a new tissue regeneration, the micro-surgical endodontics procedures such as end-root and sealing are executed to avoid infiltration of the causative microorganisms and the erasing of any foreign body related to the infection as cysts or granulomas is performed with an appropriate curettage (1,3).

4.2- Properties of the evaluated grafts:

The main purpose of using graft materials instead of the autogenous bone is to avert a secondary surgery (1). The epitome of an ideal graft material is combining osteoconductive properties and progressive bioreabsorbability in order to gradually be replaced by newly formed bone (2).

Biphasic calcium phosphate material has been reported to be a biomaterial with a very similar mineral composition to natural hard tissue providing certain regenerative properties (1). The β-TCP associated with HA has been used in apical microsurgery due to the capacity of promoting new bone formation (2) and being an aid in wound closure (23). HA is a natural biological component present in ≅65% of bone tissue (1,21). Even though the synthetic HA component provides biocompatibility and osteoconductivity, its bioreabsorbability is hardly and slowly accomplished therefore requiring a prolonged period of time to fully be replace by bone (22). In contrast, β-TCP is a ceramic natured material similar to HA and it is also osteoconductive and biocompatible (1,18). The Tricalcium Phosphate (TCP) material is resorbed 12 times faster than HA, promoting a fast new bone formation while it is being replaced (9,21). However, due to being quickly reabsorbed, it eventually leads to instability as its reabsorption rate does not match the natural bone formation rate (9). Therefore, solely, none of these materials can achieve long-term stable bone regeneration and, alternatively, biphasic associations of β-TCP to HA might present the optimal solution to find a balance between new bone formation and material resorption rate and consequently provide a long-term stable bone regeneration (9,19). The HA provides a first initial bone response while acting like a support and space maintainer and the β-TCP promotes the regeneration of the new bone (14,19). The usage of HA/β-TCP promotes the bioactivity properties of the biomaterial, showing its importance in cell proliferation, revascularization process and the presence of osteoblasts inducing the osteogenesis (1), where also the osteoclast activity on the material surface is present (active remodelling process of the bone) (19,22). The material is considered identical to the bone mineral (14) presenting mimetic bone characteristics (14,18). Generally, biphasic calcium phosphate presents 100% of crystallinity that provides cell attachment, protein adsorption and also contributes for the degradation of the biomaterial in contrast to the 90% of porosity that plays a role in the bioactive characteristics of the material and the formation of the clot (9,14,23,24). Probably, block samples could promote better kinetics in the hard tissue regeneration than with granules samples of the alloplastic material (8). According to *Di Raimondo et al.*, the complete new hard tissue replacement and graft dissolution was seen at 24 months postoperative (12). Despite the advantages, the biphasic calcium phosphate demonstrates some disadvantages such as: the occurrence of the encapsulation of the material in the surrounded tissue during the reabsorption process (21,23); the substitution by the newly formed bone doesn't happen at the same proportion of the reabsorption of the material (21). This bioceramic material plays a role for cell ingrowth and performs as a scaffold for cell maturation and development of new bone, showing its capacity to be an osteoinductive material (1). *Lindgren et al.* demonstrated that HA/β-TCP promotes soft tissue regeneration (14).

Combining the benefits of both materials adjusting the ratio, HA can counteract the instability of β-TCP by making it possible for β-TCP to create spaces to further be filled by newly formed bone, but also introducing solidity and compactness with the HA by minimizing the resorption rate of β-TCP during the healing period (1,2,8,12,19), that depends on the ratio used (19).

Bovine xenograft materials evaluated in this integrative systematic review are the well-known and widely used: Demineralized Bovine Bone Mineral (DBBM) and Bovine Derived-Hydroxyapatite (BHA). Both grafts are considered predictable biocompatible materials, presenting a great availability for the patients (11,14,15,22) and being capable to maintain the site for newly hard tissue formation with the presence of osteoconductive properties (4,6,8,9,16,18,21). However, they are not osteoinductive biomaterials (15,23). These bovine derived xenografts are fabricated by heat and chemical processes, where the purification process plays a role in removing all non-inorganic constituents in a way to avoid an immunogenic response from the host (9,11,14,23). The xenografts are based on HA crystals, the component that should be presented as the main mineral of this type of grafts, after the correct purification procedure (9,11,14). However, the transmission risk of diseases or a not well tolerated placement can't be totally excluded for the xenografts materials (9,20,23). The different temperatures in the purification procedure of each graft materials promote some alterations in their general properties that could influence the hard tissue regeneration (11). The non-sintered DBBM is a natural demineralized hard tissue (23) widely used to fill even the periapical origin lesions, acting as a scaffold not only to improve the bone formation, but also to promote the coagulum formation (5,9,16). This material appears to have mimetic qualities compared to the human hard tissue, promoting an early angiogenesis, with its capacity to promote ingrowth vessels, and it's capable to improve the osteoblast activity, as it was mentioned by *Kourkouta et al.* and *Ghezzi C et al.* in their apical studies (4,9,14,17,22). The referred authors demonstrated that DBBM placement potentiated periapical healing and the achievement of a new tissue regeneration (4,17). Its placement also improves the gains of the new soft tissue, being an aid in the soft tissue regenerative process, especially when a collagenous membrane is placed in combination with the bone graft, resulting in better outcomes (21,23). DBBM material is composed by a 75% of porosity, that stimulates proliferation of the cells and the osteoblasts (8–10,17,22). The bovine xenograft is also composed by a fibrillar surface texture and a crystallinity and size of the particles (250–1000 µm) smaller than BHA particles (8,11,15). Despite the great characteristics of the biomaterial, it was reported that the encapsulation of the particles into the soft tissue could occur (4,20). Also, DBBM presents a higher level of bone marrow spaces rather than the alloplastic material (14) and the osteoclast activity of this graft still a controversy, where just a small activity probably would be present (9,11,22). The resorption process of DBBM is the key characteristic of the material: this heterogenous graft biomaterial has a slow rate of resorption (9,12,15), in contrast to the HA/β-TCP (12), and the replacement by the newly formed bone occurs in a long extended process during the healing time (15,19,20,22), showing no remodelling mechanism in the early stages of the bone healing (19). This lack of resorption probably is due to the absence of non-collagen proteins that could contribute for a well-integrated resorption process and the high crystallinity (lesser than BHA) (11,14). Therefore, DBBM plays a role more as a space maintainer with osteoconductive properties (12,19) where its particles could be found even nine years after the surgical procedures (11,12,15), showing the ability to be a durable and a stable graft capable to promote new bone during its presence in the surgical site (8,9,11,23). However, it does not promote an accelerated and shortened bone regeneration compared to HA/β-TCP (12).

Even though the BHA was not assessed at apical level in this study, this sintered bovine-derived biomaterial, in one comparative study of *Kurkcu et al*, obtained better outcomes in osteoconductive properties than β-TCP alone, maybe due to the fast resorption rate of the synthetic material (18). Although its analogy with DBBM as well as the closeness to the properties of the human hard tissue, this xenograft material presents some different physical and chemical characteristics comparatively to DBBM due to the purification process which occurs at high temperatures (11,18). This material presents a compacted and smooth texture and it's known that 1300°C is the ideal temperature to achieve the greatest physical properties of this bone graft (11). Simultaneously, the chemical and physical properties of the material, especially the great crystallinity, are capable to influence the dissolution rate of the BHA and, for that reason, this biomaterial is identified as long-lasting scaffold due to its low rate of resorption (11,18). The main difference between these bovine xenografts, besides the highest capacity of BHA to remain stable and integrated in the surgical site (11,18), lies on the biggest quantity of multinucleated giant cells present in the material surface, promoting the release of VEGF (Vascular Endothelial Growth Factor) and HO-1 (Heme Oxygenase-1) which are directly related with the bone healing mechanisms (11,20).

4.3- The importance of the ratio in alloplastic material and the comparison with bovine xenograft materials:

In this study, it was demonstrated with both biomaterials the bone formation was successfully achieved during the healing of apical lesions regardless of the HA/β-TCP ratio used (1,2) or the bovine xenograft was used in periodontics and/or endodontics clinical situations (4–7,16,17). Nevertheless, the outcomes of the comparison articles highlight the main differences in the adjustment of ratios in the alloplastic material, when comparing with DBBM (8–10,12–15,19–23) and also with BHA (18).

The 60%HA/40%β-TCP is the ratio widely used in some dentistry procedures (9,15), and compared to bovine xenograft, in this study, similar outcomes were presented for the newly formed bone (9,12–15,21,23). Although *Dahlin et al.* referred in first three weeks of follow-up there was a difference between the alloplastic and the bovine graft, at eight weeks of follow up this difference didn't exist anymore (19). However, alterations in the HA/β-TCP ratio could alter the bioactivity properties of the synthetic material, influencing the degranulation and resorption mechanism and the regeneration process (1,9,14,19,24). In order to avoid the high resorption of β-TCP component, the increasement of HA ratio appears to be an effective choice besides the improvement that this component gives at long-term compactness to BCP (12,19), maintaining the great characteristics of the alloplastic biomaterial (1,9,12), as it happens with 60%HA/40%β-TCP. A BCP ratio with low percentage of HA and high percentage of TCP indicates that there will be an elevated resorption rate rather than when the opposite occurs (60% HA/40% β-TCP, for instance) and this demonstrates that the TCP is the principal component of the HA/β-TCP that is directly related with a fast degranulation/resorption process (14). It was demonstrated by *Kurkcu et al.,* β-TCP alone presented worst outcomes than BHA in newly formed bone might be due to the fact that the bone resorption happened excessively fast (18). However, *Sato et al.* demonstrated the opposite, where β-TCP shown superior results which might be explained by the occurrence of an adequate new bone formation while the material was being replaced, during the precise healing time (22). Despite this, although the fast replacement and new bone formation (14), the increasement of TCP ratio plays a role in decreasing cellular adhesion, as multinucleated giant cells present at the surface contributing to the beginning of angiogenesis (9,24). Furthermore, besides the percentage of each component, the elevated temperature used in the elaboration of biphasic calcium phosphate, the pH level and the replacement by calcium deficient-HA influence the behaviour of HA/β-TCP as: in the type of HA/β-TCP surface or the increase of space between the HA crystals that promotes a higher microporosity and consequently more osteoblastic cells would attach to the surface area of the material, promoting new bone formation (9,19,24). The macroporosity is also relevant in coagulum formation, angiogenesis and the nutrients transportation (9). The xenograft materials presented different temperatures of sintering: for DDBM the sintering temperature is lower (300°C) than BHA (≅1250°C), and, being both a non-fast resorbable grafts that establish powerful contact between the newly bone, they could promote, during the healing time, great bone maturation (11). Despite the opposite characteristics between TCP and BHA at resorption rate level, the formation of TCP component (α, β) comes from the decomposition of the BHA in high temperatures (11).

In fact, proving the importance in adjusting the ratio of HA/β-TCP to achieve a greatest effectiveness in the bone healing, the usage of 30%HA/70%β-TCP, in this study, resulted in better results than the bovine xenograft (DBBM) (8,10) and the same occurred with 10%HA/90%β-TCP (19), indicating that a higher percentage of β-TCP and a lesser percentage of HA could be the ideal HA/β-TCP ratio, maintaining a great replacement of newly formed bone with a better resorption rate in reduced amount of time (8,19). However, with this type of ratio (high β-TCP ratio), the wound healing could take longer since superior soft tissue gains is associated with the usage of more HA component (1).

4.4- Bone regeneration process:

The apical site defects were successfully filled by newly formed bone, showing the capacity of both type of biomaterials in promoting the bone regeneration (1,4,5,7,17). Regeneration is the process that encompasses the healing consequent to a reestablishment of the architecture and normal function of the tissue (1,20). The collagen and the HA are the extra-cellular matrix components of the hard tissue and bone tissue is capable to cure on its own in most of the cases when the lesion is considered small (11). The HA/β-TCP and the bovine xenograft placed requires an ideal ambient in order to initiate osteoblast and osteoclast activity at the lesion site which would consequently promote the remodelling mechanism, making the conversion from the resorption process into the newly formed bone, and consequent bone regeneration (10,15,17,19,22). For that reason, angiogenesis is an important step in the initial phases of the bone regeneration process, enhancing the ability in increasing the formation new vessels and acting as a stimulus for cell differentiation and migration (9,22). However, it is presumable that bovine xenograft provides a lessened remodelling process (19). Being the collagen one of the principal components of the bone, it plays a role also as a conductor for newly formed vessels and cell stimulation and proliferation by the discharge of growth factors, acting as an angiogenesis stimulator (9,20). Also, collagen is capable to promote the clot formation and it's well known its usage as a plug in a combination with the biomaterials is very common and successful (4,5,9,21,23). Therefore, there is a slight dependent connection between the processes that promote new bone formation and the one that develops and improves the bone ingrowth in order to achieve a total and well-integrated bone regeneration (22).

In this study, even though the highest bone formation with the alloplastic biomaterial in some comparison studies at the non-apical level (8,10,19), both materials are capable to improve new bone formation in different species such as mini-pigs (15,19), dogs (10,12,20,22) and humans (8,9,13,14,18,21,23). Although the similarity that both biomaterials present in the volume of the newly formed bone (9,15,19,22), the materials don't demonstrate constant similarity in the rate of residual bone graft, where the bovine xenograft demonstrated higher quantity of residual graft than the alloplastic material in two human studies (14,18). These high values of residual graft with the bovine biomaterial are maybe due to its persistent particles that are not so fastly reabsorbed than the particles of the HA/β-TCP (9,12,14,15,18). However, although the poor resorption of the bovine graft particles, this doesn't mean a delay of bone formation (18). The osteoconductive characteristics of bovine xenograft, for instance the porosity, promote the newly formed bone even if the particles aren't be reabsorbed, being this lack of resorption might constitute a benefit for the bovine xenograft (11,18).

4.5- Healing at apical level:

The complete healing of the apical lesions was successfully reached with the placement of the alloplastic and bovine xenograft biomaterials allied to the endodontic microsurgery procedure (1,2,4–7,16,17). The wound healing is a complicated arrangement of biologic procedures, some happening at the same time and some ward upon the fulfilment of earlier occasions (5). Despite the all grafts intention to enhance the wound healing and to save however much bone as could reasonably be expected (10), in some cases the apical surgery itself invigorates a progression of vascular, cellular, and biochemical systems which regularly lead to recovery or fix, where the clot commonly encloses the desired and essential factors for the tissue regeneration (5). *Taschieri et al.* reported the inexistence of a discrepancy in the healing between one group where the bovine xenograft material was implanted and the other where there wasn't a placement of any graft (6). In order to achieve better clinical outcomes, the utilization of collagen membranes in relationship with biomaterials, as a surgical complement, has been generally utilized, subsequently improving wound steadiness (4,5,9,21,23) and the recovery of certain segments of periodontum as periodontal ligament and cementum (4,17). Nevertheless, this combination with membrane is more frequently used in association with the bovine xenografts (4,9,17). The two biomaterials are fit to the enhancement of soft tissue regeneration and in this manner lead to finish tissue recovery at apical site (1,2,4,5,7,16,17). *Das et al.* indicated in their study that in only a month all out tissue recovery happened, where the HA/β-TCP was placed (2).

The minority of postoperative complications with the utilization of these two biomaterials (5,6,8,9,16–23), might be due the fact of the low level of contamination that these biomaterials provide (23). The HA/β-TCP, being a natural ceramic synthetic material, is generally safe of cross-contamination and illness transmission, demonstrating a better acceptability by the organism with no inflammatory responses and foreign bodies reactions, while with the bovine xenograft this hazard isn't completely excluded (1,14,18). Nevertheless, *Kourkouta et al.* demonstrated that after the apical microsurgery with the placement of DDBM there wasn't the necessity of medication prescription (4). Both HA/β-TCP and bovine xenograft biomaterials exhibited a great efficacy in accomplishing total healing in various types of lesions that incorporate the apical zone, as demonstrated in Table 2, in which the subsequent follow-ups showed a decent steady tissue regeneration over a long time (1,4,16,17). Only the *Taschieri et al.* and *Dietrich et al.* studies didn't demonstrate 100% effectiveness, but 83.3%, since they were clinical trials and not case reports, where a minor rate of unsuccessful cases existed (6,7). The biomaterials assessed in this study demonstrated to be suitable for bone and tissue regeneration and thus lead to the total healing of the defects.

In this integrative systematic review, the lack of clinical studies on the effectiveness of the synthetic material in the apical zone; the lack of comparative studies between the two biomaterials in their use in the apical microsurgery technique; the fact that not all studies analysed were clinical trials, were the limitations of this study. Also, it is important to mention that in certain studies, combinations of materials were used and not only the biomaterials to be assessed, so it may have influenced certain results. In addition, further studies ought to be done to assess the prolonged performance of the biomaterials as a complement in the apical microsurgery procedure.

5. CONCLUSIONS

Both bovine xenograft and HA/β-TCP ended up being potential choices for application in the apical lesion area during the endodontic microsurgery procedure, by advancing both bone and soft tissue recovery, consequently leading to total wound closure and healing.

Notwithstanding the similarities of biomaterials, the ratio of the synthetic material appears to have a great propensity in the control of the material's attributes and along these lines to the manner in which it will act in the recovery process. In this way, the proportions with lower levels of HA and higher levels of β-TCP seem to present the best outcomes, being perhaps the best option between the two types of biomaterials.

Further investigations are required so as to develop the knowledge on the regeneration mechanisms of this HA/β-TCP ratio.

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