Combination of bone substitutes and vectors in periodontology and implantology: A systematic review

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The aim of the systematic review was to analyze the use of combination of bone substitutes and vectors in periodontology and implantology among animals models and humans. Electronic databases were searched, and additional hand search was performed. The research strategy was achieved according to the PRISMA guidelines. The including criteria were: combination of bone substitutes and vectors, in vivo studies, a precise number of specimens, histological and radiographic analysis, written in English. The risk of bias was evaluated for individual studies. Thirty-two articles were selected and investigated in this systematic review. The results do not show a superiority of the use of composite biomaterial in comparison with simple biomaterial but suggest the efficacity of their utilization as a carrier of bioactive agents. Future studies need to identify the suitable association of bone substitutes and vectors and explore interest in their use such as the support of growth factors.

Keywords: Combination of bone substitutes and vectors, Periodontology, Implantology, Pre-clinical study, Clinical study

INTRODUCTION

Bone grafts are used in periodontology for the treatment of intrabony and furcation defects; they are used in implantology for alveolar ridge preservation, guided bone regeneration (GBR), or sinus lift. An ideal graft material should be biocompatible, safe, non-allergenic, non-toxic, and have no risk of disease transmission. Ideally, it should provide a role of space-maintaining, and have similar resorption rate, composition and porosity to human bone. This interconnected porosity should allow the ingrowth of blood vessels and the diffusion of bone cells and nutrients. Finally, it should have a controlled biodegradability to ensure a balance between resorption and volume maintenance during bone ingrowth and a dimensional stability to allow this adaptation in the defect. Bone grafts promote bone formation under three concepts: osteoconduction (material acts as a scaffold), osteoinduction (material contains proteins which lead to proliferation and differentiation of bone cells), and osteogenesis (material containing stem cells).

The current gold standard is still autologous graft (bone from the patient); it is the only bone graft that is osteoconductive, osteoinductive and osteogenic. This technique has several detriments, such as the necessity of a secondary operative site, which represents an augmented risk of supplementary comorbidities, or a low quantity of bone. For these reasons, some alternatives have been developed. The first alternative to autologous bone is the use of allogenic graft: tissue from a human donor or cadaver. Three types of allografts exist: fresh frozen bone (FFB), freeze-dried bone allograft (FDBA), and demineralized freeze-dried bone allograft (DFDBA). The risk of transmission of bacteria, virus, or prion cannot be excluded for this type of bone substitute. For these reasons, their uses are restricted, particularly in Europe. The second option is xenografts: transplantation of bone tissue across species. In periodontology and implantology, deproteinized bovine bone is the most commonly used. Lastly, alloplastic bone substitutes have been developed in the form of synthetic hydroxyapatites (HA), beta-tricalcium phosphate (β-TCP), biphasic calcium phosphate (BCP), and bioglasses. HA is non-resorbable biomaterial with a low resorption rate and high space-maintaining potential contrary to β-TCP. BCP is composed of different ratios of HA and β-TCP to combine the advantages of these two families. Thus, this biomaterial can have different biodegradability and stability degrees according to the bone defect.

All of these bone substitutes are available in the form of granules or blocks, which can be difficult to manipulate and set up in some clinical situations. Therefore, combination of bone substitutes and vectors (composite biomaterials) have been elaborated; they are composed of two phases, granules of bone substitutes linked together by a vector. The vectors are polymer biomaterials, mainly represented by polyglycolic acid (PGA), hydrogel, or collagen. Combination of bone substitutes and vectors are in the form of paste or injectable material. The goal of this galenic form is to allow an augmented usability for the clinician and a better stability in chirurgical sites; these materials can also be used as a support for stem cells or growth factors. However, the addition of polymer between bone particles can change the property of the biomaterial and its capacities of bone neoformation.
Nowadays, it is not yet possible to conclude at the superiority of a vector or an association vector-biomaterial. For this reason, the aims of this systematic review are to analyze relevant studies to retrieve valuable information about the interest in the use of combination of bone substitutes and vectors in periodontology and implantology and to evaluate different combinations of bone substitutes and vectors.

MATERIALS AND METHODS

The different studies concerning the use of combination of bone substitutes and vectors in periodontology and implantology on human or animal models have been collected and analyzed.

Question

Based on the PRISMA directives (Preferred Reporting Items For Systematic Reviews and Meta-Analyses), a specific question has been developed with the PICO (Participant, Interventions, Control, Outcomes) method: “Do combination of bone substitutes and vectors enhance clinical results in patients treated in periodontology and implantology?”

Information sources and search strategy

The search strategy was established according to the PRISMA guidelines. Original articles were searched using electronic databases (Medline and Cochrane Library), and relevant articles were screened by hand to potentially add relevant articles. A combination of Medical Subject Heading (MeSH) terms were used to identify appropriate studies: “combination of bone substitutes and vectors”, “periodontology”, “implantology”, “pre-clinical study”, “clinical study”. Only English articles were included and no publication dates or publication status restriction were imposed.

Study selection and inclusion/exclusion criteria

Selection was based on the inclusion and exclusion criteria defined so as to include only the most valuable articles (Table 1).

The selection process was recorded in detail to a PRISMA 2009 flow diagram (Fig. 1).

Data collection process and data items

The following data were extracted from the included studies: 1) Biomaterial (+/− membrane); 2) Animal models: species, sex, age, weight; 3) Number of defects per group; 4) Defect type, size; 5) Treatment groups; 6) Observation period; 7) Qualification of newly formed bone; 8) Result (Tables 2 and 3).

Risk of bias in individual studies

To ascertain the risk of bias in eligible articles, their methodology was evaluated by SYRCLE's Risk of Bias tool for animal intervention studies, or by Risk Of Bias Non-randomized Studies of Interventions (ROBINS-I) tool, or by the Cochrane Collaboration's tool for human randomized trials.

Data synthesis

A meta-analysis was not performed; we conducted a descriptive and systematic analysis of the studies.

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| Inclusion criteria | Exclusion criteria |
|--------------------|--------------------|
| Studies using combination of bones substitutes and vectors | *In vitro* studies |
| *In vivo* studies | Cases reports |
| Studies with the precise number of specimens | Retrospective studies |
| Studies with histological and/or radiographic analysis | Studies without control group |
| Studies written in English | Studies without statistical analysis |
| | Reviews |
Table 2 Comparative table of pre-clinical animal studies in the use of combinations of bone substitutes and vectors

| Reference          | Biomaterial                                                                 | Animal model, species, sex, age, weight | Numbers of defects per group | Defect type/size                  | Treatment groups                                                                 | Observation period | Qualification of newly formed bone (NB)                                                                                                                                                                                                 | Results                                                                                                                                                                                                                     |
|--------------------|------------------------------------------------------------------------------|-----------------------------------------|------------------------------|-----------------------------------|-----------------------------------------------------------------------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Okada et al. 2019  | β-TCP+PGLA                                                                    | Dogs, beagles Males 12 month-old 10 kg | 6 per group                 | Bucal bone defect (maxillary first premolar) 4×4×5 mm                             | Test group: β-TCP+PGLA Control group: particulate β-TCP                          | 12 weeks         | Radiographic (micro-computed tomography) Histological Histomorphometric: Amount of connective tissue was significantly greater in the control sites. The proportions of mineralized bone area and bone marrow significantly greater at the test sites. No statistically significant intergroup differences in residual β-TCP. β-TCP + PGLA seems to be more effective than conventional β-TCP for ridge preservation. |
| Fukuba et al. 2019 | Gelatin/β-TCP sponges+rh-FGF (0.3%)                                           | Dogs beagles Males 1 year old           | 6 per group                 | Saddle type bone defect 8×4 mm                                                    | a. acidic gelatin/β-TCP sponges+rh-FGF (0.3%) b. basic gelatin/β-TCP sponges+rh-FGF (0.3%) | 12 weeks         | Tomography analysis Histological: NB area significantly smaller in group a than in group b. NB height significantly lower in group b than in group a. Total tissue height not significantly different between the two groups. |
| Knabe et al. 2019  | - Si-CAOP - Si-TCP - TCP                                                      | Adults females Merino sheep 24 months   | 36 per group 6 per time point     | Critical size defect in the left scapula (8×8 mm)                                | Empty defect (ED): negative control TCP: positive control Si-CAOP Si-TCP         | Time points: -2 weeks -1 month -3 months -6 months -12 months -18 months | Histological Immunohistochemical Histomorphometric: At all the time points, defects grafted with Si-CAOP, Si-TCP or TCP exhibited a significantly higher bone area fraction than the ED. Defects grafted with Si-CAOP exhibited a significantly lower particle area fraction than defects grafted with Si-TCP and TCP. Si-CAOP displayed a better biodegradability and the greatest stimulatory effect on bone formation. |
| Ozawa et al. 2018  | Collagen sponge (ACS) Hydroxyapatite/ Collagen composite (HAP/Col)           | Rats (F344/Jcl) Males 10 weeks-old      | 10 per group                 | Circular grooves on each side of the cranium mid-suture (5 mm ø)                 | a. ACS (control) b. HAP/Col                                                     | 12 weeks (0, 4, 8 and 12 weeks for CT images) | Micro-computed analysis (CT) Histological: CT images: In collagen group, bone ingrowth started at 8 weeks. In HAP/Col it started at 4 weeks. At 12 weeks, the whole cap area was filled with NB. Number of osteoclasts and osteoblasts were not significantly different. CT analysis: NB area significantly wider in group b than in group a. These results suggested that application of HAP/Col increased the outgrowth of NB much more prominently than did collagen. |
| Study | Bone Substitute | Study Details | Control | Experimental | Treatment | Outcome | Notes |
|-------|----------------|---------------|---------|--------------|-----------|---------|-------|
| Leventis et al. 2018 | $\beta$-TCP+polylactic-co-glycolic acid (PGLA)+Biolinker® (N-methyl-2-pyrrolidone solution) | Landrace pigs Females 4 months-old 18 kg | Experimental sites: $n=10$ Control sites: $n=4$ | Fresh extraction socket (ridge preservation) | Experimental group: $\beta$-TCP granules coated with PGLA mixed with Biolinker Control group: spontaneous healing | 12 weeks | Histological Histomorphometric | Experimental sites showed less mean horizontal dimensional reduction of the alveolar bridge but not statistically significant. More NB in experimental group. No statistically difference regarding osteogenesis was demonstrated between the two groups. |
| Naenni et al. 2018 | $\beta$-TCP+PGLA | Dogs, beagles Males >1 year old 10–20 kg | Test 1: $n=22$ Test 2: $n=22$ Control: $n=18$ | Fresh extraction socket (ridge preservation) | Test group 1: $\beta$-TCP+PGLA+collagen membrane Test group 2: $\beta$-TCP+collagen membrane Control group: blood clot | 4, 8 and 16 weeks | Dental impressions Lineal and volumetric analysis | Volumetric measurements: Buccal: T1-T3 and T1-T2: no significant statistical difference between test 1 and 2. The volume decreased was significantly lower in test 1 than in control group. Occlusal: T1-T3 and T1-T2 no statistically difference between test 1 and 2 but between test 1 and control group. Linear measurements: T1-T3: no difference between test 1 and 2 but between test 1 and control. T1-T2: the higher gain found for test 1 was not significant compared to test 2 but compared to control. Majority of volume decreased is loss the firsts weeks post-extraction. Ridge preservation procedures minimized the volume loss. |
| Kim et al. 2017 | Bio-Oss Collagen® | Dogs, beagles 1 to 2 years old 10 kg | 6 per group | Combined endodontic-periodontic lesion | Control: no treatment Test 1: Bio-Oss Collagen graft Test 2: Bio-Oss Collagen graft+collagen membrane | 7 months | Micro-CT Histological | Vertical distance between buccal and lingual crest: no significant difference between C and T1; and between T1 and T2. Distance significantly smaller in T2 than in C. The amount of mineralized bone was significantly lower in T1 group than in C group. No difference between T1 and T2; C and T2. When grafts were used in the socket, quantity of mineralized bone tended to be less. |
| Benic et al. 2017 | Porcine collagenated bone substitute block (PCBB) Collagen membrane (CM) loaded with bone morphogenetic protein 2 (BMP2) | Dogs, beagles Males 12±3 months 8 kg | 6 per group | Two surgeries: Box-shaped bone defects on extraction sites (8x4x5 mm) (1) and implant placement (2) | Block Block+CM Block+BMP2 (0.5 mg/mL; 0.2 mL) Block+CM-BMP2 (0.5 mg/mL; 0.2 mL) | 20 weeks | Histological Histomorphometric | Augmented area (AA): statistically significant difference between block-BMP2 (11.8±2.9 mm²) and block+CM-BMP2 (8.5±2.2 mm²). New mineralized bone (NB): no statistically significant differences. Residual bone substitute (RS): only the difference between Block-BMP2 (6.1±2.2 mm²) and Block+CM (3.4±1 mm²) was significant. The addition of BMP2 to PCBB or CM did not render statistically significant improvement of their performance for horizontal ridge augmentation. |
| Joo et al. 2017<sup>35</sup> | CBCP - CBCP loaded with rhBMP2 | Males New Zealand whites rabbits 2.5–3.0 kg | Sinus augmentation and implant placement | Control: CBCP soaked with saline | Test: CBCP loaded with rhBMP-2 | 4 weeks | Micro-CT Histological Histomorphometric | MicroCT: The amount of newly formed bone on the apex of the implant was greater in the BMP group than in control group. The median augmented volume significantly greater in the test group. Histological and Histomorphometric: Highest point of osseointegration at the medial surface of the implant and the augmented height significantly greater in BMP group. Areas measurements did not differed between control and test groups. |
|-----------------------------|---------------------------------|-----------------------------------------------|-------------------------------------------|---------------------------------|---------------------------------|--------|----------------------------------------|------------------------------------------------|
| Thoma et al. 2017<sup>36</sup> | HA/β-TCP granules Polysterene glycol hydrogel (PEG) Arginylglycylaspartic acid (RGD) | Dogs, beagles 18 months 15 kg | Standardized box-shaped defects (4×2×4 mm) on implant site | PEG, Synthetic bone substitute+PEG PEG-RGD, Synthetic particulate bone substitute+PEG+RGD CM, synthetic bone substitute covered with collagen membrane Control, empty | 8 weeks (n=6) And 16 weeks (n=6) | Micro-CT Histomorphometric | Percentage of regenerated area within total defect area: The treatment effects were not statistically different at 8 weeks, and significant at 16 weeks. New bone formation: Statistically significantly less bone formation was observed in group empty compared with all others group. NB formation significantly greater in CM group than in PEG. First bone-to-implant contact: Group CM statistically significantly superior to all other groups at 8 weeks. PEG and CM were statistically significantly superior compared to empty controls. Micro-CT analysis: 2 mm bellow the implant shoulder: PEG significantly higher values compared to empty and CM. PEG-RGD superior compared to empty 4 mm bellow the implant shoulder: no difference. |
| Hoshi et al. 2016<sup>17</sup> | Biodegradable gelatin sponges incorporating β-TCP | Dogs, beagles Males 1 year old | Saddle-type bone defect (5×10 mm) | a. Experimental group: gelatin/β-TCP sponges+rhFGF-2 (0.3%) b. Control group: gelatin/β-TCP sponges | 8 weeks | Micro-computed tomography Histological Histomorphometric | Group a: evident large amount of NB formation continuous with host bone. Group b: NB formation limited. Total tissue height grater in group a than in group b. No statistical significant difference. Residual defect significantly smaller in group a than in group b. |
| Lee et al. 2015<sup>23</sup> | Autogenous bone Synthetic Bone Substitute (SBS=70% HA+30% βTCP)+Collagen Collagen membrane | Dogs, Mongrel Males 12–15 months 30 kg | Buccal dehiscence on implant site (3 mm) | a. SBC alone (control group) b. Inner autogenous bone; outer SBC (IAB) c. Inner SBC; outer autogenous bone (OAB) | 12 weeks | Radiographic Histological Histomorphometric | Radiographic analysis: total augmented volume did not differ significantly between IAB and OAB groups but was significantly lower in SBC group. Histological and histomorphometric analysis: Residual bone material and NB significantly greater in groups b and c than in group a. Median bone-to-implant contact significantly higher in group c than in group a. Median mineralized tissue area not significantly different between the three groups. |
Yoshida et al. 2015

β-TCP scaffold
PLGA/β-TCP scaffold
β-TCP and PLGA/β-TCP scaffolds loaded with Fibroblast Growth Factor-2 (FGF2)

Wistar Rats

Decortication (4 mm) in the cranial bone

β-TCP scaffold

β-TCP and PLGA/β-TCP scaffolds loaded with FGF-2

Histological
Histomorphometric

10 days post-surgery:
Tissue ingrowth limited to the periphery of the scaffold in groups b and c. Groups d and e: active bone formation. FGF-2 coating stimulated woven trabecular bone formation.

35 days post-surgery:
PLGA/β-TCP scaffold was more effective in bone formation than uncoated scaffold. Bone formation in group e was six-fold greater than in the control group. The open cell structure of the scaffold was adequately maintained and occupied with ingrowth tissue.

Kim et al. 2015

- CBCP
- BMP-2-loaded CBCP

Males New Zealand white rabbits 2.5-3.0 kg

4 per group

One rabbit excluded in 4wBMP group.

Sinus augmentation

2 weeks (n=8) 4 weeks (n=8)

Radiographic analysis:
Total augmented volume (TAV) larger in the BMP group than in CTL group both at 2 and 4 weeks.

Newly formed bone (NBV) larger in BMP than in CTL at 4 weeks. No significant difference at 2 weeks.

NBV and %NBV was greater in 4wBMP than in 2wBMP.

Nonmineralized tissue (NMV) larger in BMP than in CTL at 2 and 4 weeks; it decrease significantly with healing in all groups.

Histometric analysis:
At 2 and 4 weeks: TAA and NBA larger in BMP groups than in CTL groups. NBA larger in 4wBMP group than in 2wBMP group. NMA larger in 2wBMP group than in 4wBMP group.

Addition of BMP-2 to CBCP resulted in a greater initial augmented volume.

Cha et al. 2014

Bovine hydroxyapatite/Collagen (BHC)+BMP-2

Mongrel dogs 12 months 30 kg

4 per group

Sinus elevation

20 weeks

Radiographic analysis:
Differences not statistically significant.

Histomorphometric:
area and % NB significantly larger in BMP2 groups than in control group.
bone formation significantly larger in BMP2 groups
differences between BMP2 groups not significant.

Struillou et al. 2013

- BCP
- Composite hydrogel/BCP (MBCP)- Si-HPMC

Beagles 48±2 months 16±1 kg

6 per group

Dehiscence type base defects on implant site

a. no treatment
c. BCP+hydrogel
d. BCP+membrane of GBR

12 weeks

Histological
Histomorphometric:
Significant increase in bone ingrowth values in group c and d compared with control group (a).
Results no significantly different in b compared with a and between all groups with biomaterial.
| Study                  | Treatment Description                                                                 | Animals                                                                 | Procedure                                                                 | Follow-up | Assays                                                                 | Results                                                                 |
|-----------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------|------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Kim et al. 2012      | - BCP blocks<br>- BCP-Collagen blocks<br>- rh-BMP2                                     | Adults New Zealand white rabbits 3.0–3.5 kg 16 per group                | Circular graft areas in the calvarium                                       | 8 weeks   | Micro-CT Histological Histomorphometric<br>rhBMP-2 release assay       | The area of NB was significantly greater in the BMP-2-treated groups than in nontreated groups and greater in the BCP/rhBMP-2 group than in BCP-Collagen/BMP-2 group. Bone density was higher in group d than in group c. The degree of integration was highest in the BCP-Collagen/BMP-2 group. |
| Struillou et al. 2011 | Injectable composite silanized hydroxypropyl methylcellulose/BCP (Si-HPMC/BCP)        | Dogs beagles 6–8 years old                                              | Test: 4 canines+7 furcations Control: 4 canines+8 furcisions             | 12 weeks  | Histological Histomorphometric<br>Bone ingrowth more important in test group than in control group. Difference not significant. Adjunction of hydrogel did not affect new bone formation. | Bone ingrowth more important in test group than in control group. Difference not significant. Adjunction of hydrogel did not affect new bone formation. |
| Hasturk et al. 2014   | Polymethylmethacrylate Polyhydroxyethylmethacrylate, and Calcium Hydroxide (PPCH)<br>Polyanhydride (PA) | Adults minipigs 18 to 24 months old 35 kg 8 per group                   | Immediately loaded implants placed in fresh extraction socket             | 12 weeks  | Clinical and macroscopic Histological (SEM) EDX spectroscopy          | Probing depth: no significant difference. Radiographs: no significant radiolucency along the implant. Electric mobility test device (STV): no significant difference between the groups. Only maxillary implants analyzed: STV of PPCH-PA group significantly better. No difference in the mandible. Biomechanical testing: no statistical difference. NB well organized in the group a. Groups b and c: sites rich in marrow spaces. Group a: fewest microfissures between the implant and bone and the fewest fractures in the interface after pullout test. Group b and c: 10 µm of microfissures. Group d (control): 20 µm of microfissures. |
| Sato et al. 2009      | CPC : powder composed of a-tricalcium phosphate, monocalcium phosphate monohydrate, calcium carbonate+solution of sodium phosphate | Dogs, beagles 1 year-old                                                 | Second and third maxilla incisors extracted+ defect 7×6 mm created        | 6 months  | Histological Histometrical                                            | Clinical observation: In the CPC group, alveolar ridge enhanced compared with control group. Histological observations: NB which was in continuous with the host bone was larger in group a than in group b. No significant difference in the width of defect in both groups. Height of NB was significantly greater in CPC group than in control group. |
| Barbosa et al. 2002   | - Anorganic Bovine Derived Bone Matrix (ABM)<br>- Cell binding peptide (P15)<br>- Bioabsorbable membrane | Mongrel dogs Adults, males 6 per group                                  | Test 1: n=3 Test 2: n=5 Control: n=2                                      | 12 weeks  | Clinical Histological                                                 | The total amount of bone volume showed no statistically significant augmentation in control group. In test groups 1 and 2: relevant ridge augmentation was observed. Significant bone formation was histologically observed in all test areas. The association of a membrane seemed to enhance the process of bone formation. |
### Table 3 Comparative table of human studies in the use of combinations of bone substitutes and vectors

| Reference          | Biomaterial                                                                 | Animal model, sex, age, weight | Number of defects per group | Defect type/size                   | Treatment groups                        | Observation period | Qualification of newly formed bone (NB) | Results                                                                 |
|--------------------|-----------------------------------------------------------------------------|-------------------------------|-----------------------------|-------------------------------------|------------------------------------------|--------------------|----------------------------------------|------------------------------------------------------------------------|
| Hala et al. 2019   | - Autogenous Bone (ABG) - ABG/Melatonin                                   | HUMAN Female, Male 38.77±4.28 years old | 26 per group                | Immediate implants augmented       | Control group: ABG Test group: ABG/ Melatonin | 9 months           | Radiographic (CBCT)                    | Statistically significant benefit for Test group in comparison with Control group in bone density and marginal bone loss at 6 and 9 months. |
| Llanos et al. 2019 | - Deproteinized bovine bone mineral (DBBM) - DBBM with 10% collagen - Collagen matrix (CM) | HUMAN Female, Male 41.9±11.9 years old | DBBM : n=33 DBBM-C : n=32   | Ridge preservation and implant placement | Control group: DBBM-C+CM Test group: DBBM+CM | 4 months           | Radiographic (CBCT)                   | No significant difference between the groups. The DBBM demonstrated non inferiority to the DBBM-C group. |
| Lim et al. 2019    | - DBBM-C - Collagen membrane                                               | HUMAN Female, Male 54.36±9.91 years old | Test 1 group : n=11 Test 2 group : n=10 Control : n=8 | Alveolar Ridge Preservation and implant placement | Test group 1: DBBM-C+collagen membrane Test group 2: DBBM-C Control group without alveolar ridge preservation (ARP) | 4 months           | Radiographic (CBCT) Histomorphometric | CBCT Analysis: Horizontal changes values did not differ significantly between Test 1 and Test 2. Changes were greater in the control group compared with test 1 but not with test 2. Histomorphometric: The percentage of NB not differ significantly between the groups. |
| Ozawa et al. 2018  | Collagen sponge (ACS) Hydroxyapatite/ Collagen composite (HAP/Col)          | Rats (F344/ Male) 10 weeks-old | 10 per group                | Circular grooves on each side of the cranium mid-suture (5 mm ø) | a. ACS (control) b. HAP/Col | 12 weeks (0, 4, 8 and 12 weeks for CT images) | Micro-computed analysis (CT) Histological | CT images: In collagen group, bone ingrowth started at 8 weeks. In HAP/Col it started at 4 weeks. At 12 weeks, the whole-cap area was filled with NB. Histological: Number of osteoclasts and osteoblasts were not significantly different. CT analysis: NB area significantly wider in group b than in group a. This results suggested that application of HAP/Col increased the outgrowth of NB much more prominently than did collagen. |
| Lim et al. 2017    | - Porcine Bone/ Cross-linked collagen - Bovine Bone/ Non-Cross-linked collagen | HUMAN Female, Male 53.8±16.22 years old | Test group: n=12 Control group: n=14 | Ridge preservation | a) Test group: collagenated porcine bone b) Control group: collagenated bovine bone | 4 months           | Radiographic (CBCT)                    | The radiologic evaluation revealed the non inferiority of the test material compared to the control material. |
| Study (Year) | Treatments | Subjects | Control Group | Test Group | Follow-Up | Analysis Type | Findings |
|-------------|-------------|----------|---------------|------------|-----------|---------------|----------|
| Nart et al. 2017 | DBBM, DBBM-C, Collagen membrane | HUMAN Female, Male 56.76 years old | DBBM: n=11, DBBM-C: n=11 | Alveolar ridge preservation | Radiographic (CBCT), Histological and histomorphometric | CBCT analysis: Height and width decreased significantly at 5 months of healing in both groups. No significant difference between the 2 groups. Histomorphometric analysis: No statistically difference were observed between groups. |
| Serrano Mendez et al. 2017 | Deproteinized cancellous bovine bone xenograft embedded in a 10% collagen matrix (DBBM-C), Demineralized freeze-dried cortical bone allograft (DFDBA), Collagen membrane (CM) | HUMAN Female, Male 44 years old | 10 per group | Alveolar ridge preservation | Radiographic (CBCT), Histomorphometric | No statistically significant difference between the two groups. The both grafting material are suitable for the preservation of the alveolar ridge. |
| Scheyer et al. 2016 | Demineralized allograft plus reconstituted (DFDBA), Deproteinized bovine bone mineral+collagen (DBBM-C), Crosslinked collagen membrane (RECXC), Bilayer collagen membrane (NBCM) | HUMAN Female, Male | Control group: n=21, Test group: n=19 | Alveolar ridge preservation | Clinical observations, Histomorphometric | Horizontal changes: significantly more bony width in test group. Vertical changes: no significant difference. Histomorphometric: Percentage of NB formed was not significantly different between the two groups. |
| Stavropoulos et al. 2011 | rhGDF-5/β-TCP, β-TCP/autologous bone (AB) composite | HUMAN Female, Male 53.8±12.1 years old | 10 per group, Unilateral sinus augmentation and implant placement | | Histological, Histomorphometric | No statistically significant difference between the groups regarding any of the evaluated parameters. |
Table 3 continued

| Nam et al. 2011 | - Deproteinized-bovine-bone mineral | HUMAN | Control group: n=23 | Test group: n=21 | Alveolar ridge preservation | Control group: DBBM | Test group: CBM-peptide coated DBBM | 6 months | Radiographic (CBCT) | Histological |
|----------------|------------------------------------|-------|---------------------|----------------|---------------------------|----------------------|----------------------------------|----------|----------------|-------------|
| Neiva et al. 2008 | - Bioabsorbable collagen wound material | HUMAN | Control group: n=17 | Test group: n=15 | Alveolar ridge preservation and implant placement | Control group: Bioabsorbable collagen wound material | Test group: Putty P15+bioabsorbable collagen wound material | 4 months | Clinical Radiographic (CBCT) | Histomorphometric |

RESULTS

The selected results included 48 publications. The results were categorized into three groups: pre-clinical animal studies, non-randomized human trials, and randomized controlled trials. The results showed that the most common biomaterials used were alloplastic materials such as β-TCP (7 studies) and BCP (5 studies), followed by bovine bone matrix (DBM) and demineralized freeze-dried bone allograft (DFDBA) (3 studies). Autogenous bone (2 studies), bovine hydroxyapatite or Bio-Oss (3 studies), porcine bone (2 studies), and PPCH (1 study) were also used. The growth factors BMP-2 (2 studies) and rh-BMP2 (2 studies) were employed in combination with bone substitutes.

Risk of bias within studies

The results of the risk of bias assessment were viewable in Fig. 2. The adapted methodology was applied for each subgroup of studies: pre-clinical animal studies (Fig. 2.a), non-randomized human trials (Fig. 2.b), and randomized controlled trials (Fig. 2.c). The results showed that the most common biomaterials used were alloplastic materials such as β-TCP (7 studies) and BCP (5 studies), followed by bovine bone matrix (DBM) and demineralized freeze-dried bone allograft (DFDBA) (3 studies). Autogenous bone (2 studies), bovine hydroxyapatite or Bio-Oss (3 studies), porcine bone (2 studies), and PPCH (1 study) were also used. The growth factors BMP-2 (2 studies) and rh-BMP2 (2 studies) were employed in combination with bone substitutes.

Study characteristics

The studies were ranked in a comparative table (Tables 2 and 3). The studies show a high percentage of studies in the type of preparation of biomaterials. A wide variety of materials, both synthetic and natural, were used. The majority of animal studies, comprising a large variety of animal species, were performed. The majority of studies focused on the effects of biomaterials on implant stability, bone density, and alveolar ridge preservation.

The MEDLINE literature search resulted in 516 publications. After the first selection step, 54 publications were included in the systematic review. These publications included 33 articles and full-text screening. Based on the full-text analysis, 516 publications were included in the systematic review.
randomized human trials (Fig. 2.c).

1. Pre-clinical studies
Only 45% and 23% of the studies mention randomization or blinding, respectively. Our data show a high score of unclear risk of bias for the performance and detection items (59% and 77%, respectively). The majority of the studies were free from selective outcome reporting (54%).

2. Non-randomized human trials
Only two studies were included in this category. Our results show that 100% of the studies were free from pre-intervention bias, and 50% were marked for unclear risk of bias for at-intervention items and 100% for post-intervention items.

3. Randomized human trials
As expected, our results did not show a high risk of bias. Regarding selection and attrition items, 75% of the studies were free from risk of bias. Our data show that 50% and 37.5% of performance and detection items, respectively, show an unclear risk of bias. Finally, 75% of the included studies were marked for an unclear risk of bias for their reporting.

Synthesis of results
For each selected study, the significant results are shown in Table 2 for pre-clinical animal models and Table 3 for human studies.

DISCUSSION

Literature searches retrieved 32 studies. After a careful analysis, our results revealed that it was not possible to perform direct head-to-head comparisons of these studies as a result of variations between studies, in terms of bone substitute, polymer vectors, the defect type and size, and the healing time. Not surprisingly, no meta-analysis of the data could be carried out.

The majority of the selected articles in this systematic review are pre-clinical animal studies (22/32 studies) with a larger panel of biomaterials and type defects tested than in clinical studies. Indeed, the selected human studies employed biomaterials exclusively in alveolar ridge preservation and sinus augmentation; none of the studies were about intrabony periodontal defect. Consequently, the use of combination of bone substitutes and vectors in periodontal defects is based only on pre-clinical studies that have a lot of risk of bias, with randomization and blinding infrequently described. This failure makes it difficult to draw conclusions from pre-clinical studies. Nevertheless, the combined analysis of the different included studies affords the retrieval of valuable information.

The combination of bone substitutes and vectors are composed of granules bound together by vectors, which can be coated with growth factors. This discussion focuses on the different combinations used in pre-clinical and clinical studies and the utilization of growth factors.

The most used bone substitute in pre-clinical studies was β-TCP, mainly in combination with PGLA, a scaffold commonly used for tissue repair. Two studies (Leventis et al. and Naenni et al.) did not show relevant results for the use of β-TCP/PLGA combination. Okada et al. showed that β-TCP/PLGA seems to be more effective than conventional β-TCP for alveolar ridge preservation. The results show that this injected and moldable biomaterial maintains its shape, secures the regenerative space and enlarges the osteoconductive area.

Two studies used β-TCP in gelatin sponges incorporating growth factors (rh-FGF). Hoshi et al. showed that the combined use of rh-FGF and gelatin sponge/β-TCP is effective for alveolar ridge augmentation. Fukuba et al. concluded that the controlled release of rh-FGF in time induces notably more alveolar bone regeneration than short-term application of this growth factor. Here, the use of a combination of bone substitutes and vectors seemed to be essential to control
the propagation of the growth factor and optimize the bone regeneration.

Only one clinical randomized trial tested β-TCP/autogenous combination and rhGDF-5 coated β-TCP(20) and concluded in the absence of significant difference.

The relevant results obtained in animal studies with β-TCP and polymer suggest that these combinations should be tested in clinical study in human models to attest their effectiveness.

BCP is used in five animal studies combined with Si-HPMC, hydrogel and collagen. Two studies of Struillou et al.(20,21) tested BCP with hydrogel in intrabony defects and peri-implant defects. They showed that hydrogel/BCP can promote new bone formation in large defect and implant sites, the viscosity of hydrogel, allowing for increased retention capacity and mechanical strength. Three studies employed BCP in combination with collagen (CBCP) loaded with growth factor BMP2 (bone morphogenic protein) and rh-BMP2 (recombinant human bone morphogenic protein). Two of these studies(22,23) concluded that the combination of BCP/Collagen and BMP2 was favorable for the new bone formation. It is supposed that the addition of BMP2 induced post-operative swelling at the origin of an early bone formation.

The use of BMP-2 in combination with biomaterial to promote bone regeneration has been studied in numerous pre-clinical and clinical studies(24). This growth factor has the highest evidence of a positive effect on bone formation in comparison with other agents. However, plenty of growth factors act on the bone-healing process(25), which suggests that only one factor in a biomaterial may be insufficient to stimulate the regeneration. Future studies might be directed toward the combination of factors and the use of combination of bone substitutes and vectors like a delivery system for these bioactive agents.

Mainly randomized clinical trials were realized with DBBM or DFDBA in combination with collagen (5 studies), and one non-randomized clinical trial used synthetic oligopeptide as a vector. All these studies concluded that DBBM-Collagen is effective in the alveolar ridge preservation procedure, but no significant differences were observed with the control group.

One randomized clinical trial(26) employed autogenous bone (AB) in combination with melatonin in immediate implant placement. The result showed a significant benefit for AB/melatonin, and the author suggested that the addition of melatonin has a positive role in new bone formation around the implant and could protect and recover the gingival tissue integrity. However, only radiological analysis was performed in this study; further clinical trials with histological and histomorphometric analysis will be necessary to attest the efficacy of AB/melatonin combination in new bone formation.

One non-randomized study(27) in a human model used anorganic bovine-derived HA combined with putty P15. Cell-binding peptide was also used in one pre-clinical study(28). The good results suggested that HA/P15 has greater compatibility with host bone than HA alone for alveolar ridge preservation.

None of the selected clinical trials studied combination of bone substitutes and vectors in periodontal defects in contrast with pre-clinical studies. Some relevant results in the use of these bone substitutes in periodontal bony defect in animal models suggested the necessity to realize these studies in human models. Particularly for the use of combination of bone substitutes and vectors as growth factors vectors, this could be interesting to promote periodontal regeneration. Indeed, a plethora of publications brings light the valuable role of growth factors and stem cells in the bone healing process(29,24,25) and the necessity of developing sophisticated delivery systems to lead them in the defect. In that way, combination of bone substitutes and vectors may be valuable. Indeed, some studies included in this systematic review suggest this, but additional research is necessary to develop an optimal biomaterial able to support specific molecules to promote bone and periodontal system regeneration in specific clinical situations(30).

CONCLUSION

Several combinations of bone substitutes and vectors are studied with various clinical applications. The corresponding studies have heterogenous results concerning their applications in periodontology and implantology. For these reasons, a systematic approach appears essential to serve as a guide for future studies and provide data that can be generalized. The results of our systematic review indicate that combination of bone substitutes and vectors do not enhance clinical results in comparison with classical bone substitutes, but they may provide beneficial effects in combination with growth factors. The present review supports important information for the evolution of research concerning the use of growth factor in bone graft for periodontal regeneration and implantology in the future. Future studies should focus on the use of combination of bone substitutes and vectors as stem cells and bioactive molecules carrier and explore their use in complex periodontal defects to find a combination more effective than simple bone substitute.

CONFLICT OF INTEREST

None.

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