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**REVIEW**

# Alveolar bone tissue engineering in critical-size defects of experimental animal models: a systematic review and meta-analysis

Siddharth Shanbhag[1,2], Nikolaos Pandis[3], Kamal Mustafa[1], Jens R. Nyengaard[4] and Andreas Stavropoulos[2]*

[1]*Department of Clinical Dentistry, Centre for Clinical Dental Research, University of Bergen, Bergen, Norway*
[2]*Department of Periodontology, Faculty of Odontology, Malmö University, Malmö, Sweden*
[3]*Department of Orthodontics and Dentofacial Orthopedics, University of Bern, Bern, Switzerland*
[4]*Stereology and Electron Microscopy Laboratory, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark*

## Abstract

**Regeneration of large, 'critical-size' bone defects remains a clinical challenge. Bone tissue engineering (BTE) is emerging as a promising alternative to autogenous, allogeneic and biomaterial-based bone grafting. The objective of this systematic review was to answer the focused question: in animal models, do cell-based BTE strategies enhance regeneration in alveolar bone critical-size defects (CSDs), compared with grafting with only biomaterial scaffolds or autogenous bone? Following PRISMA guidelines, electronic databases were searched for controlled animal studies reporting maxillary or mandibular CSD and implantation of mesenchymal stem cells (MSCs) or osteoblasts (OBs) seeded on biomaterial scaffolds. A random effects meta-analysis was performed for the outcome histomorphometric new bone formation (%NBF). Thirty-six studies were included that reported on large- (monkeys, dogs, sheep, minipigs) and small-animal (rabbits, rats) models. On average, studies presented with an unclear-to-high risk of bias and short observation times. In most studies, MSCs or OBs were used in combination with alloplastic mineral-phase scaffolds. In five studies, cells were modified by *ex vivo* gene transfer of bone morphogenetic proteins (BMPs). The meta-analysis indicated statistically significant benefits in favour of: (1) cell-loaded vs. cell-free scaffolds [weighted mean difference (WMD) 15.59–49.15% and 8.60–13.85% NBF in large- and small-animal models, respectively]; and (2) BMP-gene-modified vs. unmodified cells (WMD 10.06–20.83% NBF in small-animal models). Results of cell-loaded scaffolds vs. autogenous bone were inconclusive. Overall, heterogeneity in the meta-analysis was high ($I^2 > 90\%$). In summary, alveolar bone regeneration is enhanced by addition of osteogenic cells to biomaterial scaffolds. The direction and estimates of treatment effect are useful to predict therapeutic efficacy and guide future clinical trials of BTE. Copyright © 2016 John Wiley & Sons, Ltd.**

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**Keywords** **bone tissue engineering; bone regeneration; mesenchymal stem cells; scaffolds; meta-analysis**

## 1. Introduction

Reconstruction of alveolar bone deficiencies, resulting from ageing, trauma, ablative surgery or pathology, remains a clinical challenge (Götz *et al*., 2015). Although autologous bone transplantation is still considered the 'gold standard' for maxillofacial bone regeneration (Corbella *et al*., 2015; Fretwurst *et al*., 2015), large defects may require volumes of bone that are locally unavailable. Moreover, the morbidity associated with bone harvesting can be a major limiting factor (Nkenke and Neukam, 2014). Alternatives have included allogeneic, xenogeneic and alloplastic bone substitutes, but no consensus currently exists on the effectiveness of one material over the other in comparison with autogenous bone, or for all indications (Al-Nawas and Schiegnitz, 2014; Milinkovic and Cordaro, 2014).

The bone tissue engineering approach involves harvesting of osteogenic cells [most commonly mesenchymal stem cells (MSCs)] from an autologous source (e.g. bone marrow, adipose tissue etc.), their *in vitro* culture expansion and combination with an appropriate carrier scaffold for implantation *in vivo* (Shanbhag and Shanbhag, 2015). Thus, the 'triad' of osteogenic cells, osteoinductive signals (growth factors released by cells), and osteoconductive scaffolds, replicates the properties of autogenous bone, without the need for invasive harvesting (Oppenheimer *et al*., 2012). The prospects for use of such tissue-engineered products for alveolar bone repair are very promising, as demonstrated by recent clinical studies (Padial-Molina *et al*., 2015; Shanbhag and Shanbhag, 2015).

Preclinical testing of new regenerative therapies in clinically relevant animal models is an important aspect of translational research and, in most cases, a requirement of regulatory health agencies before initiating human clinical trials (Pellegrini *et al*., 2009; Stavropoulos *et al*., 2015). The advantage of animal models, in addition to

*Correspondence to: A. Stavropoulos, Department of Periodontology, Faculty of Odontology, Malmö University, Carl Gustafs väg, 20506 Malmö, Sweden. E-mail: andreas.stavropoulos@mah.se

testing therapeutic safety and efficacy, is the possibility of better understanding of the underlying biological processes via methods that may be considered too invasive for human application (e.g., repeated harvesting of biological samples, biopsies, etc.) (Peric *et al*., 2015). Small-animal models (rodents and rabbits) usually constitute a starting point for proof-of-principle or feasibility studies, while studies in large-animal models (dogs, pigs, sheep, and non-human primates) attempt to simulate clinical conditions more closely and predict therapeutic efficacy (Stavropoulos *et al*., 2015).

The 'calvarial critical-size defect' (CCSD) is a widely used experimental model for screening bone biomaterials in small and large animals. This is the smallest-size experimental defect in the cranium of the animal that will not spontaneously and completely regenerate with bone in a defined time-frame without intervention (Schmitz and Hollinger, 1986; Vajgel *et al*., 2014). However, CCSD may reflect poorly the clinical scenario of alveolar bone defects, given the variation in development and healing pattern between different skeletal sites (Quarto *et al*., 2010; Ichikawa *et al*., 2015), and the additional influence of dental and masticatory factors on alveolar bone physiology (Liebschner, 2004; Bagi *et al*., 2011).

For this reason, critical-size defect (CSD) models have been developed involving the maxillary and mandibular bones of small and large animals. The aim of the present study was to systematically review the available literature to answer the focused 'PICO' (population, intervention, comparison, outcome) question: In alveolar CSD of experimental animals, does a tissue engineering approach (implantation of osteogenic cells seeded on biomaterial scaffolds), enhance histomorphometric bone regeneration, compared with grafting with only biomaterial scaffolds or autogenous bone? Based on the nature of the data retrieved, it was also aimed to perform a meta-analysis of the efficacy of 'cell-based' vs. 'cell-free' approaches, to determine the estimates and, more importantly, the direction of treatment effect for guiding future human clinical trials.

## 2. Material and methods

### 2.1. Study design

A review protocol was developed based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Moher *et al*., 2009), and predetermined inclusion/exclusion criteria.

Inclusion criteria were:

1. English language studies.
2. Randomized or non-randomized controlled animal experimental studies with two or more experimental groups.
3. Use of experimental CSD in the maxillae or mandibles of small- or large-animal models (CSD were defined by the inclusion of an untreated or 'empty defect' control group in which the defects did not heal throughout the observation period, or if the reported model was based on a referenced previous confirmatory study).
4. Transplantation of cultured autologous, allogeneic or human-derived osteogenic cells [MSCs or osteoblasts (OBs)] seeded on biomaterial scaffolds in at least one experimental group.
5. A control group receiving 'cell-free' biomaterial scaffolds or autogenous bone.
6. Reporting of quantitative histomorphometric new bone formation (%NBF), which was selected as the primary outcome (Vajgel *et al*., 2014). Studies reporting quantitative radiographic assessments of bone formation via computerized tomography (CT) or micro-CT were considered separately.

Exclusion criteria were:

1. *In vitro* studies.
2. *In vivo* animal studies reporting CSD in other anatomical sites (calvarial or non-craniofacial), ectopic (e.g. subcutaneous) models or systemic cell-delivery.
3. Absence of a control group.
4. *In vivo* animal studies reporting alveolar bone CSD with only qualitative or semiquantitative histological analyses.

### 2.2. Search strategy, screening and study selection

Electronic databases of MEDLINE (via PubMed) and EMBASE were searched for relevant English-language literature up to and including June 2015. Unpublished literature was searched via the Google and Google Scholar search engines. Bibliographies of the studies selected and relevant review articles were checked for cross-references. A specific search strategy was developed for MEDLINE (see the Supplementary material online) and adapted for other databases.

Titles and abstracts of the search-identified studies were screened by two authors (S.S. and A.S.) and full texts of all eligible studies were obtained. Uncertainty in the determination of eligibility was resolved by discussion with the other authors. Two authors (S.S. and A.S.) reviewed the selected full texts independently and final inclusion was based on the aforementioned inclusion criteria. A summary of the screening process is presented in Figure 1.

### 2.3. Data extraction

Data was extracted from the full texts of selected articles on: author(s), study design, animal species, model type, number of animals/defects, number of procedures, inclusion criteria, observation time(s), outcome(s), method (s) of outcome evaluation, main findings and conclusions. Descriptive summaries of studies included were entered into tables. Quantitative histomorphometric data regarding %NBF was extracted for possible meta-analysis. Standard errors of mean, when reported, were converted



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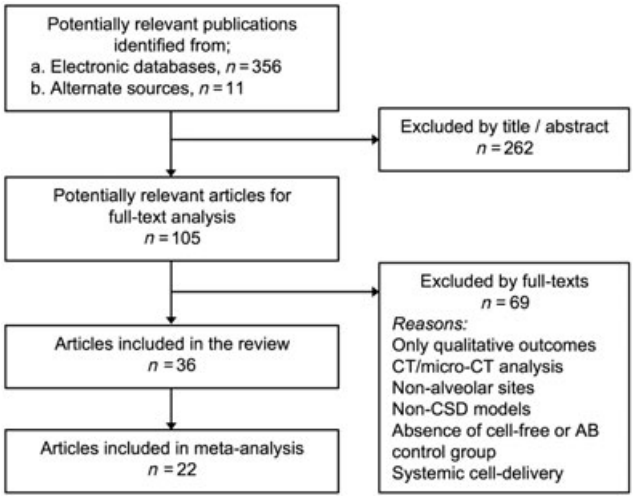


**Figure 1. Flowchart for study screening and selection. AB, autogenous bone; CSD, critical-size defect; CT, computed tomography; 'n', number of articles**

to standard deviation (SD) for analysis. If data were only expressed graphically, numerical values were requested from the authors, and if no response was received digital ruler software was used to measure graphical data (ImageJ; National Institutes of Health, Bethesda, MD, USA). When studies reported outcomes at multiple time-points, data from similar time-points of different studies were pooled for meta-analysis.

### 2.4. Quality assessment and risk of bias

Reporting quality assessment of all studies was performed based on a modification of the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines, regarding relevant items (Kilkenny *et al*., 2010; Berglundh and Stavropoulos, 2012). Compliance with the guidelines was evaluated using a predefined grading system applied to each of the 20 items (Schwarz *et al*., 2012; Supporting information). Reporting quality was judged as 'high', 'moderate' or 'low'. Risk of bias (RoB) assessment was performed using a modification of SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) RoB tool for animal studies, and judged as 'high', 'low' or 'unclear' (Hooijmans *et al*., 2014b; Yan *et al*., 2015; see the Supplementary material online). Any disagreement between the reviewers during study selection, data extraction, and quality assessment was resolved by discussion and consensus.

### 2.5. Meta-analysis

Meta-analysis was performed to compare the effectiveness of cell-loaded [experimental (Ex)] and cell-free [control (Co)] scaffolds using histomorphometric data [means and SD of %NBF and number of animals/defects ($n$) per group]. Studies were pooled based on homogeneity regarding PICO and observation time. Subgroup analyses were performed at the level of animals and observation time, using the DerSimonian and Laird random effects model (Deeks *et al*., 2008) and STATA Statistical Software (StataCorp LP, College Station, TX, USA). When studies included multiple Ex-groups and one Co-group, the number of animals/defects ($n$) in the Co-group was divided by the number of Ex-groups (Vesterinen *et al*., 2014). In several studies, the design included multiple interventions per animal (e.g., 'split-mouth' design), for which a correlation coefficient ($r$) was calculated for between treatment group measurements. The $r$-value, calculated from one split-mouth study (Haghighat *et al*., 2011) using the $p$-value provided (Higgins and Deeks, 2008), was close to 0.80 and this was used for the meta-analysis. For parallel group studies, the $r$-value was set at 0, and thus, both parallel and split-mouth studies were included in the same analysis, if appropriate (Higgins *et al*., 2008). To assess robustness of the findings, sensitivity analyses were performed using $r$-values of 0.50 and 0.20. Pooled estimates of treatment effect [weighted mean differences (WMD)] were calculated along with 95% confidence interval (CI), and the $I^2$ statistic was used as a measure of inconsistency of results across studies (Deeks *et al*., 2008).

## 3. Results

### 3.1. Search results and study characteristics

Of the 367 search-identified studies, 36 studies reporting quantitative histomorphometric outcomes from 6 different species and 636 animals were included in the review. A list of studies excluded along with reasons for exclusion is reported in the Supplementary material online. Large-animal models included monkeys (one study, $n$ = 24), dogs (14 studies, $n$ = 94), sheep (one study, $n$ = 8) and minipigs (four studies, $n$ = 38) (Table 1). Small-animal models included rabbits (eight studies, $n$ = 179) and rats (eight studies, $n$ = 293) (Table 2). Sample sizes ranged from 2–24 and 9–75 for the large- and small-animal models, respectively. Observation times varied between species: monkeys (6 months), dogs (4 weeks to 12 months), sheep (5 months), minipigs (8–12 weeks), rabbits (4–24 weeks) and rats (4–8 weeks). Nine studies – six in dogs, one in pigs, and one each in rabbits and rats – included a control group receiving autogenous bone.

### 3.2. Quality assessment and risk of bias

Most studies provided adequate information regarding title, abstract, introduction, study objectives, ethical approval, experimental design and procedures (Figure 2). Information regarding experimental animals, and their housing and husbandry, was generally inadequate; the majority of studies lacked complete information regarding animals' age and gender (Tables 1 and 2). No studies provided information on sample-size calculation or baseline characteristics of the animals. In 19 studies (52.7%), animals or defects were randomly allocated to different treatment groups to minimize 'selection bias', although no



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**Table 1. Summary of study characteristics in large-animal models**

| Study | Strain, age, gender | Defect model, dimensions | *n* | Time | Cells | Source, induction | Cell number | Scaffold, AB if used |
|---|---|---|---|---|---|---|---|---|
| **Monkeys** | | | | | | | | |
| Chanchareonsook *et al*. 2014b | *Macaca fascicularis*, adult male | Mandible SD, 15 mm, length | 24 | 6 months | BMSC | Femur, auto; no | $5 \times 10^6$ | PCL–HA ± BMP-2 |
| **Dogs** | | | | | | | | |
| De Kok *et al*. 2003 | Beagle, adult | Mandible CSD (s), 20 × 6.5 mm | 14 | 4 weeks, 9 weeks | BMSC | Iliac, auto or allo | $1 \times 10^6$ | HAβ–TCP |
| Yamada *et al*. 2004 | Hybrid, adult | Mandible CSD (s), 10× 10 mm | 4 | 8 weeks | BMSC | Iliac, auto; yes | $1 \times 10^7$/ml | PRP gel, iliac AB |
| Yoshimi *et al*. 2009 | Hybrid, adult | Mandible CSD (s), 10× 10 mm | Unclear | 8 weeks | BMSC | Iliac, auto; yes | $1 \times 10^7$/ml | Peptide ECM ± PRP |
| Yamada *et al*. 2011 | Hybrid, adult | Mandible CSD (s), 10× 10 mm | Unclear | 8 weeks | BMSC or DPSC | Iliac, auto; auto or puppy; yes | $1 \times 10^7$/ml | PRP gel |
| Jafarian *et al*. 2008 | Mongrel, adult | Mandible CSD (s), 10 mm, diameter | 4 | 6 weeks | BMSC | Humerus, auto; no | $5 \times 10^5$ | HAβ–TCP–Col or Bio–Oss–Col® |
| Vahabi *et al*. 2012 | Hybrid, 1 year, male | Mandible CSD (s), 10 mm, diameter | 5 | 8 weeks | BMSC | Iliac, auto; no | $5 \times 10^5$ | HAβ-TCP |
| Khojasteh *et al*. 2013 | Mongrel, adult male | Mandible CSD (s), 20× 10× 10 mm | 4 | 8 weeks | BMSC | Humerus, auto; no | $5 \times 10^5$ | PCL-TCP |
| Haghighat *et al*. 2011 | NR, 3y | Mandible CSD (s), 9 mm, diameter | 4 | 6 weeks | ADSC | Thoracic, auto; no | $5 \times 10^6$ | Collagen |
| Behnia *et al*. 2014 | Mixed, adult male | Mandible CSD (s), 9 mm, diameter | 4 | 12 weeks | SHED | Human; no | $1 \times 10^6$ | Collagen |
| Zhao *et al*. 2009 | Mongrel, adult male | Mandible SD (s), 20× 10 mm | 14 | 12 months | BMSC | Iliac, auto; yes | $5 \times 10^7$ | Silk-polymer ± apatite, mandible AB |
| Wang *et al*. 2015 | Beagle, adult, male | Mandible SD, 30 mm, length | 16 | 12 mo | OB, fresh or cryo | Mandible, auto; yes | $2 \times 10^7$ | β-TCP, mandible AB |
| Zhang *et al*. 2011 | Beagle, 24 weeks, male | Maxilla cleft (s), 10× 5× 15 mm, ortho. Movement | 7 | 20 weeks | BMSC | Iliac, auto; yes | $2 \times 10^7$ | β-TCP, iliac AB |
| Pourebrahim *et al*. 2013 | Mongrel, adult | Maxilla cleft (s) 15 mm, width, 2 months' healing | 4 | 15 days, 60 days | ADSC | Scapula, auto; no | $5 \times 10^6$ | HAβ–TCP (no scaffold only) tibial AB |
| Huang *et al*. 2015 | Beagle, 24 weeks, male | Maxilla cleft, 15 mm, width, RME | 14 | 12 weeks | BMSC | Iliac, auto; yes | Unclear | β-TCP, Iliac AB |
| **Sheep** | | | | | | | | |
| Schliephake *et al*. 2001 | NR, adult female | Mandible SD, 35 mm, length | 8 | 5 months | OB | Iliac bone, auto; no | $1–5 \times 10^6$ | Bovine bone |
| **Minipigs** | | | | | | | | |
| Zheng *et al*. 2009 | Inbred, 4–6 months, female | Mandible CSD, 25× 15× 15 mm | 16 | 6 months | DPSC | Deciduous, auto; no | $4 \times 10^8$ | β-TCP |
| Pieri *et al*. 2009 | NR, adult | Mandible CSD (s), 3.5× 8 mm | 8 | 3 months | BMSC | Iliac, auto; no | $4 \times 10^7$ | HA-PRP, mandible AB |
| Konopnicki *et al*. 2015 | Yucatan | Mandible CSD (s), 20 × 20 mm | 2 | 8 weeks | BMSC | Iliac, auto; yes | $30 \times 10^6$ | PCL–TCP |
| Kuo *et al*. 2015 | Lanyu, 3 months | Mandible CSD (s), 6 mm, diameter | 12 | 8 weeks | DPSC | Commercial, human; no | $2 \times 10^6$ | α-CSH, α-CSH/ACP or α-CSH/β-TCP |

CSD, critical-size defect; SD, segmental defect; (s), split-mouth design; n, number of animals; ortho. Movement, orthodontic tooth movement; RME, rapid maxillary expansion; AB, autogenous bone; BMSC, bone marrow MSC; ADSC, adipose tissue-derived MSC; OB cryo, cryopreserved osteoblasts; DPSC, dental pulp stem cells; SHED, stem cells from human exfoliated deciduous teeth; Auto, autologous; Allo, allogeneic; Human, human-derived; PRP, platelet-rich plasma; HA, hydroxyl-apatite; β-TCP, beta-tricalcium phosphate; Col, collagen; PCL, poly-caprolactone; ECM, extracellular matrix; CSH, calcium sulphate hemihydrate.

details of the randomization procedure were reported. Ten studies (27.7%) reported blinding of outcome assessors to treatment groups, to minimize 'detection bias'. All studies reported detailed outcome evaluation, including statistical analyses, but few reported any information on adverse reactions or complications. Overall, RoB in most studies was judged to be 'unclear' (Figure 3). Information regarding study limitations and implications for translation to human models was limited, and none of the studies referred to the '3R's' principle (replacement, refinement and reduction) for experimental animals (Kilkenny *et al*., 2010).

### 3.3. Characteristics of animal models

Studies reported the use of CSD more frequently in the mandible (83.3%) than the maxilla. A majority of studies included bilateral CSD or a 'split-mouth' design (55.5%). Dental extraction was commonly performed in large-animal models and adequate healing time allowed before defect preparation. Following general anaesthesia, irrigated trephine drills were used to prepare unilateral or bilateral CSD, most often in the mandibular body or ramus, using either an intra-oral (large animals) or extra-oral (small animals) approach. The CSD ranged from 4 mm in diameter (rats) to 15 mm in length (rabbits) in small animals, and from 6 mm in diameter (pigs) to 20 mm in length (dogs) in large animals. Four studies reported the use of 'segmental' defects in monkeys (15 mm; Chanchareonsook *et al*., 2014b), dogs (20–30 mm; Zhao *et al*., 2009; Wang *et al*., 2015) or sheep (35 mm; Schliephake *et al*., 2001), where a portion of the mandibular body was resected by either disrupting or preserving mandibular continuity. When continuity was disrupted a titanium plate was fixed with screws on either end for stabilization. Three studies reported the repair of experimental maxillary 'clefts' in dogs, with



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**Table 2. Summary of study characteristics in small-animal models**

| Study | Strain, age, gender | Defect model, dimensions | *n* | Time | Cells | Source, induction | Cell number | Scaffold, AB if used |
|---|---|---|---|---|---|---|---|---|
| **Rabbits** | | | | | | | | |
| Jiang *et al*. 2006 | NZ, female | Mandible CSD (s), 15 × 6 mm | 14 | 4 weeks | BMSC, BMSC-BMP-4, or BMSC-EGFP | Femur, auto; yes | $50 \times 10^6$ | Porcine bone |
| Li *et al*. 2010 | NZ | Mandible CSD, 12 × 8 mm | 54 | 4, 8, 16 weeks | BMSC, BMSC-BMP-7 | Tibia, allo; yes | $2 \times 10^6$ | nHA–PA |
| Liu *et al*. 2011 | NZ, mature female | Mandible CSD, 10 × 4 × 3 mm | 36 | 12 weeks | DPSC | Permanent, allo; yes | $1 \times 10^8$ | nHA–PLA ± BMP-2, Iliac AB |
| Sun *et al*. 2013 | NZ, adult | Mandible CSD (s), 10 × 6 mm | 18 | 4, 8, 12 weeks | POC or POC-BMP-2 | Mandible, allo; yes | $1 \times 10^7$ | Bioglass–ceramic |
| Park *et al*. 2013 | NZ | Mandible CSD; 5-week healing, 6 × 4 × 3 mm | 9 | 4 weeks | ABMSC | Mandible, Auto; No | $1 \times 10^6$ | Bio-Oss® |
| Saad *et al*. 2015 | NZ, adult male | Mandible CSD, 15 × 10 mm | 16 | 4, 12, 24 weeks | BMSC | Femur, auto; no | $5–7 \times 10^6$ | β-TCP |
| Su *et al*. 2015 | NZ, male | Mandible CSD, 10 × 5 × 4 mm | 20 | 12 weeks | PDLSC or PDLSC-OPG | Impacted, allo; no | $5 \times 10^6$ | β-TCP |
| Wei *et al*. 2015 | NZ, male | Mandible CSD (s), 8 mm, diameter | 12 | 4 weeks, 12 weeks | ADSC | Inguinal pad, auto; no | $1.5 \times 10^6$ | Antler cancellous bone |
| **Rats** | | | | | | | | |
| Arosarena *et al*. 2003 | Fischer, male | Mandible CSD, 4 mm, diameter | 37 | 8 weeks | BMSC | Femur, allo; no | $1 \times 10^7$ | HA–collagen ± BMP-3, TGFβ-2 |
| Jiang *et al*. 2009 | Fischer, 12 weeks male | Mandible CSD, 5 mm, diameter | 24 | 8 weeks | BMSC, BMSC-BMP-2, or BMSC-LacZ | Femur, allo; yes | $2 \times 10^7$ | HA–Silk polymer |
| Schliephake *et al*. 2009 | Athymic nude, 5–7 weeks | Mandible CSD (s), 5 mm, diameter | 30 | 6 weeks | OB | Femur, Human; No | $5 \times 10^6$ | Biocoral®, HA-Collagen or TCP |
| Zhao *et al*. 2010 | Fischer, 6 weeks male | Mandible CSD (s), 5 mm, diameter | 11 | 8 weeks | BMSC, BMSC-BMP-2, or BMSC-EGFP | Femur, allo; yes | $2 \times 10^7$ | β-TCP |
| Mohammadi & Amini 2015 | Wistar, male | Mandible CSD, 4 mm, diameter | 75 | 1, 2, 3, 4 weeks | ADSC (SVF) | Omentum, allo; no | $2 \times 10^7$ | Chitosan |
| Raposo-Amaral *et al*. 2014 | Wistar, adult male | Maxilla CSD, 5 mm, diameter | 28 | 8 weeks | MMSC | Muscle, human; no | $1 \times 10^6$ | Bio-Oss-Col® or α-TCP, calvarial AB |
| Jiawen *et al*. 2014 | Sprague–Dawley, 6–8 weeks | Maxilla CSD, 4 × 4 × 3 mm | 16 | 4 weeks, 8 weeks | AESC | Amnion, human; no | $2–3 \times 10^8$ | β-TCP |
| Korn *et al*. 2014 | Lewis, female | Maxilla CSD, 3 mm, diameter | 72 | 1, 3, 6 weeks | BMSC, induced or non-induced | Femur, allo; yes | $5 \times 10^4$ | HAβ–TCP–Silica |

CSD, critical-size defect; (s), split-mouth design; *n*, number of animals; AB, autogenous bone; NZ, New Zealand; BMSC, bone marrow MSC; BMSC-BMP-4/7/2, bone morphogenetic protein-4/7/2-modified BMSC; EGFP, enhanced green fluorescent protein; DPSC, dental pulp stem cells; POC, periosteal stem cells; PDLSC, periodontal ligament stem cells; LacZ, beta-galactosidase; ADSC, adipose tissue-derived MSC; SVF, stromal vascular fraction; MMSC, muscle-derived MSC; AESC, amniotic epithelial stem cells; Auto, autologous; Allo, allogeneic; Human, human-derived; HA, hydroxyl-apatite; nHA, nano-HA; β-TCP, beta-tricalcium phosphate; TGFβ-2, transforming growth factor beta-2; Col, collagen.

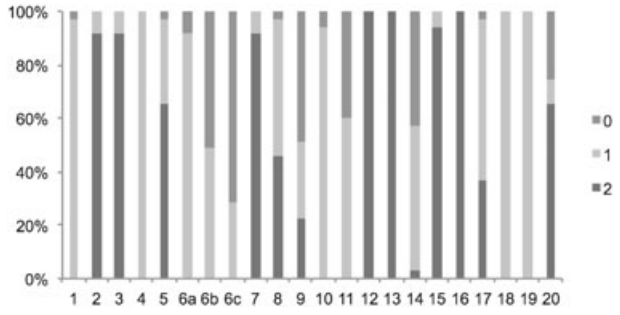


**Figure 2. Frequency distribution (%) of the scores assessed for each item of the modified ARRIVE guidelines in all studies included. Items 2, 3, 5, 7–10, 12, 13 and 15–20 were scored 0, 1 or 2 (clearly inadequate, possibly adequate or clearly adequate). All other items scored 0 or 1 (no or yes)**

(Zhang *et al*., 2011; Huang *et al*., 2015) or without (Pourebrahim *et al*., 2013) additional orthodontic procedures.

## 3.4. Characteristics of tissue engineering strategies

### *3.4.1. Cells*

All but four studies in large-animal models reported the use of autologous cells; two studies reported either the use of allogeneic adult (De Kok *et al*., 2003) or puppy-derived cells (Yamada *et al*., 2011) in dogs and two studies reported the use of human dental-derived cells [dental pulp stem cells (DPSCs) or stem cells from human exfoliated deciduous teeth (SHED)] in dogs (Behnia *et al*., 2014) or minipigs (Kuo *et al*., 2015). Among the small-animal models, nine studies reported the use of allogeneic cells, including DPSCs (Liu *et al*., 2011), periosteal- (Sun *et al*., 2013) or periodontal-ligament-derived stem cells (PDLSCs) (Su *et al*., 2015). Three studies reported implantation of human bone-, amnion- or muscle-derived

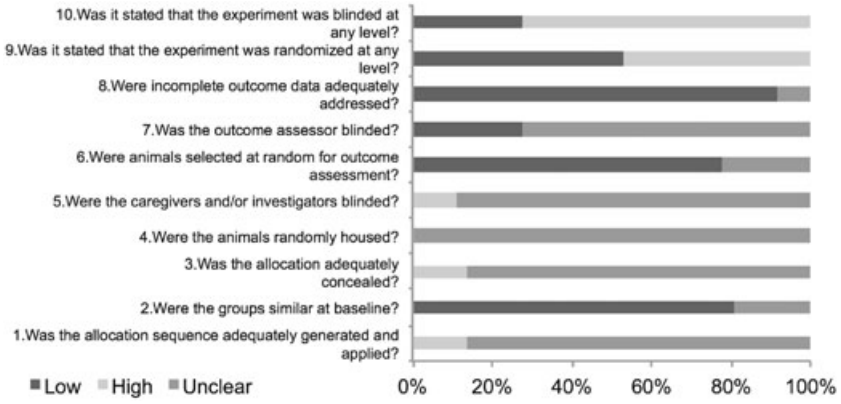


**Figure 3. Frequency distribution (%) of the risk of bias assessment for each item of the modified SYRCLE RoB tool in all studies included. Items 1–8 were judged as 'yes', 'no', or 'unclear'; items 9 and 10 were judged as 'yes' or 'no' (risk of bias; yes = low, no = high, unclear = unclear). Item 6 was always judged as 'yes' if all animals in both test and control groups were analysed at the same time-point**

cells in rats (Schliephake *et al*., 2009; Jiawen *et al*., 2014; Raposo-Amaral *et al*., 2014). No immunological reactions were reported in studies using allogeneic or human-derived cells, in either immunosuppressed or immunocompetent animals.

Most studies (55.5%) reported the use of bone marrow MSC; three studies used osteoblasts. Other MSC sources included dental pulp, alveolar bone and adipose tissue. Mesenchymal stem cells were used in early (1–6) passages, with (15 studies) or without osteogenic preinduction. One study compared the efficacy of osteogenically differentiated and undifferentiated MSCs in rats alveolar clefts: a trend towards superior regeneration with undifferentiated cells was observed (Korn *et al*., 2014). Primary cell cultures were expanded *ex vivo*; seeding densities ranged from $1 \times 10^4$ to $4 \times 10^8$ cells per scaffold. Cells were cultured on scaffolds for a specified period (range 30 min to 2 weeks), in basal or osteogenic media, before implantation.

Six studies reported the use of 'gene-modified' cells in rabbits or rats; cells were altered via viral vector-mediated gene transfer of osteogenic growth factors [bone morphogenetic proteins (BMPs)] (Jiang *et al*., 2006, 2009; Li *et al*., 2010; Zhao *et al*., 2010; Sun *et al*., 2013) or osteoclast inhibitors [osteoprotegerin (OPG)] (Su *et al*., 2015). Control groups in these studies included 'reporter' gene-modified cells [cells infected with adenovirus expressing enhanced green fluorescent protein (EGFP) (Jiang *et al*., 2006; Zhao *et al*., 2010) or β-galactosidase (LacZ) (Jiang *et al*., 2009)], unmodified cells and/or scaffold-only groups.

### *3.4.2. Scaffolds*

A majority of studies (58.3%) reported the use of mineral-phase alloplastic [hydroxyapatite (HA), alpha−/beta-tricalcium-phosphate (α−/β-TCP), bioglass or coral] or xenogeneic (bovine, porcine or antler bone) scaffolds, used in the block, disc or particulate form. Five studies reported the use of non-mineral-phase scaffolds [platelet-rich plasma (PRP), polypeptides or collagen]. Seven studies reported the use of composite scaffolds, composed of a mineral- and non-mineral [(co)polymer] phase. Five studies reported the addition of growth factors [BMP-2, BMP-3 or transforming growth factor-β2 (TGF-β2)] known to stimulate osteogenesis, to the scaffolds in at least one experimental group. However, for the sake of homogeneity with regard to the property of 'defect-space maintenance', and to minimize any confounding influence of growth factors, only studies reporting mineral-phase, polymeric or composite scaffolds, without additional growth factors, were considered for the meta-analysis.

## 3.5. Meta-analysis

Twenty-two studies reporting histomorphometric data of cell-loaded vs. cell-free scaffolds in dogs (CSD or segmental defects), minipigs, rabbits (at 4 or 12 weeks) and rats (mandibular or maxillary CSD) were included in the meta-analysis (Figure 4). Separate analyses were performed for three studies in rabbits and two in rats, comparing BMP-gene-modified and 'unmodified' cell-groups (Figure 5), and in two studies in dogs comparing cell-loaded scaffolds and autogenous bone (Figure 6). As there were fewer than 10 studies in each meta-analysis, publication bias via funnel plots or statistical testing was not assessed because of the lack of power to distinguish chance from real asymmetry (Sterne *et al*., 2008).

Overall, the meta-analyses revealed three main findings: (1) a statistically significant effect in favour of cell-loaded vs. cell-free scaffolds [pooled estimate (WMD) range: 15.59–49.15% and 8.60–13.85% NBF in large- and small-animal models, respectively]; (2) a statistically significant effect in favour of BMP gene-modified cells vs. unmodified or EGFP/LacZ-modified cells (WMD range: 10.06–20.83% NBF in small-animal models); and (3) a marginally significant effect in favour of autogenous bone vs. cell-loaded scaffolds (WMD: 4.05% NBF in dogs). Heterogeneity in most cases was very high ($I^2 > 90\%$, $p < 0.05$). Robustness of findings of the meta-analysis, were confirmed by observation of similar 95% CI values in the sensitivity analyses, which excluded 0 for all comparisons except cell-loaded scaffolds vs. autogenous bone. Pooled WMD with 95% CI

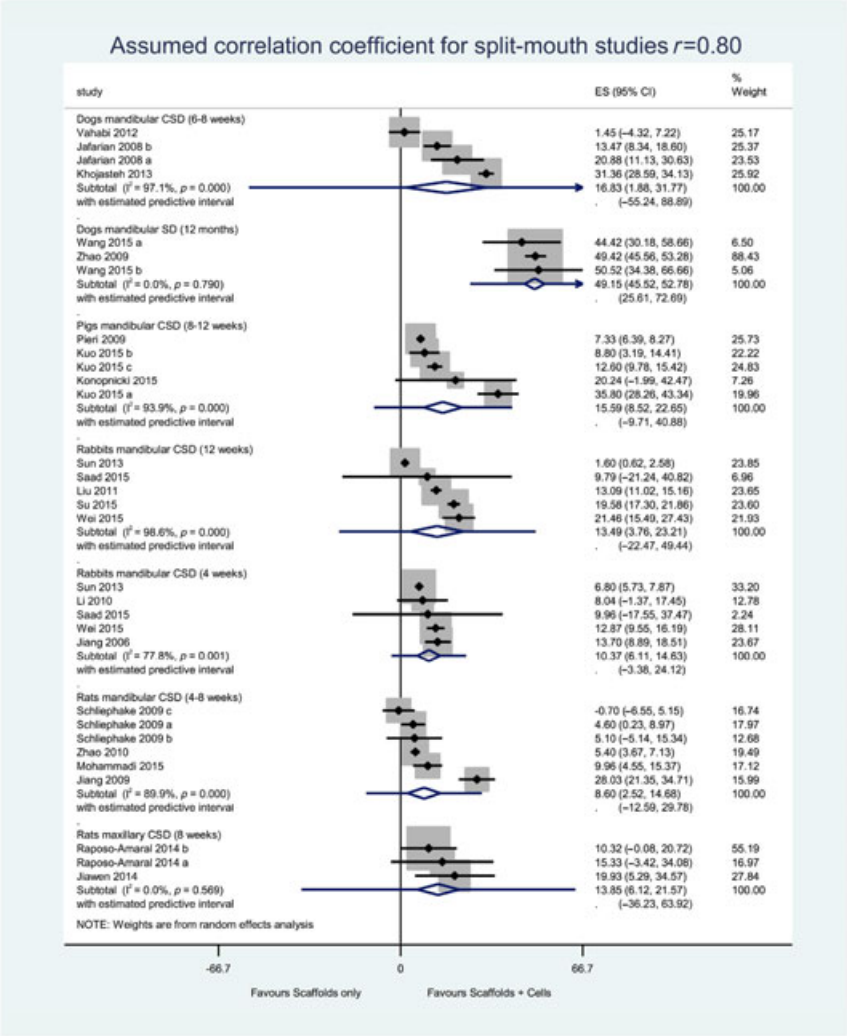


**Figure 4. Forest plot for the comparison cell-loaded vs. cell-free scaffolds. The forest plot displays relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval, for the outcome percentage of new bone formation (%NBF). A diamond indicates the pooled estimate and its 95% CI. SD, segmental defect; CSD, critical-size defect. References on the left give first author and year. The letters a, b, and c represent different comparison groups within the same study. [Colour figure can be viewed at wileyonlinelibrary.com]**

and measures of heterogeneity for each of the subgroups are presented in the Supplementary material online, along with an example for interpretation.

## 4. Discussion

The aim of the present study was to systematically review the preclinical *in vivo* evidence for cell-based bone tissue engineering (BTE) strategies for alveolar bone regeneration. Systematic reviews and meta-analyses of animal studies can be useful for guiding the design of future clinical trials, detecting heterogeneity between studies and treatment effects, and improving the methodological quality of future studies (Hooijmans *et al*., 2014a). Recent systematic reviews of animal studies have reported favourable effects of BTE approaches for skeletal (Liao *et al*., 2014) and periodontal regeneration (Bright *et al*., 2015; Yan *et al*., 2015). Similar findings have been reported in systematic reviews of human clinical trials evaluating the effectiveness of BTE approaches for alveolar bone regeneration (Padial-Molina *et al*., 2015; Shanbhag and Shanbhag, 2015). However, because of the large variation in the methodology of studies, especially with regard to the nature of cells and biomaterial scaffolds used, no conclusive statements regarding the effectiveness of BTE exist in the literature. In addition, concerns regarding ethical aspects and cost-effectiveness have limited large-scale clinical application of BTE, and a need for further, more standardized, preclinical research on this topic has been highlighted (Cancedda *et al*., 2007).

Guidelines for designing preclinical animal models in BTE have been proposed; the model should: (1) simulate the target clinical and biological environment; (2) allow the use of quantifiable parameters to evaluate success and functional performance of regenerated tissues; and (3) allow detection of clinically relevant differences in biological performance between the regenerative therapies assessed (Muschler *et al*., 2010). The wide variation in bone anatomy, composition, biomechanics, size and biology between and within



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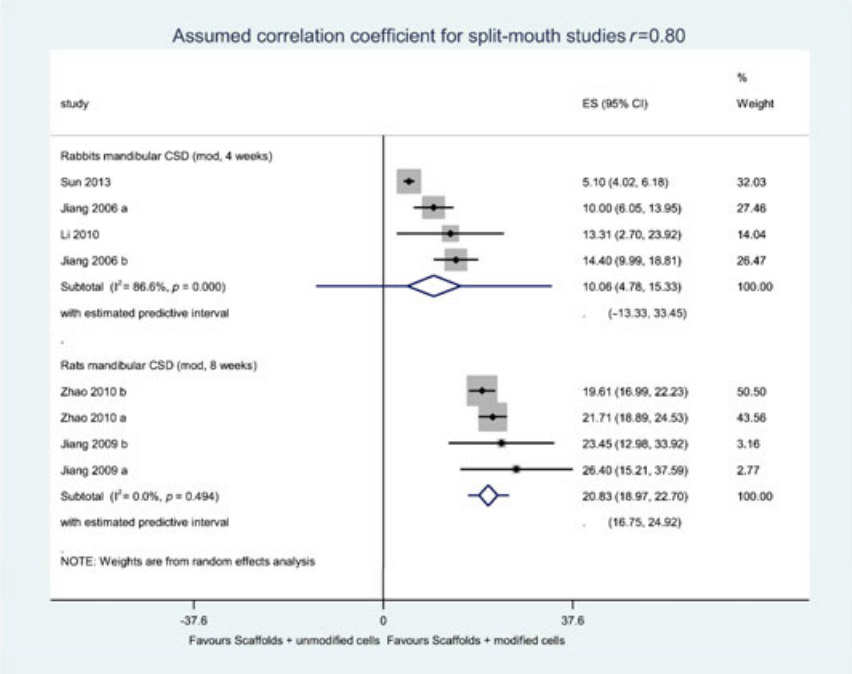


**Figure 5.** Forest plot for the comparison bone morphogenetic protein (BMP)-modified vs. unmodified cells. The forest plot displays relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval, for the outcome percentage of new bone formation (%NBF). A diamond indicates the pooled estimate and its 95% CI. CSD, critical-size defect. References on the left give first author and year. The letters a, b, and c represent different comparison groups within the same study. [Colour figure can be viewed at wileyonlinelibrary.com]

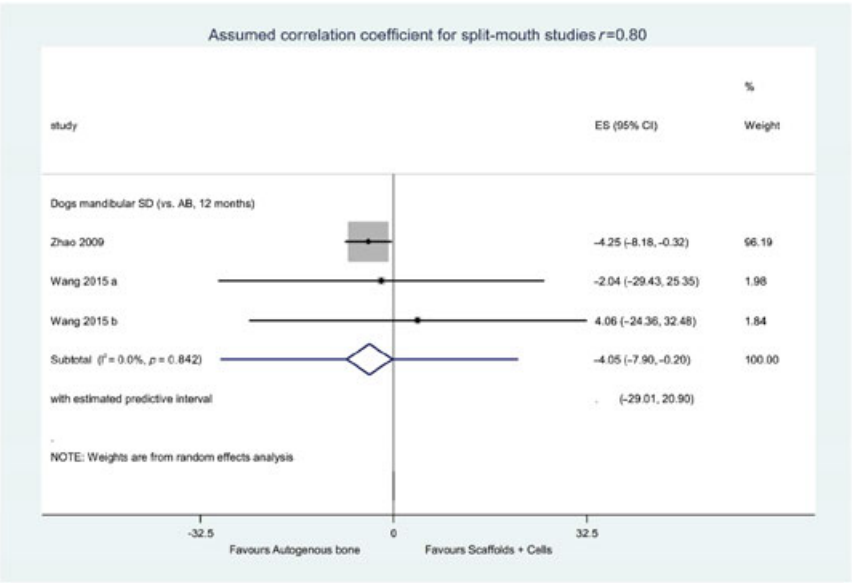


**Figure 6.** Forest plot for the comparison cell-loaded scaffolds vs. autogenous bone. The forest plot displays relative weight of the individual studies, the estimates of treatment effect (ES) expressed as weighted mean differences (WMD), 95% confidence intervals (CI) and a predictive interval, for the outcome percentage of new bone formation (%NBF). The diamond indicates the pooled estimate and its 95% CI. SD, segmental defect. References on the left give first author and year. The letters a, b, and c represent different comparison groups within the same study. [Colour figure can be viewed at wileyonlinelibrary.com]

species, and in comparison with humans, often complicates translationability of the results in animal models. Generally, small-animal models constitute a starting point for proof-of-principle or feasibility studies before 'clinical modelling' and efficacy testing in larger animals (Pellegrini *et al.*, 2009; Li *et al.*, 2015). Therefore, the results herein are discussed in the context of small- and large-animal models.

### 4.1. Small-animal models

Small-animal models used in musculoskeletal research include primarily two species, rodents (rats or mice) and rabbits (O'Loughlin, 2008). Rodent models are often preferred over larger animals because of the significantly lower costs, easier housing and handling, and minimal social concern (Gomes and Fernandes, 2011). Rodents



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also have a well-defined and controlled genetic background, with less variation among individual animals in terms of biological response, which implies that fewer experimental units may be required to achieve statistically valid data, compared with larger animals (Stavropoulos *et al*., 2015). Rabbits, like rodents, provide advantages of small size and easy handling. Additional advantages include the achievement of skeletal maturity by 6 months of age and larger volumes of mandibular bone tissue, which allow the creation of more reliable CSD than in rodents (Stübinger and Dard, 2013).

In the present meta-analysis, a statistically significant effect in favour of cell-based approaches was observed in rats' maxillary and mandibular CSD after 4–8 weeks, and in rabbits' mandibular CSD after 4 weeks and 12 weeks. However, the significant differences in structure, composition and physiology of rodent, rabbit and human bone (e.g. trabecular content, metabolic rate, remodelling, etc.) must be considered when extrapolating results from these studies (Pearce *et al*., 2007).

### 4.2. Large-animal models

Advantages of large-animal models include the ability to easily create multiple CSD with clinically relevant dimensions (i.e. both Ex- and Co-groups within the same jaw of the animal), thus limiting inter-animal variation and the number of animals needed. Further, large-animal models allow longer observation times; for example, the longest observation time in the present review (12 months) was in studies involving canine segmental CSD. Biopsies of the regenerated sites can be obtained at the end of observation periods without the need for euthanasia (Pourebrahim *et al*., 2013; Behnia *et al*., 2014), which is consistent with the '3R's' principle (Russel and Burch, 1959). Importantly, for BTE research, large-animal models allow preparation of defects with clinically relevant diffusion distances, so that the influence of mass transport, hypoxia and vascularization on the survival of transplanted cells can be evaluated in a simulated clinical setting (Muschler *et al*., 2010).

A majority of studies (55.5%) included in the present review reported data from large-animal models (i.e., monkeys, dogs, sheep and minipigs); data from dogs and minipigs were included in the meta-analysis. Dogs and pigs are widely used animal platforms in musculoskeletal research, given the similarities in structure, composition and physiology between canine/porcine and human bone (Aerssens *et al*., 1998). Although some differences in the bone remodelling process do exist between the three species, both canine and porcine models are considered to be highly relevant: the rate of remodelling in pigs (1.2–1.5 μm/day) is comparable to that in humans (1.0–1.5 μm/day) but slower than that in dogs (1.5–2.0 μm/day) (Pearce *et al*., 2007). However, limitations of large animals include high costs, ethical issues in the case of dogs, and handling difficulty in the case of pigs. In context, minipigs represent a more suitable model because of more morphological similarities to human bone than other large-animal models (Mardas *et al*., 2014).

In the present meta-analysis, significantly greater bone regeneration was observed in favour of cell-based vs. cell-free approaches in mandibular CSD of dogs and minipigs. A similar result was reported in one study of sheep mandibular defects (Schliephake *et al*., 2001). Another recent study in sheep, which was excluded from the present analysis because of the use of uncultured autologous bone marrow (BM; see the Supplementary material online), also reported greater regeneration in mandibular defects augmented with BM-TCP vs. TCP-blood constructs; this was attributed to the possible 'osteopromotive' effects of MSC within the BM (Russmueller *et al*., 2015). These results are in agreement with a recent meta-analysis of the effectiveness of stem cell therapy for histological bone regeneration in all anatomical skeletal defects of large-animal models (Liao *et al*., 2014).

Non-human primates are considered the closest experimental model to humans, given their anatomical and biological similarities (Muschler *et al*., 2010). Only one study included herein used a mandibular segmental defect model in monkeys and found no significant benefit of autologous MSC-loaded polycaprolactone (PCL) scaffolds, over BMP-2-loaded PCL or PCL scaffolds alone, after 6 months of healing (Chanchareonsook *et al*., 2014b). However, previous studies, which were not included in the present review because they reported only qualitative outcomes (see the Supplementary material online), have observed superior regeneration, and even complete 'bridging', of mandibular segmental defects in monkeys following implantation of autologous bone marrow/BMSC-loaded PLGA or collagen scaffolds impregnated with BMP-2, compared with implantation of only BMSC- or BMP-2-loaded scaffolds (Seto *et al*., 2001, 2006). The combined delivery of osteogenic (BMSC) and osteoinductive (BMP-2) agents may have contributed to superior outcomes in the latter studies. Moreover, the choice of scaffold and its biological (osteoconductivity) and mechanical (load-bearing) properties, and cell–scaffold interactions are critical for the regenerative outcome.

### 4.3. Use of gene-modified cells

Five studies reported *ex vivo* gene transfer of BMP-2, −4 or −7 into cells via adenoviral vectors before implantation. The BMPs are osteoinductive growth factors that have been well established to regenerate CSD *in vivo* (Khojasteh *et al*., 2013). Gene transfer is a method by which growth factors can be introduced, either directly or via cells, into defect sites to enhance *in vivo* bone regeneration (Kofron and Laurencin, 2006). Gene transfer into cells is usually performed using viral or non-viral (e.g. liposomes) vectors. In the present meta-analysis, a significant effect in favour of BMP (viral-mediated)



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gene-modified cell groups over unmodified and control (EGFP/LacZ) gene-modified cell groups was observed in rabbit and rat mandibular CSD. Similarly, in one study, OPG-modified PDLSC enhanced regeneration compared with unmodified PDLSC in rabbits; OPG, also known as osteoclastogenesis inhibitory factor (OCIF), is an inhibitor of osteoclast differentiation and function (Su *et al*., 2015).

Implantation of gene-modified MSC has also been evaluated in studies of alveolar CSD, which were not included in the present analysis because they reported only qualitative outcomes (see the Supplementary material online). Use of BMP-2 gene-modified MSC has been reported in minipigs (Chang *et al*., 2003), mice (human BMSC; see the Supplementary material online, Steinhardt *et al*., 2008), and in normal (Park *et al*., 2003) and osteoporotic rats (Tang *et al*., 2008). In one of these studies, superior regeneration with BMSC modified by viral-mediated vs. liposome-mediated BMP-2 gene transfer, was observed (Park *et al*., 2003). Other studies that were excluded reported gene transfer of osteoinductive factors such as LIM mineralization protein-3 (LMP-3) to dermal fibroblasts in rats (see the Supplementary material online: Lattanzi *et al*., 2008; Parrilla *et al*., 2010), and basic fibroblast growth factor (bFGF) to BMSC in rabbits (see the Supplementary material online: Yang *et al*., 2013). Gene-mediated suppression of osteo-inhibitory factors, e.g., *noggin*-suppression in adipose MSC (*noggin* is an inhibitor of BMP-signalling), was also reported (see the Supplementary material online, Fan *et al*., 2014). All the above studies consistently reported superior bone regeneration in gene-modified vs. unmodified and/or control (EGFP/LacZ) gene-modified cell-groups, in small- and large-animal models (see the Supporting information online).

### 4.4. Tissue-engineered vs. autogenous bone

Among the studies involving large-animal models, only one study (Pourebrahim *et al*., 2013) reported significantly greater regeneration with autogenous bone compared with MSC/HA–β-TCP constructs, in a canine alveolar cleft defect; all other studies reported no significant differences between cell/scaffold constructs or autogenous bone in CSD or cleft defects. For canine segmental defects, a marginally significant effect in favour of autogenous bone was observed in the meta-analysis, but disappeared in the sensitivity analyses, suggesting insufficient evidence to detect true differences between the groups (Hooijmans *et al*., 2014a). In smaller animals, one study in rabbits reported significantly greater regeneration with autogenous bone compared with DPSC/nano-HA–PLA constructs (Liu *et al*., 2011), while another study in rats reported no significant differences between AB and MSC/α-TCP or HA constructs (Raposo-Amaral *et al*., 2014). In summary, the current evidence seems to indicate that tissue-engineered constructs may result in comparable alveolar bone regeneration with what is achieved with the 'gold standard' autogenous bone; however, the evidence is limited and thus it should be considered inconclusive regarding the effectiveness of this approach.

### 4.5. Implantation of human-derived cells in experimental animals

Three studies reported implantation of human muscle- (Raposo-Amaral *et al*., 2014), amnion- (Jiawen *et al*., 2014) or femoral bone-derived cells (Schliephake *et al*., 2009) in either immunocompetent or immunosuppressed rats, with no remarkable inflammatory or immunological reactions. In one study, an 'immunomodulatory' effect of amnion-derived cells was observed when implanted with β-TCP scaffolds in immunocompetent rats, via suppression of the physiological host response and milder macrophage infiltration, compared with cell-free scaffolds (Jiawen *et al*., 2014). Interestingly, two studies reported implantation of SHED or DPSC in large animals – dogs (Behnia *et al*., 2014) and minipigs (Kuo *et al*., 2015) – without adverse reactions. Similar results were reported in other studies of alveolar CSD (not included in the present review because they reported only qualitative outcomes; see the Supplementary material online), following implantation of human-derived cells in minipigs (placenta-MSCs; Lee *et al*., 2010), rabbits (adipose-MSCs; see the Supplementary material online, Linero and Chaparro, 2014), rats (adipose-MSCs; see the Supplementary material online, Streckbein *et al*., 2013; and gingiva-MSC; see the Supplementary material online, Wang *et al*., 2011), and mice (maxillofacial-BMSCs; see the Supplementary material online, Steinhardt *et al*., 2008). These data are consistent with previous reports of uneventful implantation of human MSC in CSD of non-immunosuppressed animals (de Mendonça Costa *et al*., 2008; Bueno *et al*., 2009; Daei-Farshbaf *et al*., 2014).

The biocompatibility of MSC within and across species can be attributed to their hypoimmunogenic, immunomodulatory and anti-inflammatory properties. Mesenchymal stem cells are reported to exert these effects via three broad mechanisms: (1) their lack or limited expression of major histocompatibility complex (MHC)-I and MHC-II molecules; (2) via direct and indirect modulation of T-cell responses; and (3) secretion of various anti-inflammatory cytokines, making them a promising resource for allogeneic transplantation in regenerative therapies (De Kok *et al*., 2003; Ryan *et al*., 2005). A recent randomized controlled trial reported favourable 2-year outcomes and no adverse reactions in patients after transplantation of allogeneic BMSCs for knee meniscus regeneration (Vangsness *et al*., 2014).

### 4.6. Meta-analysis and heterogeneity

A random effects model was chosen for the present meta-analysis to account for the expected between-study variance (Hooijmans *et al*., 2014a). The distribution of effect sizes was provided by WMD and measures of $I^2$. The



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$I^2$-value is a measure of 'true' inconsistency between the study results, owing to between-study differences and not simply chance (Hooijmans *et al*., 2014a), and was found to be very high within most categories in the meta-analyses. A large heterogeneity may have questioned the validity of the results, if the direction of the effects varied greatly, as was not the case in the present meta-analyses. This heterogeneity could be due to biological factors related to the animals and/or methodological differences between the studies. Biological factors may include the animals' species, gender, age, immunological status, etc., while methodological differences in the study design (e.g. sample sizes, randomization), nature of interventions (e.g. use of autologous vs. allogeneic or human cells, osteogenic induction of cells before implantation), and outcome evaluation (e.g. methods of bone histomorphometry) could possibly explain the large heterogeneity observed in the meta-analyses.

All attempts were made to minimize heterogeneity when performing the meta-analyses. Care was taken to pool only those studies with similar characteristics in terms of PICO, observation times, nature of experimental models and interventions (e.g. type of scaffold used). Subgroup analyses were performed for each animal model. Although sample sizes were generally small and sample size calculation was never reported, the majority of studies involved split-mouth designs, which is a more efficient design in terms of sample size. Split-mouth and 'parallel group' studies were combined using recommended statistical methods (Higgins *et al*., 2008; Smaïl-Faugeron *et al*., 2014), thus increasing the overall power to detect treatment effects. It should be noted that sample size has an impact only on the precision of the estimates and heterogeneity during the synthesis. Baseline differences are not applicable and are irrelevant in terms of bias in split-mouth designs. Finally, comparisons of gene-modified cell groups were evaluated separately from those of 'unmodified' cell-groups, to avoid the influence of confounders (BMP gene-transfer) on the outcomes.

### 4.7. Outcome measures

Histomorphometry is considered the 'gold standard' method for evaluation of bone structure (Vidal *et al*., 2012; Rentsch *et al*., 2014). All studies included in the meta-analysis reported relatively consistent methods for calculating the main parameter of interest (i.e. %NBF), which is calculated as the percentage of newly formed bone tissue relative to the total defect space (i.e. area or volume). A majority of studies (66.7%) reported decalcified paraffin-embedded preparation of samples for histology. Microscopic images of central sections (three, on average) were analysed by computerized software for quantitative estimation of new bone and residual graft material. No remarkable variation in terms of bone regeneration should be expected among studies because of the method of histological analysis (decalcified or non-decalcified); in a recent report, similar relative amounts of calcified tissue components within augmented periodontal intrabony defects were calculated from decalcified and non-decalcified histological sections (Park *et al*., 2015). However, variation in the studies regarding processing methods (e.g. section thickness, number of sections analysed per implant, software used for analysis, etc.; Chappard *et al*., 1999; Kopp *et al*., 2012; Stewart *et al*., 2013), difficulty in differentiating between mineralized scaffolds and regenerated mineralized bone (Schliephake *et al*., 2009) and investigator-related factors (e.g. inter-observer/intermethod variation, lack of blinding, etc.; Wright *et al*., 1992), may have introduced heterogeneity in the meta-analysis.

Conventional histomorphometry is considered to be destructive, time-consuming and limited to two-dimensional assessment of tissue sections; a third dimension can be added on the basis of stereology (Müller *et al*., 1998). Recently, micro-computed tomography (micro-CT) has been proposed as an alternative method for assessing three-dimensional bone microarchitecture with high resolution and accuracy, in a fast and non-destructive manner. Several studies have reported high correlation between micro-CT and histomorphometry (Müller *et al*., 1998; Thomsen *et al*., 2005; Vandeweghe *et al*., 2013). For this reason, 11 studies reporting quantitative micro-CT-based or CT-based outcomes were also considered in the present review (see the Supplementary material online). Although a wide variation was observed in the parameters evaluated, a majority of studies (63.6%) reported significantly greater regeneration in defects implanted with cell–scaffold constructs compared with scaffolds alone. Moreover, in three studies, no significant differences in regeneration were observed between cell–scaffold constructs and autogenous bone. However, care should be taken when interpreting outcomes of CT or micro-CT because of the difficulties in differentiating between mineralized scaffolds and newly formed bone.

### 4.8. Experimental models

Unlike calvarial CSD, alveolar CSD models have not been well characterized in the literature in terms of defect location, size and morphology. Defect dimensions varied between studies for the same animal model/species, and, in many cases, selection of a particular model appeared to be based on one previously established by the same, or related, research group(s). Only 16 studies reported inclusion of an 'empty' or untreated control group to determine whether the defects were truly of critical size, as demonstrated by minimal or no bone formation at the end of the observation period, although many studies based their CSD models on previous reports. To place this in context, even a very small size of defect would be of critical size, provided that the experiment is of short enough duration; meaningful results regarding the ability of an intervention to enhance bone formation



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can be produced only if the defects have relevant dimensions. A relatively large variation in the location, size and morphology of alveolar CSD was observed within and between animal models, which could likely have also contributed to heterogeneity in the present meta-analysis. Indeed previous studies have highlighted the influence of alveolar CSD characteristics, such as defect site(s) (e.g. 'marrow-rich' vs. 'marrow-poor' sites; Guo *et al*., 2012), preservation or removal of bony cortices (e.g. 'partial-thickness' vs. 'full-thickness' defects; Young *et al*., 2008) and preservation vs. removal of the periosteum (Huh *et al.*, 2005; Ma *et al*., 2009) on regenerative outcomes.

The results of the present review can also be discussed in light of CSD models in other skeletal sites, more frequently reported in the orthopaedic literature. These commonly include CSD in the tibiae or femur of small animals, or more extensive CSD in the long bones of larger animals (Li *et al*., 2015). A recent study reviewed various large-animal defect models, mostly in the extremities (tibial, radial, ulnar and femoral) for cell-based BTE (Liao *et al*., 2014). The meta-analysis identified: (1) a significant effect in favour of cell-based vs. other therapies for histological new bone formation (WMD 17.79%, 95% CI 10.54, 25.03, $I^2$ 99%); (2) a superior effect of cells in combination with matrix scaffolds vs. direct cell injection; and (3) no variation in effects based on the type of animal or cells, such as BMSC vs. other cell types (Liao *et al*., 2014).

### 4.9. Quality of reporting

The reliability of results of meta-analyses directly depends on the quality of the primary studies (Hooijmans *et al*., 2014a). The overall methodological quality of the studies included, as assessed by compliance with the ARRIVE guidelines (Kilkenny *et al*., 2010), was found to be moderate. The ARRIVE guidelines have been developed to improve the reporting quality of animal studies and have been widely used for assessment of preclinical research in implant dentistry (Berglundh and Stavropoulos, 2012). Moreover, the SYRCLE tool, which addresses particular aspects of bias that play a role in animal experimental studies, was also utilized (Hooijmans *et al*., 2014b). Nevertheless, a clear need for more standardized reporting of animal studies was identified herein, to allow reliable future reproduction and synthesis.

### 4.10. Clinical relevance

Clinical meta-analyses aim to obtain a combined estimate or size of treatment effect, while preclinical meta-analyses aim to summarize the effect of an intervention, where the direction rather than size is meaningful, because of the large inherent variations in animal studies (Hooijmans *et al*., 2014a; Vesterinen *et al*., 2014). Thus, although numerical values from the present meta-analysis should not be directly translated to the human situation, it can be inferred that a similar response, or direction of treatment effect, could also be expected in humans (Stavropoulos *et al*., 2015). For example, the ~17% additional bone regeneration observed in dogs' mandibular CSD augmented with cell-loaded biomaterial scaffolds compared with scaffolds alone, would not translate to 17% (or '*x*-times' 17%) more bone, if the same procedure was performed in humans. Other inherent limitations of animal models that must be considered, are: (1) underestimation of clinical variation, with regard to both local (defect size, morphology, mass transfer, etc.) and systemic (age, co-morbidities, etc.) biological environments; and (2) overestimation of clinical performance, especially in the context of CSD, where uniform defects are surgically created most often in healthy animals with sound surrounding tissues and generally uncompromised blood supply, which is often not the case in clinical scenarios (Faggion *et al*., 2010; Muschler *et al*., 2010). In perspective, meta-analyses of animal studies tend to be exploratory rather than confirmatory. Standardization of alveolar CSD models to better represent the clinical scenario and standardization of study reporting should be important considerations in future studies of alveolar BTE.

Several reviews of clinical BTE strategies in humans have recently been published (Chanchareonsook *et al*., 2014a; Gamie *et al*., 2014; Gothard *et al*., 2014; Shanbhag and Shanbhag, 2015; Roux *et al*., 2015). Overall, the findings suggest that BTE, especially cell-based, approaches have shown promising clinical results with minimal adverse reactions in orthopaedic and maxillofacial applications. However, the evidence is based on few controlled studies, usually with small sample-sizes and short observation times. Large heterogeneity between studies regarding the nature of BTE approaches in terms of the cells, scaffolds and/or growth factors used, and *in vitro* processing methods, limit the drawing of reliable conclusions. Long-term evaluations of the safety of cell therapy appear to be lacking (Lalu *et al*., 2012). Notably, adverse effects have been reported following clinical use of recombinant human BMP (Carreira *et al*., 2014). Finally, further research is needed to evaluate: (1) the safety and efficacy of allogeneic 'off-the-shelf' cell-based products; (2) strategies to enhance vascularization of constructs, especially in large defects; (3) optimization of the *ex vivo* expansion process and it's duration; and (4) the cost-effectiveness of cell-based therapy, to facilitate clinical translation.

## 5. Conclusions

The pre-clinical *in vivo* evidence reviewed can be summarized as follows:

1. Based on results of both small- and large-animal models, the addition of osteogenic cells (MSCs or OB) to biomaterial scaffolds can enhance histomorphometric alveolar bone regeneration.



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2. Based on results of small-animal models, *ex vivo* BMP gene-transfer to MSCs and OB can enhance their *in vivo* osteogenic potential.
3. Limited evidence suggests that tissue-engineered constructs may result in comparable alveolar bone regeneration with what is achieved with the 'gold standard' (i.e. autogenous bone).
4. The results should be interpreted with caution because of the large heterogeneity between studies resulting from biological and methodological variability.

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## Conflict of interest

The authors declare no conflict of interest and no external funding was obtained for performing the current review.

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## Supporting information on the internet

The following supporting information may be found in the online version of this article:

Table S1. Search strategy for MEDLINE.
Table S2. Checklist for quality assessment of studies according to modified ARRIVE guidelines and a predefined grading system.
Table S3. Assessment of risk of bias (RoB) in studies included using a modified SYRCLE's RoB tool.
Table S4. Summary of results of the meta-analysis and sensitivity analyses.
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