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Performance of Mesenchymal Cell-Scaffold Constructs in Human Oral Reconstructive Surgery: A Systematic Review

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Abstract

Background: Different sources of cultured cells combined with different scaffolds (allogenic, xenogeneic, alloplastic or composite materials) have been tested extensively *in vitro* and in preclinical animal studies, but there have been only a few clinical trials involving humans.

Aim: This study reviewed all of the English language literature published between January 1990 and December 2015 to assess the histological performance of different mesenchymal cell-scaffold constructs used for bone regeneration in human oral reconstructive procedures.

Methods: An electronic search of the MEDLINE and Cochrane Central Register of Controlled Trials databases complemented by manual searching was conducted to identify studies involving histological evaluation of mesenchymal cell-scaffold constructs in human oral surgical procedures. The methodological quality of randomized controlled clinical trials and controlled clinical trials was assessed using the Cochrane Collaboration tool for assessing the risk of bias. Heterogeneity was assessed using Review Manager software. Considering the heterogeneity, the data collected were reported by descriptive methods and a meta-analysis was applied only to the articles that reported the same outcome measures. The articles were classified and described based on the material scaffolds used.

Results: The search identified 1030 titles and 287 abstracts. Full-text analysis was performed for 32 articles, revealing 14 studies that fulfilled the inclusion criteria. Three randomized controlled clinical trials were identified as potentially eligible for inclusion in a meta-analysis. The studies were grouped according to the scaffold materials used: bone allograft (three studies), polyglycolic-polylactic scaffold (four studies), collagen sponge (two studies), and bovine bone matrix (five studies). The stem cells used in these studies had been sourced from the iliac crest, periosteum, dental pulp and intraoral sites.

Conclusions: The very small amount of available data makes it impossible to draw any firm conclusions regarding the increase in bone formation in human oral reconstructive procedures when using graft materials engineered with autogenous stem cells.

Keywords: Mesenchymal stem cell; Tissue scaffold; Bone regeneration; Tissue engineering; Ridge augmentation; Maxillary sinus lift

Introduction

Critical-size bony defects represent a major clinical challenge in oral reconstructive surgery because they jeopardize physiological bone healing to an extent that may prevent complete regeneration. In achieving bone repair of oral tissue lost due to congenital defects, degenerative disease, infections, cysts, trauma or surgical procedures, autogenous bone grafting still remains the gold standard due to its osteogenic, osteoinductive and osteoconductive properties [1,2]. However, the use of autogenous bone has significant drawbacks such as a limited intraoral supply, the requirement for an additional operation under general anaesthesia in cases of an extraoral donor site, the tendency for partial resorption, and donor-site discomfort and morbidity. For these reasons many different biomaterials have been investigated for use in bone regeneration over the years, but all of them have demonstrated poor clinical performance when used alone.

An important opportunity is offered by tissue-engineering approaches, which involve the use of adequately differentiated cells, and provide the ability to produce extracellular matrix with mineralizing capability, the presence of communications and interactions between cells and matrix, the production and release of growth factors in the regeneration area, and the presence of a scaffold, which mimics the three-dimensional (3D) structure of the bone [3].

In this field, different sources of cultured cells combined with different scaffolds (allogenic, xenogeneic, alloplastic or composite materials) are being extensively tested *in vitro* and in preclinical animal studies, but only a few clinical trials have been performed in humans.

The present study reviewed all English-language literature published between January 1990 and December 2015 with the aim of determining the histological performance of different mesenchymal cell-scaffold constructs used for bone regeneration in human oral reconstructive procedures.

Material and Methods

The protocol used in this systematic review was based on the

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PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [4,5].

Focus question

This study attempted to address the following question: what is the clinical and histological performance of different mesenchymal cell-scaffold constructs used for bone regeneration in oral surgical procedures in humans?

Search strategy

A comprehensive and systematic electronic search in the Cochrane Central Register of Controlled Trials (CENTRAL) and MEDLINE via OVID was carried out for articles published in the English language between January 1990 and December 2015. Only human studies were selected. The following combination of the MeSH terms was used: "stem cells", "sinus floor augmentation", "alveolar ridge augmentation", "oral surgery", "maxillary sinus", "oral surgery procedures" and "jaw diseases".

A supplementary manual search was performed of the following peer-reviewed journals for articles published between January 2005 and December 2015: Clinical Oral Implant Research, Journal of Oral and Maxillofacial Surgery, Clinical Implant Dentistry and Related Research, British Journal of Oral and Maxillofacial Surgery, Tissue Engineering and Biomaterials. In addition, the bibliographies of all selected articles were checked so that other potentially relevant studies were also included in the analysis.

Selection criteria

Only studies involving histological evaluations of mesenchymal cell-scaffold constructs in human oral surgical procedures were considered. The following exclusion criteria were applied:

- Case report.
- Case series with fewer than 10 surgical sites.
- Letters and narrative or retrospective reviews.
- Studies without histological results.
- Periodontal or maxillofacial procedures.
- The use of mesenchymal stromal cells (MSCs) in general surgery.
- *In vitro* and animal studies.

In addition, in cases of duplicate publications, the article with the most recent data was preferred.

Study selection

The screening procedure of all titles and abstracts retrieved by the electronic and manual searches was carried out by two of the authors independently. To avoid excluding potentially relevant articles, articles whose abstracts described unclear results were included in the full-text analysis.

The full texts of all potentially relevant articles were obtained, and eligible studies were identified by two reviewers. Any disagreement was checked by an independent reviewer and resolved through discussion.

Data collection

Two reviewers used specially designed data extraction forms to independently extract the following information from the included studies: year of publication, type of study, characteristics of the scaffold, source of stem cells, patient's sample, surgical procedures and clinical, radiographic and histological data related to changes in mineralized bone. Any disagreement between them was discussed, and a third review author was consulted where necessary. The articles were classified and described based on the scaffold material utilized.

Quality assessment

A methodological quality assessment was performed in the randomized controlled clinical trials (RCTs) and in the controlled clinical trials using the Cochrane Collaboration tool for assessing the risk of bias.

Assessment of heterogeneity

Heterogeneity was assessed using Review Manager (RevMan) software [6]. The significance of any discrepancies in the estimates of the treatment effects from the different trials was assessed using Cochran's test for heterogeneity and the I^2 statistic. The chi-square test was used to evaluate the percentage of total variation across studies that were due to heterogeneity rather than chance. Heterogeneity would have been considered to be significant if the probability value was less than 0.1.

Data synthesis

A descriptive method was applied to report the data of selected articles, considering the heterogeneity in the study design, stem-cell population, surgical procedures, study period, methods for assessing the quality and quantity of regenerated bone, and the time required to perform the histological evaluation.

A meta-analysis was applied only to articles that reported the same outcome measures for the bovine bone matrix (BBM) scaffold. The mean differences were combined for continuous data using either fixed-effects models or, if the presence of heterogeneity between the studies was established, random-effects models. However, if there was a high degree of heterogeneity, the data were explored further to determine if they should be excluded from the meta-analysis [7].

Mean ± standard deviation values were calculated using the method outlined in Pudar-Hozo et al. [8]. If sufficient data were available, point estimates and 95% confidence intervals for the specific interventions were calculated.

Results

Study selection

The electronic search identified 1017 studies, while an additional 13 were collected from the manual search and 9 from the references of selected articles and reviews. The full text of 32 of these 1039 articles was screened. Fourteen studies fulfilled the inclusion criteria, while 19 were excluded (Table 1) from the descriptive analysis and 2 from the meta-analysis (Figure 1). The 14 included studies comprised 3 case series, 7 case-control studies, 1 retrospective cohort study and 3 RCTs. The 3 RCTs were identified as potentially eligible for inclusion in the meta-analysis.

Study characteristics

The augmentation procedure was a sinus lift in 12 studies [9-20]. In one study [21], bone grafting was performed in a mandibular defect after extracting the third molar. Another study [22] applied ridge augmentation and sinus lifting in the posterior maxilla and ridge augmentation in the anterior maxilla. The stem-cell populations had been harvested from the iliac crest (bone marrow stem cells) [11,13,15-

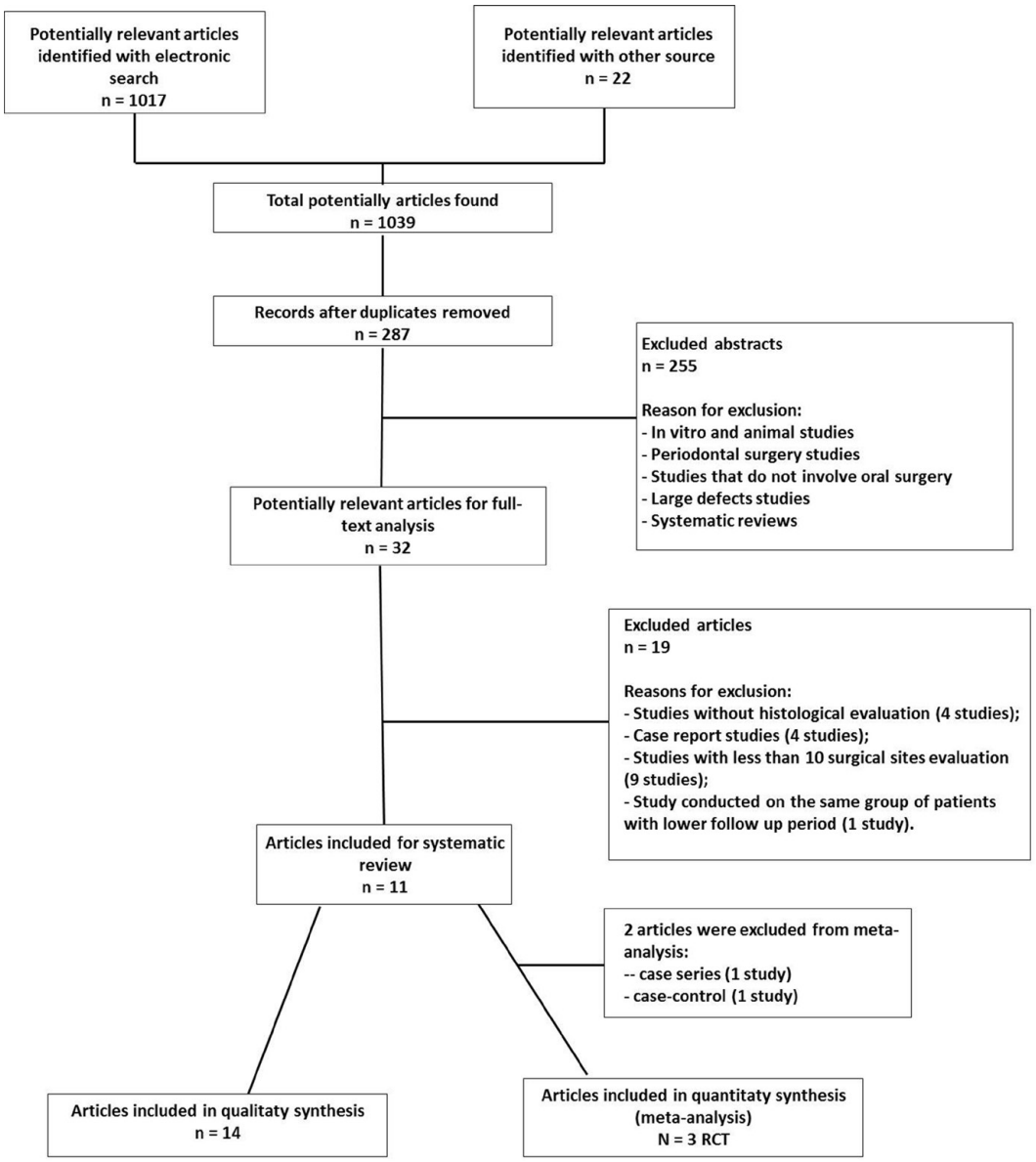

Figure 1: Flowchart of the selection of the studies for the review.

17,20,22], periosteum (mesenchymal stem cells) [9,10,14,18], dental pulp (dental pulp stem cells) [21] and intraoral sites (autogenous culture-expanded bone cells) [12,19].

Bone biopsies were performed at different time periods after surgery: 3-4 months [9,13-17,19,20], 6 months [10-12,18], 8 months [22] or 3 years [21]. Histological findings were reported in terms of percentages of newly formed bone (NB) [10-13,15-17,19] or as descriptive results [9,14,18,20-22]. Radiographic evaluations were performed using computed tomography (CT) in seven studies [11-13,15,17,18,22] and by orthopantomography (OPT) in three studies [9,14,21].

The studies were divided into four groups based on the scaffold materials used: bone allograft [15,20,22], polyglycolic-polylactic (PLGA) scaffold [9,12,14,18], collagen sponge [10,21] and BBM [11,13,16,17,19]. The main characteristics of the included studies are summarized in Table 2.

Bone allograft

Bone allografts from tissue banks were used in three studies [15,20,22]. In a case-series study, Cerruti et al. [22] treated 32 patients aged 45–83 years (median age 65 years) with 32 anterior or posterior maxillary defects to increase the amount of bone available for placing the dental implants. All procedures were performed using iliac bone allografts with autologous mononuclear cells obtained from the iliac crest or sternum bone-marrow aspirate, and platelet-rich plasma (PRP). Bone biopsies and CT were performed at 8 months after surgery, and the implants were placed in the grafted area.

Thirty of the 32 bone grafts (94.7%) were well-integrated, and all of the placed implants were functional and exhibited almost no bone loss after 2-4 years. Radiographic examinations revealed that the amount of bone was at least 6 mm in width and 10 mm in height, peaking at 14 mm in the anterior maxilla; in the posterior maxilla the width increase

Reference	Rationale for exclusion
MacAllister et al. [30]	< 10 surgical sites
Ueda et al. 2005 [31]	No histological evaluations
Ueda et al. 2008 [32]	No histological evaluations
D'Aquino et al. [23]	Redundant publication (Giuliani et al. [21])
Yamada et al. [33]	Case report
Smiler et al. [34]	< 10 surgical sites
Kim et al. [35]	Case report
Soltan et al. [36]	< 10 surgical sites
Zizelmann et al. [27]	No histological evaluations
Schmelziesen et al. [37]	< 10 surgical sites
Beaumont et al. [38]	< 10 surgical sites
Hibi et al. [39]	Case report
Behnia et al. [40]	< 10 samples
Yamada et al. [41]	No histological evaluations
Shayesteh et al. [42]	< 10 surgical sites
Montesani et al. [43]	Case report
Strietzel et al. [44]	Case report
Behnia et al. [45]	< 10 surgical sites
Meijer et al. [46]	< 10 surgical sites

Table 1: Reasons of excluded studies.

was not significant, and the bone height ranged between 9 mm and 15 mm. The bone biopsies reportedly showed "lines of bone formation and the presence of osteoblasts around the bone trabecula" [22].

In a case-control study, Gonshor et al. [15] compared bone formation in a two-step maxillary sinus lift procedure using either an allograft cellular bone matrix containing MSCs and osteoprogenitors (Osteocel, ACE Surgical and NuVasive) in 14 test sites, or conventional allografts (alloOss, ACE Surgical) in 7 control sites. CT scans were performed prior to and immediately following surgery and at the time of implant placement. Bone biopsy samples were harvested after 3.7 ± 0.6 months, during implant insertion. Histomorphometric evaluations revealed that the amounts of both vital and residual bone differed significantly between the two grafts. The amount of vital bone was 32.5 ± 6.8% for the allograft cellular bone graft and 18.3 ± 10.6% for the conventional allograft, and the amounts of residual graft were 4.9 ± 2.4% and 25.8 ± 13.4% in the test and control allografts, respectively.

Bertolai et al. [20] evaluated bone regeneration in 40 two-stage sinus augmentation procedures performed bilaterally in 20 patients (mean age 55.2 years). In accordance with the split-mouth design, the test side of each patient was grafted with freeze-dried bone allograft (FDBA) absorbed with MSCs, harvesting from the iliac crest and PRP,

First author, year	Study design	Scaffold	Stem cell	Sample size (control/ test)	Surgical procedure	Rx Evaluation	Bone biopsy
Schimming et al. [9]	Case series	PLGA	MSCs from periosteum	27	Sinus lift	OPT after 3 months.	After 3 months.
Springer et al. [10]	Case-control	Collagen sponge/BBM	MSCs from periosteum/ ACBCs from tuberosity	8/12	Sinus lift	No	After 6 months in test sites and 8 months in control sites.
Filho Cerruti et al. [22]	Case series	Bone allografts block	hBMSCs from iliac crest + PRP	32	Anterior-posterior augmentation + sinus lift	CT before and 8 months after surgery.	After 8 months.
Fuerst et al. [11]	Case series	BBM	ACBCs from iliac crest	22	Sinus lift	CT after sinus lift, after implant placement and after implant uncovery.	After 6 months.
Mangano et al. [12]	Split-mouth case-control	PLGA	ACBCs from the posterior area of the mandible	5/5	Sinus lift	CT before and 6 months after surgery.	After 6 months.
Sauerbier et al. [13]	Case-control	BBM	hBMSCs from iliac crest	6/12	Sinus lift	CT cone beam	After 3 months.
Voss et al. [14]	Case-control	PLGA	MSCs from periosteum	63/50	Sinus lift	OPT before surgery, before and after implant placement, after the final prosthesis.	After 6 months.
Gonshor et al. [15]	Case-control	Allograft cellular bone matrix	hBMSCs	21	Sinus lift	CT before and 3-4 months after surgery.	After 3-4 months.
Rickert et al. [16]	RCT	BBM	hBMSCs from iliac crest	12/12	Sinus lift	No	After 3-4 months.
Sauerbier et al. [17]	RCT	BBM	hBMSCs from iliac crest	11/34	Sinus lift	CT before and after c3.5 months.	After 3.5 months
Trautvetter et al. [18]	Retrospective cohort	PLGA	MSCs from periosteum	17	Sinus lift	CT before, after 4,12,24 and 60 months after surgery.	After 6 months.
Hermund et al. [19]	RCT	BBM	ACBCs from tuberosity	10/10	Sinus lift	No	After 4 months.
Giuliani et al. [21]	Case-control	Collagen sponge	hDPSCs	7/7	Socket grafting	OPT before, 6 months, 1 and 3 years after surgery.	After 3 years.
Bertolai et al. [20]	Split-mouth case control	Freeze-dried bone allograft	hBMSCs from iliac crest + PRP	20/20	Sinus lift	No	After 3 months.

PLGA = Polyglycolic–polylactic scaffold; MSCs = Mesenchymal stem cells; PRP = Platelet-rich plasma; OPT = Orthopantomography; BBM = Bovine bone matrix; ACBCs = Autogenous culture-expanded bone cells; hBMSCs = Human bone marrow-derived mesenchymal stem cells; CT = Computer Tomography; RCT = Randomized controlled trial; hDPSCs = Human dental pulp stem cells

Table 2: Characteristics of the included studies.

and the control side was grafted with FDBA alone. Bone biopsies were performed after 3 months at the time of implant placement. Histological analyses revealed that the control samples showed substantial persistence of the FDBA particles, separated from the trabeculae of NB bone by large amounts of fibrous connective tissue; in the test samples, the graft was adjacent to or embedded within the NB, without the interposition of fibrous connective tissue.

Polyglycolic-polylactic scaffold

Four articles had reported on the use of PLGA scaffold in the sinus lifting procedures. In a non-randomized clinical study, Shimming et al. [9] carried out 41 sinus lift procedures (17 one-stage and 24 two-stage procedures) in 27 patients (45-57 years old) using PLGA fleece (Ethisorb®, Ethicon, Norderstedt, Germany), and used cultures of osteogenic cells deriving from mandibular periosteum. In all cases of the one-step procedure, radiologic (OPT) and clinical assessments were performed 3 months after surgery, which revealed excellent results in 66.6% of the patients. CT was carried out in selected cases. Bone biopsy samples were obtained at the time of implant placement, but only in 16 of the two-stage procedures, because in 8 patients no NB was detected at clinical inspections performed 3 months after augmentation. Histological analyses revealed mineralized trabecular bone, and the presence of residual biomaterial between trabeculae and osteocytes in lacunae within the bone substance.

In a split-mouth case-control study, Mangano et al. [12] compared the outcomes of 10 two-stage sinus augmentations that were performed bilaterally in 5 patients (45-65 years old) using autogenous osteoblasts seeded on PLGA scaffold (Oral Bone®, BioTissue Technologies, Freiburg, Germany) in the test sites and blocks of coral-derived porous hydroxyapatite (Biocoral, Novaxa Spa, Milan, Italy) in the control sites. CT data were acquired at baseline and at 6 months after surgery. Bone biopsies were performed at the time of implant placement after a healing period of 6 months. A radiographic comparison of the test and control sites showed vertical bone gains of 6.47 ± 1.39 mm and 9.14 ± 1.19 mm, respectively. Bone biopsies performed at both sites showed the presence of mature bone with compact and cancellous areas. Histomorphometrically the biopsy samples obtained from engineered bone revealed 37.32 ± 19.59% bone spaces and 62.67 ± 27.71% medullar spaces. Sinus grafted with hydroxyapatite comprised 54.65 ± 21.17% NB, 17.56 ± 5.03% medullar spaces and 27.78 ± 16.31% remaining material particles. Biopsies performed at both sites showed the presence of mature bone with compact and cancellous areas. From these results the authors concluded that PLGA scaffold plus autogenous osteoblasts showed a poor efficacy in promoting cellular activity and bone regeneration.

In a non-randomized clinical study, Voss et al. [14] evaluated bone regeneration in sinus lift procedures comparing the use of periosteum stem cells and PLGA scaffold (Ethisorb®, Ethicon) in 35 patients (35-69 years old) and autogenous iliac bone in 41 patients (38-73 years old). Among the 35 patients in the study group, 15 underwent a bilateral sinus lift procedure (50 test sites) and 17 received a two-stage procedure. In the control group, 22 patients underwent augmentation of both sinuses (65 control sites). Bone biopsy samples were obtained after a healing period of 15 weeks in selected two-stage cases during the insertion of the implants. Radiologic evaluations were performed using OPT before surgery, before and after implant placement, and after the final prosthetic restoration. The clinical and radiologic follow-up lasted at least 24 months. Biopsy specimens obtained from 7 patients in the study group showed mineralized trabecular bone with remnants of biomaterial and the presence of osteocytes within the bony lacunae, while in 10 patients (16 sinuses) the grafts had acquired a connective-tissue-like consistency, and so an additional augmentation procedure with autologous bone and bone substitutes was required. No patient in the control group required a second operation, and only 1 implant failed, compared to 11 in the study group. The rate of complications (sinusitis, abscesses, loss of augmented material and loss of implants) was higher in the study group than in the control group.

Trautvetter et al. [18] evaluated the use of the same polymer scaffold in a 5-year retrospective cohort. Ten patients were treated with 17 one-step sinus lifts (bilaterally in 7 patients) using autologous tissue-engineered periosteal bone grafting (Oral Bone®, BioTissue Technologies). Radiologic examinations (OPT and CT) and clinical evaluations were carried out preoperatively and at 4, 12, 24 and 60 months post-surgery. Bone biopsy specimens were obtained in two cases after 6 months. The median bone height, measured in 27 regions of interest, increased from 6.9 mm, preoperatively, to 16.0 mm at the 4-month follow-up, and then remained significantly greater ($P<0.05$, median 14.2 mm) during the 5-year observation period. Histological examinations performed at 6 months confirmed the formation of full grown/mature bone, with osteocytic cells and/or osteocytes embedded in the trabecular bone lacunae and osteoblasts actively forming NB. No remnants of the biomaterial, no formation of connective tissue and no signs of necrosis or cell apoptosis were seen.

Collagen sponge

Only two studies had used collagen as a scaffold material [10,21]. Springer et al. [10] reported on a comparative study of three regenerative approaches for the two-stage sinus lift procedure. In group 1 (test group), 12 sinuses in 8 patients (51.4-65.2 years old) were augmented with periosteum-derived stem cells seeded on 4 sheets of collagen matrix (Lyostyp, Braun); in group 2A (control group), 3 sinuses in 2 patients (43 and 56 years old) were regenerated with a combination of cultured autogenous osteoblasts (taken from the maxillary tuberosity) and BBM blocks (Bio-Oss®, Geistlich Pharma, Wolhusen, Switzerland); and in group 2B (another control group), 5 sinuses in 3 patients (46-58 years old) were treated with BBM blocks alone. Bone biopsy samples were obtained during implant placement at 6 months in group 1 and at 8 months in groups 2A and 2B. None of the implants were lost and no adverse effects were seen throughout the observation period: 12–38 months in group 1 and approximately 7 years in groups 2A and 2B. In group 1 the core biopsies showed vital woven, partly lamellar bone, and small remnants of collagen matrix and an NB density of 38% (range 30.5-51%), which did not differ significantly from that in group 2A (range 32–43%).

Giuliani et al. [21] assessed the stability and quality of regenerated bone in bilateral post-extractive third molar sockets, filled with equine collagen I sponge (Gingistat, Vebas, San Giuliano Milanese, Italy) with (test site) or without (control site) third molar dental pulp stem cells, at 3 years after grafting. The sample (7 patients aged 24-40 years) was the same as that in a previously published study [23]. Clinical and radiologic evaluations were performed, and bone biopsy samples were obtained from all patients at 3 months after augmentation, using the replacement jigs used in the previous study.

The clinical assessment showed no signs of infection or morbidity in the surgical areas, and periodontal probing gains of 6.3 ± 2.1 mm and 4.5 ± 1.4 mm at the test and control sites, respectively. The completeness of regeneration and the improved vertical bone height in the test sites relative to the control sites were confirmed by OPT. Histological analyses revealed that the collagen sponge used as a scaffold was always

completely reabsorbed, and that the regenerated bone at the test sites was characterized by a compacted architecture, with Haversian canals surrounded by several lamellae and osteocyte-containing lacunae. In contrast, the bone at the control sites was characterized by a cancellous (spongy) structure, with interrupted lamellae surrounding numerous large marrow-filled spaces arranged in a more-or-less regular pattern.

Bovine bone matrix scaffold

BBM was used as a scaffold in five studies: two [11,13] fulfilled the criteria for inclusion in the systematic review, and three [16,17,19] were RCTs and could be included in the meta-analysis.

In a prospective clinical study, Fuerst et al. [11] described the healing process in 22 two-stage sinus lift procedures applied to 12 patients (age 56.2 ± 9.3 years) with a mixture of bovine bone mineral granules (Bio-Oss®, Geistlich Pharma) and autogenous bone cells harvested from the anterior iliac crest (9 patients) or from the chin (3 patients). CT scans were performed after sinus grafting (CT1), after implant placement (CT2) and after uncovering the implant (CT3). Bone biopsy samples were obtained after 6 months during implant insertion. The post-operative healing period was uneventful, but 3 of the 82 placed implants were removed due to implant mobility after uncovering the implant. The graft volume was 2218.4 ± 660.9 mm^3 at the time of CT1, 1694.0 ± 470.4 mm^3 at CT2 and 1347.9 ± 376.3 mm^3 at CT3, with significant progressive decreases of 23.62%, 20.45%, and 39.24% from CT1 to CT3. Histologically the BBM and bone were unevenly distributed: NB was woven and vital, and some of the BBM granules were surrounded by NB and some by connective tissue. A histomorphometric analysis revealed that NB and BBM were 17.9 ± 4.6% and 19.4 ± 10.1%, respectively, and that 26.8 ± 13.1% of the BBM surface was in contact with NB.

Sauerbier et al. [13] compared the NB formation in the two-stage sinus lift procedure using BBM granules (Bio-Oss®, Geistlich Pharma) and bone-marrow-aspirate-derived mesenchymal stem cells harvested from the pelvis, and processed by a FICOLL (Sigma, St Louis, MO, USA) or BMAC (Bone Marrow Procedure Pack, Harvest Technologies Corporation, Plymouth, MA, USA) method.

In total, 18 sinuses (6 in the FICOLL group and 12 in the BMAC group) were augmented in 11 patients (4 in the FICOLL group and 7 in the BMAC group). In a second-stage procedure performed after 3 months, bone biopsy samples were obtained and implants were placed. Of the 50 implants inserted (17 FICOLL and 33 BMCA), only 1 implant in the BMCA group failed before applying prosthetic loading. Histological specimens from the FICOLL and BMCA groups showed similar results: the newly formed osseous lamellae appeared as vital bone tissue containing osteocytes inside the bone lacunae, which connected the BBM particles and stabilized the graft complex. The histomorphometric analysis produced the following estimated values: 19.9% NB for BMCA and 15.5% NB for FICOLL, significantly more residual biomaterial in BMCA (31.9%) than in FICOLL (19.7%), and a significantly smaller marrow space in BMCA (47.4%) than in FICOLL (64.8%).

The three RCTs included in the meta-analysis comprised two with a parallel-group design [17,19] and one with a split-mouth design [16].

In a randomized controlled split-mouth study, Rickert et al. [16] analysed histomorphometrically the percentage of NB in 12 consecutive edentulous patients (48-69 years old) after performing bilateral maxillary sinus floor elevation. On one side the augmentation procedure was performed with BBM (Bio-Oss®, Geistlich Pharma) seeded with bone-marrow-aspirate autogenous bone cells harvested from the posterior iliac crest (test group), while on the contralateral side the augmentation procedures were performed with BBM mixed with autogenous bone that had been harvested from the retromolar area (control group), with the two sides allocated randomly. Biopsy samples were obtained after 13–16 weeks, and 66 implants were placed. One patient was excluded from the histological analysis because the biopsy sample of the control side was not obtained from an augmented site. The amount of NB formation was significantly greater in the test group (17.7 ± 7.3%) than in the control group (12.0 ± 6.6%). At 3 months after sinus augmentation, the percentage of BBM present in the samples was comparable in the test and control groups. Histological analysis showed that NB lamellae surrounded the biomaterial particles and stabilized the graft complex. No signs of inflammatory reaction were observed.

Sauerbier et al. [17] performed 45 two-stage sinus lift augmentation procedures in 26 of 40 randomized patients (38.9–67.7 years old). Thirty-four sinuses of 25 patients (test arm) were augmented with BBM (Bio-Oss®, Geistlich Pharma) in combination with pelvic bone-marrow concentrate aspirate, while 11 sinuses (control group) in 11 patients were grafted with a mixture of 70% BBM and 30% autologous bone harvested from the retromolar area. Each sinus was randomly assigned to either the test or control arm, and in 10 patients with a double sinus lift, the randomization resulted in a split-mouth model. Bone biopsy samples were obtained after a healing period of 3.41 ± 0.39 months at the time of implant insertion. The histological findings were similar in all of the specimens. No signs of inflammation were detected, vital bone tissue containing osteocytes inside the bone lacunae was observed, and the NB connected the biomaterial particles and stabilized the grafted complex. In a histomorphometric analysis the amount of NB formation did not differ significantly between the control (14.3 ± 1.8%) and test (12.6 ± 1.7%) groups. Cone-beam CT performed on 28 test and 9 control sinuses revealed that the volume of augmented bone was significantly greater in the test group (1.74 ± 0.69 mL) than in the control group (1.33 ± 0.62 mL).

Hermund et al. [19] evaluated histologically the bone formation in two-stage sinus floor augmentation procedures, comparing the use of composite graft (BBM and autogenous bone harvested using a scraper) alone (control group) or supplemented with cultivated autogenous bone cells derived from the tuberosity area (test group). Twenty maxillary sinus lift procedures were carried out in 20 patients randomly assigned to the test group (10 patients, age 60.4 ± 11.2 years) or the control group (10 patients, age 58.5 ± 8.1 years).

Implants (n=39) were placed after 4 months, and bone biopsies were performed at the same time. All implants were osseointegrated and loaded. There were no remarkable differences between the bone specimens in the two groups. In biopsy specimens from caudal portions, the mostly woven and occasionally lamellar NB was in contact with the BBM particles, which occasionally were completely incorporated within the new osseous tissue. In contrast, only a small amount of NB was found in the more apical aspects, where the biomaterial was mostly embedded within fibrovascular connective tissue.

The potential risks of bias in the studies included in the meta-analysis are summarized in Figure 2. Two trials [16,19] were judged to be at low risk of bias, whereas one [17] was judged to be at high risk of bias due to the specific study design used. This meta-analysis found insufficient evidence for determining whether there was a difference in NB formation after sinus floor augmentation performed with BBM or BBM and stem cells (Figure 3).

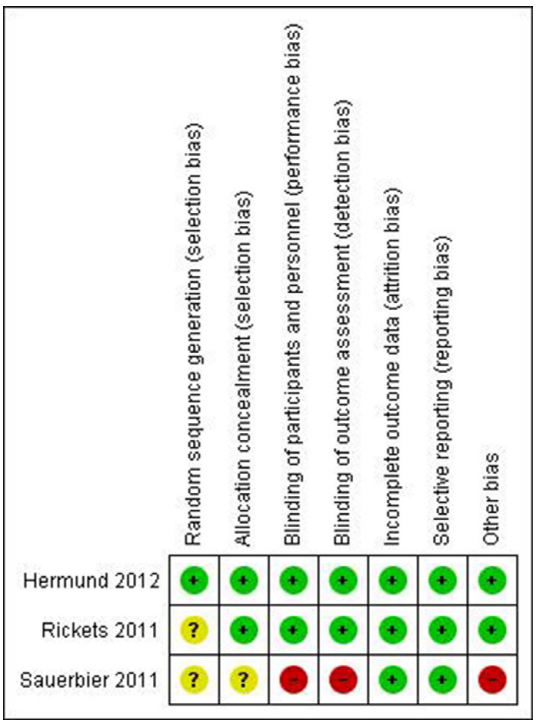

Figure 2: Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Discussion

The purpose of this review was to determine the clinical and histological performances of different mesenchymal cell-scaffold constructs used for oral bone regeneration in human subjects. The systematic search revealed a paucity of publications and high heterogeneity among the various studies. Indeed, while there have been numerous *in vitro* and animal studies of engineered stem-cell scaffolds, few researches have involved human subjects, probably due to the difficulty of obtaining consent from the relevant ethics committees. Moreover, the lack of blinding and randomization, and differences between model protocols, study designs, the included human and cellular populations, and surgical techniques made it difficult to compare the results and draw significant conclusions.

This review found that the most commonly used surgical procedure for testing the outcome of tissue-engineered scaffolds is the two-stage sinus lift [9,20]. This procedure is a good clinical model for evaluating bone regeneration, because bone formation occurs within an enclosed space, and hence with a minimal interference from external factors [17]. In addition, the two-stage sinus lift procedure is more predictable than vertical bone regeneration and allows bone biopsy specimens to be collected during implant insertion, thereby avoiding any additional discomfort for the patients [24].

While many different materials for promoting bone regeneration have been tested, none of them has satisfied all of the requirements for an ideal scaffold. Based on the current literature, an ideal scaffold should maintain an adequate 3D shape after implantation, facilitate cell adhesion and proliferation, promote cell growth, and be mechanically strong [1,3,24-26]. Furthermore, it should be biocompatible, biodegrade into no toxic by-products, easily handled and easily processed during manufacture. The scaffold should have an appropriate macrostructure (in terms of the surface geometry and a porous structure with a pore size of 100–700 μm^2) and microstructure to induce cell attachment, and an adequate molecular structure to induce specific tissue responses [1,3,24-26]. Finally, it should exhibit osteoinductive/osteoconductive properties and a resorption time compatible with tissue regeneration [1]. No ideal scaffold has yet been developed, and the evidence for the use of different investigated materials remains controversial.

The three included studies [15,20,22] that used an allogenic graft as a scaffold for mesenchymal stem cells produced encouraging findings in terms of NB formation. However, it is impossible to compare their results due to differences in the types of allograft, sources of stem cells, laboratory procedures, surgical interventions and methods used to analyze NB. Indeed, the properties of allografts change significantly during decellularization, sterilization and storage processes. When the method of processing removes the viable cells (osteogenic and osteoinductive) and leaves the extracellular matrix (osteoconductive), the allograft is osteoconductive, whereas when leaving factors such as bone morphogenetic proteins and transforming growth factor-β, the demineralized bone matrix can be osteoinductive and able to recruit mesenchymal stem cells and stimulate their differentiation into osteoprogenitor cells [1].

PLGA scaffold is a widely investigated biomaterial in bone regeneration [2,9,12,14,18,27,28], although the few studies that have investigated its utility as a scaffold in tissue engineering have produced conflicting results. Insufficient clinical success in the sinus lift–procedures due to poor efficacy in promoting cellular activity and bone regeneration was reported by Mangano [12] and Voss [14], whereas good clinical and radiologic results were found in the studies of Trautvetter [18] and Shimming [9]. Insufficient bone regeneration and a high resorption rate have been reported when two-stage sinus lift procedures were performed with stem cells and PLGA scaffolds [9,14,18,27,28]. Augmentation failure could be caused by the supply of oxygen and nutrients being insufficient to sustain the survival and proliferation of cells embedded within a large polymer construct, and the low pH produced by polymer resorption, which could prevent the survival of osteoblasts. Furthermore, an increase in fibrous tissue encapsulation during healing might be due to foreign-body reactions to acidic polymer degradation products induced by the hydrolysis of

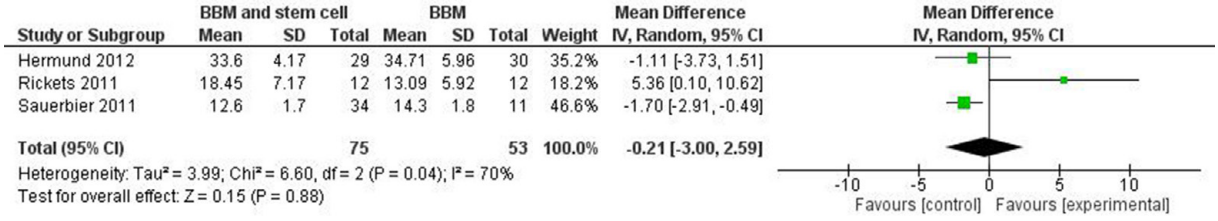

Figure 3: Forest plot of comparison: BBM versus BBM and stem cell.

PLGA bulk erosion *in vivo* [1]. Therefore, such tissue-engineered bone should be used only in sinus lift procedures with simultaneous implant placement and at sites that provide sufficient bone.

Major advantages have been reported when using collagen sponge scaffolds, including biocompatibility, biodegradability and the ability to bind growth factors critical for osteoconduction [10,21,23]. A tissue-engineered combination of a collagen matrix with autologous cells, derived from periosteum or dental pulp, tested in two of the included studies, was shown to be capable of creating NB tissue and exhibit a high mineralization rate. However, the reported data relate to small numbers of procedures and patients and short observation periods, and so they must be considered with extreme caution [10,21,23,28].

BBM is one of the most-used and well-studied grafting materials in bone reconstructive procedures. BBM exhibits excellent osteoconductive properties due to its morphological structure and mineral composition, which are similar to those of human cancellous bone, and its widespread interconnecting pore system promotes angiogenesis and the migration of osteogenic cells [29].

The present review found that more studies have investigated the use of BBM as a scaffold construct with mesenchymal cells. The availability of three RCTs made it possible to perform a meta-analysis, but this found that the available evidence is insufficient for determining whether NB formation after sinus floor augmentation differed between using BBM or BBM and stem cells. Indeed, the results were contradictory. Rickets et al. [16] reported an improved bone formation when the biomaterial was mixed with stem cells, whereas other studies found that the addition of autogenous stem cells to a graft of BBM [11,17] or to a composite graft of BBM and autogenous bone [19] did not exert statistically significant effects on the amount of NB formed.

Conclusions

The results of the present review should be interpreted with caution since few studies have histologically evaluated the performance of mesenchymal cell-scaffold constructs in human oral reconstructive procedures. The very small amount of available data makes it impossible to draw firm conclusions regarding any increase in bone formation achieved by using graft materials engineered with autogenous stem cells.

Further well-designed trials involving larger samples and longer follow-up periods are required to improve the level of evidence in order to understand whether mesenchymal cell-scaffold constructs offer significant long-term benefits to patients.

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