The Role of Three-Dimensional Scaffolds in Treating Long Bone Defects: Evidence from Preclinical and Clinical Literature—A Systematic Review

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Long bone defects represent a clinical challenge. Bone tissue engineering (BTE) has been developed to overcome problems associated with conventional methods. The aim of this study was to assess the BTE strategies available in preclinical and clinical settings and the current evidence supporting this approach. A systematic literature screening was performed on PubMed database, searching for both preclinical (only on large animals) and clinical studies. The following string was used: "(Scaffold OR Implant) AND (Long bone defect OR segmental bone defect OR large bone defect OR bone loss defect)." The search retrieved a total of 1573 articles: 51 preclinical and 4 clinical studies were included. The great amount of preclinical papers published over the past few years showed promising findings in terms of radiological and histological evidence. Unfortunately, this in vivo situation is not reflected by a corresponding clinical impact, with few published papers, highly heterogeneous and with small patient populations. Several aspects should be further investigated to translate positive preclinical findings into clinical protocols: the identification of the best biomaterial, with both biological and biomechanical suitable properties, and the selection of the best choice between cells, GFs, or their combination through standardized models to be validated by randomized trials.

1. Introduction

Traumatic long bone defects still represent a clinical challenge for orthopaedic surgeons. In fact, a critical size defect requires invasive surgical procedures to reconstitute the structural integrity of the collapsed bone. This does not provide fully satisfactory results entailing a significant socioeconomic burden [1, 2]. Despite all recent innovations in bone repair techniques, autologous bone grafting (ABG) is still considered the “gold standard” treatment for long bone defects. However, ABG presents major limitations due to related drawbacks such as longer operating time, little availability of material, and significant morbidity [3–8]. Other options could be the treatment with allografts or xenografts, but some disadvantages have also been reported for these methods, such as immune rejection, slow and only partial integration, absorption and substitution with new bone, graft sequestration, and failures [9].

The concept of bone tissue engineering (BTE) has been developed to overcome problems associated with conventional methods. The typical paradigm of BTE is constituted by the four biological prerequisites which include osteogenic cells, osteoinductive stimulus, osteoconductive matrix scaffolds, and mechanical environment (the diamond concept) [10]. These promote signalling cascades such as osteogenesis, chondrogenesis, and angiogenesis in an orchestrated spatiotemporal manner, leading to bone regeneration [10]. In this context, it is crucial for the scaffold to have a proper...
macroporous structure, good degradability, and osteoconductive properties [11–13]. Thus, three-dimensional (3D) scaffolds have been developed with hierarchically organized structures similar to healthy bone and with the ability to yield well-organized bone regeneration [14, 15]. Moreover, the important role of growth factors (GFs) in bone remodelling and osteogenesis, by accelerating chemotaxis, proliferation, and differentiation of bone cells, has been largely described [16–18]. In this light, it has become common to add augmentation strategies such as osteoinductive factors like bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet rich plasma (PRP), and bone marrow derived stem cells (BMSCs) to further stimulate bone healing in critical size defects. However, many questions remain unanswered (choice of scaffold, cell source and concentration, type of GFs, etc.) thereby causing uncertainty on the potential of available technologies as well as on the choice of the most suitable strategy.

The main objective of this systematic review was to assess the BTE strategies available in preclinical and clinical settings, in order to analyse the current evidence supporting the use of this approach for the treatment of long bone defects.

2. Methods

A systematic literature screening was performed by two independent reviewers (GS and NG) on the PubMed database, searching for both preclinical and clinical studies on 3D synthetic scaffolds with organized structures for long bone defects developed to treat defects of the upper/lower extremities. In particular, the research criteria included studies published in English language until February 2017. The following string was used: “(Scaffold OR Implant) AND (Long bone defect OR segmental bone defect OR large bone defect OR bone loss defect)” (Figure 1). Among preclinical publications, only studies on large animal models were selected. After an initial screening of all abstracts, selected full texts were analysed and separated into preclinical and clinical studies. Reference lists were also screened to identify further papers. All articles dealing with other types of bone defects not involving upper/lower extremities or studies without scaffolds were excluded. Moreover, biomaterials in form of granules, sponges, or powders were excluded and studies with only autografts or allografts were also excluded.

3. Results

The PubMed search analysis retrieved a total of 1573 articles and, following the inclusion criteria, 51 preclinical studies [19–69] and 4 clinical studies [70–73] were identified and included in the present analysis. Details of preclinical studies are reported in Table 1 (scaffold alone) and Table 2 (augmented scaffold) and clinical papers in Table 3.

3.1. Preclinical Studies. In the past, few years there has been a progressive increase in the number of publications for scaffold treatments in the preclinical field, as shown in Figure 2. The most commonly investigated large animal model was sheep 30/51, followed by dog 13/51, goat 6/51, and monkey 2/51. A composite scaffold derived from a combination of different biomaterials was the most common investigated...
### Table 1: Completedetailsof12preclinicalpapersidentifiedinthissystematicreviewfocusingontheusefulnessofscaffolds-aloneintreatinglongbonedefects.

| Authors                  | Biomaterials                  | Animal model                        | Results          | Effects |
|--------------------------|-------------------------------|-------------------------------------|------------------|---------|
| Boyde et al., 1999 (Bone) [19] | (1) HA                        | Sheep tibial defect (3.5 cm)         | SEM: +           | +       |
|                          |                               |                                     | BSE: +           | +       |
| Maracci et al., 1999 (Cal Tiss Int) [20] | (1) HA                        | Sheep tibial defect (3.5 cm)         | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
| Zhang et al., 2001 (J Biomat Mat Res) [21] | (1) HA-TCP                    | Dog femoral defect (1.5 cm)          | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
|                          |                               |                                     | 𝜇CT: +           | +       |
| Mastrogiacomo et al., 2006 | (1) Si-TCP                    | Sheep tibial defect (4.8 cm)         | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
|                          |                               |                                     | SEM: +           | +       |
| Sarsilmaz et al., 2007 (Acta of Bioeng & Biomech) [23] | (1) HA-PE                     | Dog radial defect (1.5 cm)           | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
| Schneiders et al., 2009 (J Orth Res) [24] | (1) HA-COL (2) HA-COL-CS       | Sheep tibial defect (3 cm)           | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
|                          |                               |                                     | 𝜇CT: +           | +       |
| (for HA-COL-CS)          |                               |                                     |                  |         |
| Nandi et al., 2009 (Res Vet Sci) [25] | (1) Untreated (2) Bioactive glass | Sheep radial defect (1.2 cm)         | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
| Nair et al., 2010 (J Tiss Eng Pt A) [26] | (1) HAsi                      | Goat femoral defect (2 cm)           | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
|                          |                               |                                     | 𝜇CT: +           | +       |
| Reichert et al., 2011 (Int Ortho) [27] | (1) Untreated (2) mPCL-TCP (3) PLDLLA-TCP-PCL (4) ABG | Sheep tibial defect (2 cm) | X-ray: + | +       |
|                          |                               |                                     | Hist: +          | +       |
|                          |                               |                                     | 𝜇CT: +           | +       |
|                          |                               |                                     | Mech: +          | +       |
| (similar results of ABG) |                               |                                     |                  |         |
| Rentsch et al., 2012 (Biomatter) [28] | (1) PCL-Coll I-CS             | Sheep tibial defect (3 cm)           | X-ray: +         | +       |
|                          |                               |                                     | Hist: +          | +       |
|                          |                               |                                     | 𝜇CT: +           | +       |
|                          |                               |                                     | Mech: +          | +       |
| Kim et al., 2015 (Biomed Res Inter) [29] | (1) HA/alumina (2) HA/alumina-medullary canal (3 mm) | Dog tibial defect (2 cm) | X-ray: = | +       |
|                          |                               |                                     | 𝜇CT: =           | +       |
|                          |                               |                                     | Fluorescent labelling: + |         |
| (for HA/alumina-medullary canal) |                               |                                     |                  |         |
| Li et al., 2016 (Biomed Mat) [30] | (1) Baghdadite (2) Baghdadite-PCL-nBG | Sheep tibial defect (3 cm) | X-ray: = | +       |
|                          |                               |                                     | Hist: =          | +       |
|                          |                               |                                     | 𝜇CT: =           | +       |
|                          |                               |                                     | Mech: +          | +       |
| (for Baghdadite-PCL-nBG) |                               |                                     |                  |         |

| HA: hydroxyapatite, HA-TCP: hydroxyapatite-tricalcium phosphate, Si-TCP: silicon stabilized tricalcium phosphate, HA-PE: hydroxyapatite-polyethylene, HA-COL: hydroxyapatite-collagen, HA-COL-CS: hydroxyapatite-collagen-chondroitin sulphate, HASi: calcium silicate, tricalcium phosphate, and hydroxyapatite, mPCL-TCP: medical grade polycaprolactone-tricalcium phosphate, (PLDLLA)-TCP-PCL: poly(L-lactide-co-D,L-lactide)- polycaprolactone-tricalcium phosphate, ABG: autologous bone graft, PCL-Coll I-CS: polycaprolactone-collagen-chondroitin sulphate, HA-alumina: hydroxyapatite-alumina, Baghdadite: Ca₃Zr₂Si₄O₁₄, Baghdadite-PCL-nBG: Ca₃Zr₂Si₄O₁₄-polycaprolactone-bioactive glass nanoparticles, SEM: scanning electron microscopy, BSE: backscattered electron imaging, Hist: histological analysis, 𝜇CT: microcomputed tomography, Mech: mechanical analysis, X-ray: radiological analysis, +: positive effects, −: negative effects, and =: no significant difference. |
Table 2: Complete details of 39 preclinical papers identified in this systematic review focusing on the usefulness of scaffolds with augmentation in treating long bone defects.

| Authors | Biomaterials | Animal model | Cells/Gf's type and dose | Analysis | Scaffold results |
|---------|--------------|--------------|--------------------------|----------|-----------------|
| Grundel et al., 1999 (Clin. Orthop. Relat. Res) [31] | (1) Untreated (2) HA-TCP-Granular-BMCs (3) HA-TCP-Block-BMC (4) ABG (1) TCP (2) TCP-BMCs (3) HA-COL (4) HA-COL-BMCs (5) ABG | Dog ulna defect (2.5 cm) | BMSCs | X-ray: + Hist: + Mech: + | + (For HA-TCP-Block BMC) |
| Johnson et al., 1996 (J. Orth. Res) [32] | Dog radial defect (2.5 cm) | BMSCs | X-ray: + Hist: + Mech: + | + (For TCP-BMCs) |
| Bruder et al., 1998 (J. Bone & Joint Surg) [33] | Dog femoral defect (7.5 × 10^6/ml) | BMSCs | X-ray: + Hist: + | + |
| Kon et al., 1999 (J. Biomed. Mat. Res) [34] | Sheep tibial defect (2.5 cm) | BMSCs | X-ray: + Hist: + SEM: + | + |
| Arinzeh et al., 2003 (J. Bone & Joint Surg) [35] | Dog femoral defect (7.5 × 10^6/ml) | BMSCs | X-ray: + Hist: + | + |
| Bensaïdet al., 2005 (J. Tiss. Eng. A) [36] | Sheep metatarsus defect (1 × 10^7/ml) | BMSCs | X-ray: + Hist: + | + |
| Mastrogiacomo et al., 2005 (Orthod. Craniofac. Res) [37] | Sheep tibial defect (5 cm) | BMSCs | X-ray: + Hist: + | + |
| Viateau et al., 2006 (J. Orth. Res) [38] | Sheep metatarsus defect (2.5 cm) | BMSCs | X-ray: + Hist: + | + |
| Zhu et al., 2006 (J. Tiss. Eng) [39] | Goat femoral defect (2.5 cm) | BMSCs | X-ray: + Hist: + Mech: + | + |
| Mastrogiacomo et al., 2007 (J. Biomat) [40] | Sheep tibial defect (4 cm) | BMSCs | X-ray: + Hist: + μCT: + | + |
| Liu et al., 2008 (J. Mat Sci: Mat Med) [41] | Goat tibia defect (2.6 cm) | BMSCs | X-ray: + Hist: + μCT: + | + |
| Giannoni et al., 2008 (J. Tiss. Eng. Regen. Med) [42] | Sheep tibial defect (4.5 cm) | BMSCs | X-ray: + Hist: + | + |
| Nair et al., 2008 (J. Biomed. Mater. Res. A) [43] | Goat femoral defect (2 cm) | BMSCs | X-ray: + Hist: + μCT: + SEM: + | + |
| Authors                        | Biomaterials                                      | Animal model                  | Cells/GFs type and dose | Analysis  | Scaffold results          |
|-------------------------------|---------------------------------------------------|-------------------------------|-------------------------|-----------|---------------------------|
| Niemeyer et al., 2010         | (1) Untreated                                     | Sheep tibial defect (3 cm)    | BMSCs (2 x 10^7/ml)     | X-ray: +  | + (For allogenic BMSCs)   |
| (J. Tiss. Eng. A) [44]        | (2) HA-COL-BMSCs (allogenic)                      |                               |                         | Hist: +   |                           |
|                               | (3) HA-COL-BMSCs (xenogenic)                      |                               |                         |           |                           |
|                               | (1) CHA                                           |                               |                         |           |                           |
|                               | (2) CHA-BMSCs (vascularized)                      |                               |                         |           |                           |
|                               | (3) CHA (vascularized)                            |                               |                         |           |                           |
|                               | (4) CHA-BMSCs                                     |                               |                         |           |                           |
| Cai et al., 2011              | Dog fibula defect (1 cm)                          | BMSCs (20 x 10^6/ml)          | Hist: +                |           | +                         |
| (J. Biomat) [45]              |                                                   |                               | μCT: +                 |           |                           |
|                               |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | +                      |           |                           |
| Manassero et al., 2013        | Sheep metatarsus defect (2.5 cm)                   | BMSCs (7.5 ± 1.2 x 10^6/implant) | X-ray: +       |           |                           |
| (J. Tiss. Eng. A) [46]        |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | μCT: +                 |           | (Similarly for both autologous and allogenic cells) |
| Berner et al., 2013           | Sheep tibial defect (3 cm)                         | BMSCs (35 x 10^5/500 μl)     | X-ray: +       |           |                           |
| (Acta. Biomater) [47]         |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | μCT: =                |           |                           |
| Fan et al., 2014              | Monkey tibial defect (2 cm)                        | BMSCs (5 x 10^6/implant)      | X-ray: +       |           |                           |
| (J. Biomat) [48]              |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | SPECT: +              |           |                           |
|                               |                                                   |                               | MRI: +                 |           |                           |
| Yoon et al., 2015             | Dog ulna defect (1.5 cm)                          | ADMSCs (1 x 10^5/50 μl)       | X-ray: +       |           | + (For TCP-β-BMSCs-saphenous vascular) |
| (J Vet Sci) [49]              |                                                   |                               | Hist: +                |           |                           |
| Berner et al., 2015           | Sheep tibial defect (3 cm)                         | BMSCs (100 x 10^6)           | X-ray: +       |           |                           |
| (Stem cells Trans Med) [50]   |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | μCT: +                |           |                           |
|                               |                                                   |                               | SEM: +                 |           |                           |
|                               |                                                   |                               | Mech: +                |           |                           |
| Masaoka et al., 2016          | Monkey femur defect (5 cm)                         | BMSCs (1.3–4.1 x 10^6/ml)    | X-ray: +       |           |                           |
| (The Open Biomed Eng J) [51]  |                                                   |                               | Hist: +                |           |                           |
| Smith et al., 2017            | Sheep tibial defect (3.5 cm)                       | BMSCs (1 x 10^7/3 implant)    | Hist: +       |           |                           |
| (J Tiss Eng Reg Med) [52]     |                                                   |                               | μCT: +                |           |                           |
|                               |                                                   |                               | Mech: +                |           |                           |
| Kirker-Head et al., 1995      | Sheep femoral defect (2.5 cm)                      | BMP-2 (2 mg and 4 mg)         | X-ray: +       |           | + (For both concentrations of BMP-2) |
| (Clin. Orthop. Relat. Res) [53]|                                                   |                               | Hist: +                |           |                           |
| Scidini et al., 1997          | Dog radial defect (2.5 cm)                         | BMP extract (3 mg/implant)    | X-ray: +       |           | +                         |
| (J. Orth. Res) [54]           |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | Mech: +                |           |                           |
| Gao et al., 1997              | Sheep tibial defect (1.6 cm)                       | BMP extract (100 mg/implant)  | X-ray: +       |           | +                         |
| (Int. Ortho) [55]             |                                                   |                               | Hist: +                |           |                           |
|                               |                                                   |                               | Mech: +                |           | + +                       |
### Table 2: Continued.

| Authors                  | Biomaterials                  | Animal model          | Cells/Growth factors and dose | Analysis | Scaffold results |
|--------------------------|--------------------------------|-----------------------|------------------------------|----------|-----------------|
| Tuominen et al., 2001    | (1) HA                         | Dog ulna defect (2 cm) | BMP extract                  | X-ray: − | − (With or without BMP but inferior to ABG) |
|                          | (2) HA-BMP                     |                       |                              | Hist: −  |                 |
|                          | (3) ABG                        |                       |                              | Mech: −  |                 |
|                          | (56)                            |                       |                              |          |                 |
| Hu et al., 2003          | (1) Untreated HA-COL-PLA        | Dog radial defect (2 cm)| BMP extract (30 mg/implant)  | X-ray: + |                 |
|                          | (3) HA-COL-PLA-PLA-BMP         |                       |                              | Hist: +  |                 |
|                          | (57)                            |                       |                              | DEXA: +  |                 |
| Cook et al., 2005        | (1) 3.5 mg BMP-7               | Dog ulna defect (2.5 cm) | BMP-7 (3.5 mg/implant and 1.75 mg BMP-7-CMC) | X-ray: = |                 |
|                          | (3) 3.5 mg BMP-7-CMC            |                       |                              | Hist: =  |                 |
|                          | (4) 1.75 mg BMP-7-CMC           |                       |                              | Mechanical: = |                 |
|                          | (58)                            |                       |                              |          |                 |
| Maissen et al., 2006     | (1) Untreated PLA               | Sheep tibial defect (1.8 cm) | rhTGFβ-3 (269.4 μg/implant) | μCT: −  | − (Inferior to ABG) |
|                          | (2) PLA                        |                       |                              | X-ray: − |                 |
|                          | (3) PLA-rhTGFβ-3               |                       |                              | Hist: +  |                 |
|                          | (4) ABG                        |                       |                              | μCT: +  |                 |
|                          | (59)                            |                       |                              | BSE: +   |                 |
|                          |                                 |                       |                              | SAXS: +  |                 |
|                          |                                 |                       |                              | Mech: +  |                 |
| Cipitria et al., 2015    | (1) mPCL-TCP                   | Sheep tibial defect (3 cm) | BMP-7 (3.5 mg/implant)      | X-ray: = |                 |
|                          | (2) mPCL-TCP+BMP-7             |                       |                              | Hist: +  |                 |
|                          | (60)                            |                       |                              | μCT: +   |                 |
|                          |                                 |                       |                              | BSE: +   |                 |
|                          |                                 |                       |                              | SAXS: +  |                 |
|                          |                                 |                       |                              | Mech: +  |                 |
| Petite et al., 2000      | (1) Coral-BMSCs                | Sheep metatarsal defect (2.5 cm) | BMSCs (3.25 ± 0.25 × 10^7 cells/ml) | X-ray: + | + (For BMSCs) |
|                          | (2) Coral-BMSCs                |                       |                              | BMSCs (7 × 10^6 ± 1 × 10^6) | Hist: + |
|                          | (3) Coral                      |                       |                              |          |                 |
| den Boer et al., 2003    | (1) Untreated                  | Sheep tibial defect (3 cm) | BMP-7 (2.5 mg/implant BMPs) | X-ray: + | + (For both HA + BMP-7 and HA + BMSCs) |
|                          | (2) ABG                        |                       |                              | Hist: =  |                 |
|                          | (3) HA                         |                       |                              | Mech: +  |                 |
|                          | (4) HA-BMP-7                   |                       |                              |          |                 |
|                          | (5) HA-BMSCs                   |                       |                              |          |                 |
| Comparisons              | (61)                            |                       |                              |          |                 |
| Filar do et al., 2014    | (1) BioSiC(HA-COL)             | Sheep metatarsal defect (2 cm) | BMSCs (4 ± 2 × 10^5/ml) | X-ray: = | + (For BMSCs) |
|                          | (2) BioSiC(HA-COL) + PRP       |                       |                              | Hist: +  |                 |
|                          | (3) BioSiC(HA-COL) + BMSCs     |                       |                              |          |                 |
| Berner et al., 2015      | (1) mPCL-TCP-PRP               | Sheep tibial defect (3 cm) | MPCs, mOB, tOB (35 × 10^6 cells) | X-ray: + | + (For mPCL-TCP-allogenic MPC) |
|                          | (2) mPCL-TCP-allogenic-MPC     |                       |                              | Hist: +  |                 |
|                          | (3) mPCL-TCP-allogenic-mOB     |                       |                              | μCT: +   |                 |
|                          | (4) mPCL-TCP-allogenic-tOB     |                       |                              | BSE: +   |                 |
|                          | (64)                            |                       |                              | SAXS: +  |                 |
|                          |                                 |                       |                              | Mech: +  |                 |
| Nair et al., 2009        | (1) HASi                       | Goat femoral defect (2 cm) | BMSCs (1 × 10^5 cm^2) | X-ray: + | + (For HASi + BMSCs + PRP) |
|                          | (2) HASi + BMSCs                |                       |                              | Hist: +  |                 |
|                          | (3) HASi + BMSCs + PRP         |                       |                              |          |                 |
| Zhu et al., 2009         | (1) Coral-BMSCs                | Goat femoral defect (2.5 cm) | BMSCs (5 × 10^7/ml) | X-ray: + | + (For Coral-AdBMP-7-BMSCs) |
|                          | (2) Coral-AdBMP-7-BMSCs        |                       |                              | Hist: +  |                 |
|                          | (65)                            |                       |                              | Mech: +  |                 |
| Combinations             | (66)                            |                       |                              |          |                 |
| Reichert et al., 2012    | (1) Untreated                  | Sheep tibial defect (3 cm) | BMSCs (35 × 10^6 cells/ 250 μl) | X-ray: + | + (For mPCL-TCP-BMP-7) |
|                          | (2) mPCL-TCP                   |                       |                              | Hist: +  |                 |
|                          | (3) mPCL-TCP-BMSCs + PRP       |                       |                              | μCT: +   |                 |
|                          | (4) mPCL-TCP-BMP-7             |                       |                              | BSE: +   |                 |
|                          | (5) ABG                        |                       |                              | SAXS: +  |                 |
|                          | (67)                            |                       |                              | Mech: +  |                 |
Table 2: Continued.

| Authors | Biomaterials | Animal model | Cells/Gf type and dose | Analysis | Scaffold results |
|---------|--------------|--------------|------------------------|----------|-----------------|
| Li et al., 2014 (Orthop) [68] | (1) TCP-β-OCs-ECs | Sheep femoral defect (3 cm) | OCs and ECs (2 × 10^6/ml) | X-ray: + | (For TCP-β OCs-ECs) |
| Ronca et al., 2014 (J Biomat Appl) [69] | (1) HYAFF11 * | Sheep metatarsus defect (2 cm) | BMSCs (1 × 10^6/ml) | Hist: + | (For HYAFF11 + BMP-7) |

HA: hydroxyapatite, HA-TCP: hydroxyapatite-tricalcium phosphate, HA-COL: hydroxyapatite-collagen, TCP: tricalcium phosphate, ABG: autologous bone graft, PLGA: poly(DL-(lactic-co-glycolide)), HA-COL-PLA: hydroxyapatite-collagen-poly(L-lactic acid), CMC: carboxymethyl cellulose, CHA: coral hydroxyapatite, PLA: poly(L/DL-lactide), Si-TCP: silicon stabilized tricalcium phosphate, β-TCP: beta tricalcium phosphate, HA-Si-TCP: hydroxyapatite silicon stabilized tricalcium phosphate, HASi: calcium silicate, tricalcium phosphate, hydroxyapatite, mPCL-TCP: medical grade polycaprolactone-tricalcium phosphate, BioSic(HA-COL): biomorphic silicon carbide hydroxyapatite-collagen, HYAFF11: poly(ε-caprolactone-poly-L-lactic acid) with hyaluronan derivatives, PLLA-PCL: poly(L-lactic acid)-poly(ε-caprolactone), ASA: autologous serum albumin, PCL-HA: polycaprolactone-hydroxyapatite, AdBMP-7: adenovirus mediated bone morphogenetic protein 7, ADMSCs: adipose derived mesenchymal stem cell, BMPs: bone marrow concentrates, MPCs: mesenchymal progenitor cells, tOBs: axial skeleton osteoblasts, mOBs: orofacial skeleton osteoblasts, BMP: bone morphogenetic protein, BMP-2: bone morphogenetic protein-2, BMP-7: bone morphogenetic protein-7, BMSCs: bone marrow derived mesenchymal stem cell, rhTGF-β3: recombinant transforming growth factor beta 3, PRP: platelet rich plasma, OCs: osteoblast cells, ECs: endothelial cells, SEM: scanning electron microscopy, SAXS: small angle X-ray scattering, DEXA: dual-energy X-ray absorptiometry, Hist: histological analysis, μCT: microcomputed tomography, Mech: mechanical analysis, X-ray: radiological analysis, +: positive effects, -: negative effects, and =: no significant difference.

Table 3: Complete details of 5 clinical papers identified in this systematic review focusing on the usefulness of scaffolds with or without augmentation in treating long bone defects.

| References | Study type | Pathology | Scaffold | Augmentation | Number of patients | Follow-up | Results |
|------------|------------|-----------|----------|--------------|-------------------|----------|---------|
| Werber et al., 2000 (J Hand Surg) [70] | Case series | Distal radius fracture | HA ceramic from bovine spongiosa (Merck Biomaterials) | — | 14 | 15 m | Bone healed around the graft material and fibrovascular ingrowth within the scaffold observed |
| Quarto et al., 2001 (N Engl J Med) [71] | Case series | Tibia, humerus, and ulna defect | Porous HA ceramic (Fincemarica) | BMSCs (2 × 10^7 cells/mL) | 3 | 15–27 m | Limb function recovered for all patients; good integration with the host bones by the second month after surgery in all cases |
| Arai et al., 2005 (Clin Orthop Relat Res) [72] | Case series | Fibula resections for use as autograft for reconstruction of large segmental defects of tibia | TCP (Osferion Olympus) | — | 14 | 4–42 m (mean 17 m) | In 12 patients scaffold was absorbed and replaced by newly formed bone at an average 9.3 months after surgery. In all children, new bone formation was at 3.2 months; only one patient had complete regeneration of the fibula |
| Marcacci et al., 2007 (Tissue Engineering) [73] | Case series | Tibia, humerus, and ulna defect | Porous HA ceramic (Fincemarica) | BMSCs (2 × 10^7 cells/mL) | 4 | 1.25–7 y | In all patients, good integration of the implants with host bone; no late fractures in the implant zone |

HA: hydroxyapatite, TCP: tricalcium phosphate, and BMSCs: bone marrow derived mesenchymal stem cell.

The use of scaffolds with GFs augmentation was reported in 8/39 papers. Out of these, 7/8 papers reported the use of BMPs (freshly extracted BMPs from bone in 4/7, BMP-2 in 1/7, and BMP-7 in 2/7) and 1/8 paper reported the use of rhTGFβ-3. Results of 5/8 articles reported positive effects, mainly emphasizing that GFs largely assisted the healing process of critical sized defects due to their osteoinductive properties.

On the other hand, 2/8 papers, 1 on freshly extracted BMPs from bone and 1 on rhTGFβ-3, reported inferior and similar results, respectively, when compared to ABG treatment.
Four papers out of 39 compared the use of different cell sources, or cells versus GFs. Among these, 2 papers reported superior results for BMSCs when compared to PRP or BMC augmentation. One paper showed no significant differences between BMC and BMP-7 added to a HA scaffold, with better results compared to the scaffold alone and similar results compared to a ABG. Interestingly, one paper comparing the effects of PRP, mesenchymal progenitor cells (MPCs), orofacial skeleton osteoblasts (mOBs), and axial skeleton osteoblasts (tOBs) found that the MPCs group produced better results when compared to other biological enhancers.

The use of cells and GFs in combinations was reported in 5/39 papers with different study designs, which prevents us drawing an overall conclusion. The combination of scaffolds, cells, and GFs (either PRP or AdBMP-7) provided superior results compared to the scaffold/cell construct in 2/5 papers. On the contrary, 2/5 studies showed worse results for scaffold/BMSCs/PRP compared to a scaffold/BMP-7 construct. Finally, one study showed superior results combining 2 cell sources, such as OBs (osteoblasts cells) and ECs (endothelial cells) compared to the use of a single cell type (ECs).

3.2. Clinical Studies. The literature search identified 4 clinical papers that met the inclusion criteria. Two of the 4 clinical trials used scaffolds without any augmentation and the other 2 reported a cell augmentation approach. An HA scaffold was used in 3/4 papers followed by beta-TCP in 1 study.

In 2000 Werber et al. [70] presented a study about the treatment of distal radius fractures with HA ceramic from processed bovine spongiosa. The scaffold was implanted in 14 patients followed up for up to 15 months after surgery. Magnetic resonance imaging (MRI) scans showed integration of the biomaterial with the surrounding tissue and bone regeneration in 13 patients, without any adverse events (MRI was nondiagnostic in 1 case where a broken screw caused extensive artifacts). However, only in 1 patient complete radius regeneration was documented. One year later, a case series performed by Quarto et al. in 2001 [71], followed up by Marcacci et al. in 2007 [73], described the treatment of tibia, humerus, and ulna segmental defects with porous HA ceramic scaffold seeded with BMSCs, expanded by culture with fetal calf serum and FGF-2, and suspended in fibrin glue activated with thrombin to form the final ceramic-cell composite. Radiographic and computed tomography (CT) analyses reported complete integration between the scaffold and host bone starting from 5 to 7 months after surgery in all 4 patients. In 3 of them, whose evaluations were available at longer follow-up times, this trend was confirmed until 6 to 7 years after surgery. Additionally, no major complications were reported in the early or late periods after surgery. In 2005, Arai et al. [72] investigated the use of beta-TCP scaffold for the treatment of 14 patients who had fibula resections to be used as autogenous bone grafts. These were used for the reconstruction of large segmental defects in benign bone tumours of the extremities and pseudarthrosis of the tibia. At an average time of 9.3 months after surgery, scaffold absorption and new bone formation were observed. However, according to the radiographic evaluation, complete regeneration of the fibula occurred only in one case. In 2 paediatric patients, implant replacement by neotissue was noticed already at 3.2 months after surgery.

4. Discussion

In this systematic review, the interest on the scaffold based strategy to treat long bone defects was documented by the great amount of preclinical papers (even though highly heterogeneous) published over the past few years, showing overall promising findings in terms of radiological and histological evidence, with the ability to treat segmental defects in large animal models. Unfortunately, this in vivo situation is not reflected by a corresponding clinical impact of this treatment approach, with few published clinical papers, highly heterogeneous and presenting small patient populations.

In orthopaedic surgery, critical size bone defects derived from nonunion, trauma, or tumours are a challenging problem, from both a social and economic perspective. In fact, in Europe, the total cost of treatment of nonunion defects is between 10000 and 100000 € per patient, with an amount of around 1 million bone operations every year [74]. Bone grafting as an ABG still represents a gold standard for regenerating bone defects. Approximately, 2.2 million bone grafting procedures are performed worldwide every year and the majority involve ABG followed by allograft [74]. Although ABG has been widely used for the treatment of long bone defects with a high success rate reported to range between 70 and 95%, a 50% failure rate has also been reported [74, 75]. These failures of autologous grafting procedures can be related to morbidity, pain, and many other perioperative and postoperative complications caused by the harvesting process. Allograft can be a good substitute of ABG by avoiding donor-site morbidity and pain, but immunorejection, bacterial infections, and viral transmission are limitations of this procedure [76, 77], which still offers not optimal outcomes. In fact, the internal repair (revascularization and substitution of the original graft bone with new host bone) progresses slowly and seems to be confined only to the superficial surface and the ends of the graft [78, 79]. Furthermore, the rate of complications increases proportionally to the size of the defect that have to be replaced [79], and among these, allograft fractures are the major drawback [80]. Moreover, the cost of allo- or autografting can be high, which further prompts the development of other strategies such as BTE [74].

In the last 20 years, the number and variety of biomaterials developed for the treatment of segmental bone defects have been increasing, especially in preclinical setting. This review was focused on solid biomaterials, with 3D scaffolds that can mimic bone structure and composition when implanted in vivo into the defects, with results documented in studies on large animals (to better reproduce human conditions) and in clinical settings.

HA and ceramic calcium phosphates, such as TCP that resemble mineral components of bone, are the most used materials in both preclinical and clinical settings, offering a biological response similar to that of natural bone [81]. This ability is probably due to their suitable chemical composition, porosity, and mechanical properties [81], which may differ among scaffolds. TCP ceramics possess sufficient porosity,
which may be adjusted to favour neotissue in-growth; however, their biomechanical resistance is limited compared to HA [76, 81]. On the other hand, a key aspect that could affect the final clinical outcome is the degradation time of the scaffold: in this light, the mechanical properties of HA are counterbalanced by its slow degradation by osteoclasts, which is approximately 2–5 years, while a faster biodegradation, as in the case of TCP that is degraded in 1 year, could lead to a faster loss of mechanical strength [81]. Indeed, slow biodegradation of the HA scaffold was observed in the clinical trial of Werber et al. where at the 15-month follow-up porous HA was not completely resorbed and replaced by new bone [70]. Moreover, in the clinical study performed by Maracci et al., HA ceramics were not absorbed even after 7 years [73]. On the contrary, Arai et al. used a degradable beta-TCP scaffold and observed its absorption and deposition by new bone in 12 out of 14 patients 9 months after surgery, although its regeneration was mainly incomplete, with only one adult patient presenting a complete regenerated fibula [72]. Therefore, the design of a mechanically stable material, suitable for load-bearing in segmental defects, which is also bioabsorbable, remains challenging [74–77].

Another interesting aspect in the field of biomaterials is related to the diamond concept, which involves a combined approach by combining osteoinductive factors (cells or GFs) with 3D scaffolds and was the most investigated option in preclinical settings. Due to the heterogeneity of these studies (different cell sources or GFs used, application protocol, dosage, . . .), it is difficult to draw a conclusion about the best augmentation procedure able to enhance bone healing. In fact, overall positive results have been reported, but only few papers compared the different augmentation strategies in the preclinical model, thus leaving literature findings inconclusive. Among GFs, the most exploited strategy involves the BMPs family, whose discovery dates back to the end of the nineteenth century and drew an increasing attention in the scientific community, with a large literature including also the overall good results of the clinical application, although documented adverse events and some controversial reported outcomes limited their impact in the clinical practice [82]. While other isolated GFs have been explored as well, the currently most exploited strategy to deliver GFs is the use of blood derivatives such as platelet concentrates [5]. PRPs are proposed as powerful tools for tissue healing, thanks to the many GFs contained in their alpha granules, which can be delivered concentrated but in physiologic proportions [5]. The evidence for PRP osteogenic potential has been suggested by several in vitro studies. PRP addition in culture medium promoted the proliferation and differentiation of MSCs, PRP can improve cell chemokinesis and chemotaxis through cytoskeleton reorganization and accelerate cell migration, thus influencing cells mobility, and antimicrobial effects have been suggested as well, which are highly desirable in relation to a surgical bone application [5]. Nonetheless, besides the aforementioned beneficial roles, in vitro studies have also shown controversial results on PRP potential to favour bone healing, which remains a debated aspect [5]. Among augmentation strategies, MSCs represent an exciting and promising cell population for bone regeneration, especially when tissue engineering or biomaterials are applied [83]. Their potential of “natural system of tissue repair” has been suggested by studies in different fields of medical application, and they have been extensively investigated also for bone tissue engineering. MSCs have been firstly identified in bone marrow, but nowadays they have been isolated also from other human sources, which are explored in terms of potential applicability in the clinical practice. In this light, considering that cell amplification by culture is not free from the dangers of bacterial contamination and entails economic and regulatory limitations, the use of concentrates is gaining increasing interest, despite the lower number of cells with respect to cell expansion process [83]. While autologous cells have been preferred up to now in the clinical scenario, the possibility of simplifying the procedure by taking advantage of allogeneic cells seems attractive and is currently explored in terms of potential and risks as well. This preclinical review documented many studies applying allogeneic cells, but only one study directly compared autologous and allogeneic sources, showing overall similarly good results [47]. Finally, in light of future advancement of the augmentation strategies potential, gene therapy is investigated to improve the repair of tissues by providing a temporarily and spatially defined expression of therapeutic genes at the site of injury [84]. In fact, adapting tissue engineering platforms to gene transfer approaches mediated by viral vectors is an attractive tool to circumvent both the limitations of the current therapeutic options to promote an effective healing of the tissue. Several gene transfer vehicles have been developed to modify human cells and tissues from musculoskeletal system, and future studies should demonstrate whether this technology might provide an effective solution compared to the other available augmentation strategies for bone healing [84]. Therefore, while cells and GFs are highly attractive for the healing of segmental defects, the identification of the best application strategy still requires investigation with specifically designed studies to compare cells from different sources and with different manipulation, GFs, and their combinations.

The clinical scenario does not reflect the high research activity documented by the preclinical literature: only 4 papers were found, all with a small patient number, different study design, and heterogeneous pathologies treated. The search identified 2 clinical trials using scaffolds without any augmentation, while another 2 reported a cell augmented scaffold approach. Among different sources of MSCs, bone marrow was the most commonly used for BTE in orthopaedic surgery [75, 76]. BMSCs can be easily isolated from the iliac crest, immediately injected or implanted with the carrier into the defect or expanded in vitro before implantation. In two clinical studies presented in this review, expanded BMSCs were seeded on a HA porous scaffolds, both showing satisfactory results [71, 73]. Nevertheless, lack of suitable controls did not allow verifying whether the positive clinical outcome is derived from the added regenerative potential of the implanted cells or from the implanted scaffold itself, by promoting the body own regenerative potential. Thus, while there is a huge demand to increase the regenerative potential of scaffolds, the difficulties in translating preclinical findings into clinical practice leave many questions still
unanswered \[77\]. Further studies are needed to develop strategies with scaffold, cells, and GFs combined to overcome the results of autografts and offer a suitable treatment option to rapidly regenerate bone segmental defects.

5. Conclusion

This systematic research of the literature documented a growing interest in scaffold based approaches applied in preclinical settings to promote tissue regeneration in long bone defects of critical size. However, this evidence did not translate into a similar interest in the clinical scenario, characterized by only 4 papers published, with low quality and heterogeneous study designs. Several interesting aspects have been underlined by preclinical literature, in particular with regard to the benefit of an augmentation strategy to enhance the regenerative potential of the biomaterial. These should be further investigated in order to translate positive preclinical findings into clinical protocols: first of all, to identify the best biomaterial for long bone defects, with both biological and biomechanical suitable properties, and then to select the best choice between cells, GFs, or their combination, in order to provide the best treatment option for patients affected by long bone defects.

Conflicts of Interest

Dr. Elizaveta Kon is (1) paid presenter or speaker at Zimmer-Biomet (USA); (2) paid consultant, stock or stock options, at Cartiheal (Israel); (3) paid presenter or speaker at Fidia (Italy); (4) paid presenter or speaker at Fincerasma (Italy); (5) board or committee member of International Cartilage Repair Society; and (6) editorial or governing board member of Journal of Experimental Orthopedics. Dr. Giuseppe Filardo (1) received institutional support from Zimmer-Biomet (USA); (2) is consultant and received institutional support from Cartiheal (Israel); (3) is consultant and received institutional support from Fidia (Italy); (4) is consultant and received institutional support from Fincerasma (Italy); (5) is consultant and received institutional support from Green Bone (Italy); (6) received institutional support from DSM Biomedical (USA); (7) received institutional support from IGEA Clinical Biophisic; and (8) received institutional support from PIRAMAL/Smith-Nephew. All the other authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

[1] C. S. Molina, D. J. Stinner, and W. T. Obremskkey, “Treatment of traumatic segmental long-bone defects: a critical analysis review,” Journal of Bone and Joint Surgery Reviews, vol. 2, no. 4, article e1, 2014.
[2] E. A. Horner, J. Kirkham, D. Wood et al., “Long bone defect models for tissue engineering applications: criteria for choice,” Tissue Engineering Part B, Reviews, vol. 16, no. 2, pp. 263–271, 2010.
[3] T. J. Blokhus, B. W. Wippermann, F. C. Den Boer et al., “Resorbable calcium phosphate particles as a carrier material for bone marrow in an ovine segmental defect,” Journal of Biomedical Materials Research, vol. 51, no. 3, pp. 369–375, 2000.
[4] W. Zhi, C. Zhang, K. Duan et al., “A novel porous bioceramics scaffold by accumulating hydroxyapatite spherulites for large bone tissue engineering in vivo. II. Construct large volume of bone grafts,” Journal of Biomedical Materials Research - Part A, vol. 102, no. 8, pp. 2491–2501, 2014.
[5] A. Roffi, B. Di Matteo, G. S. Krishnakumar, E. Kon, and G. Filardo, “Platelet-rich plasma for the treatment of bone defects: from pre-clinical rational to evidence in the clinical practice. A systematic review,” International Orthopaedics, vol. 41, no. 2, pp. 221–237, 2017.
[6] D.-X. Wang, Y. He, L. Bi et al., “Enhancing the bioactivity of Poly(lactic-co-glycolic acid) scaffold with a nano-hydroxyapatite coating for the treatment of segmental bone defect in a rabbit model,” International Journal of Nanomedicine, vol. 8, no. 1, pp. 1855–1865, 2013.
[7] J. R. Field, M. McGee, R. Stanley et al., “The efficacy of allogeneic mesenchymal precursor cells for the repair of an ovine tibial segmental defect,” Veterinary and Comparative Orthopaedics and Traumatology, vol. 24, no. 2, pp. 113–121, 2011.
[8] L. Bi, B. Zobell, X. Liu, M. N. Rahaman, and L. F. Bonewald, “Healing of critical-size segmental defects in rat femora using strong porous bioactive glass scaffolds,” Material Science Engineering C Materials for Biological Applications, vol. 42, pp. 816–824, 2014.
[9] J. C. Reichert, S. Saijfzadeh, M. E. Wüllschleger et al., “The challenge of establishing preclinical models for segmental bone defect research,” Biomaterials, vol. 30, no. 12, pp. 2149–2163, 2009.
[10] P. V. Giannoudis, T. A. Einhorn, and D. Marsh, “Fracture healing: the diamond concept,” Injury, vol. 38, 4, pp. S3–S6, 2007.
[11] E. L. Smith, J. M. Kanczler, D. Gothard et al., “Evaluation of skeletal tissue repair, part 1: assessment of novel growth-factor-releasing hydrogels in an ex vivo chick femur defect model,” Acta Biomaterialia, vol. 10, no. 10, pp. 4186–4196, 2014.
[12] L. F. Amorosa, C. H. Lee, A. B. Aydemir et al., “Physiologic load-bearing characteristics of autografts, allografts, and polymer-based scaffolds in a critical sized segmental defect of long bone: an experimental study,” International Journal of Nanomedicine, vol. 8, no. 1, pp. 1637–1643, 2013.
[13] Y. Guo, R. T. Tran, D. Xie et al., “Citrate-based biphasic scaffolds for the repair of large segmental bone defects,” Journal of Biomedical Materials Research - Part A, vol. 103, no. 2, pp. 772–781, 2015.
[14] A. Tampieri, G. Celotti, S. Sprio, A. Delcogliano, and S. Fracese, “Porosity-graded hydroxyapatite ceramics to simulate natural bone,” Biomaterials, vol. 22, no. 11, pp. 1365–1370, 2001.
[15] M. Griffin, S. A. Iqbal, and A. Bayat, “Exploring the application of mesenchymal stem cells in bone repair and regeneration,” The Journal of Bone & Joint Surgery—British Volume, vol. 93, no. 4, pp. 427–434, 2011.
[16] E. Argintar, S. Edwards, and J. Delahay, “Bone morphogenetic proteins in orthopaedic trauma surgery,” Injury, vol. 42, no. 8, pp. 730–734, 2011.

[17] Z. Gu, X. Zhang, L. Li, Q. Wang, X. Yu, and T. Feng, “Acceleration of segmental bone regeneration in a rabbit model by strontium-doped calcium polyphosphate scaffold through stimulating VEGF and bFGF secretion from osteoblasts,” Material Science Engineering C Materials for Biological Applications, vol. 33, no. 1, pp. 274–281, 2013.

[18] S. Kotev-Emeth, N. Savion, S. Pri-chen, and S. Pitaru, “Effect of maturation on the osteogenic response of cultured stromal bone marrow cells to basic fibroblast growth factor,” Bone, vol. 27, no. 6, pp. 777–783, 2000.

[19] A. Boyde, A. Corsi, R. Quarto, R. Cancedda, and P. Bianco, “Osteoconduction in large microporous hydroxyapatite ceramic implants: evidence for a complementary integration and disintegration mechanism,” Bone, vol. 24, no. 6, pp. 579–589, 1999.

[20] M. Maracci, E. Kon, S. Zaffagnini et al., “Reconstruction of extensive long-bone defects in sheep using porous hydroxyapatite sponges,” Calcified Tissue International, vol. 64, no. 1, pp. 83–90, 1999.

[21] C. Zhang, J. Wang, H. Feng, B. Lu, Z. Song, and X. Zhang, “Replacement of segmental bone defects using porous bioceramic cylinders: a biomechanical and X-ray diffraction study,” Journal of Biomedical Materials Research, vol. 54, no. 3, pp. 407–411, 2001.

[22] M. Mastrogiacomo, A. Corsi, E. Francioso et al., “Reconstruction of extensive long bone defects in sheep using resorbable bioceramics based on silicon stabilized tricalcium phosphate,” Tissue Engineering, vol. 12, no. 5, pp. 1261–1273, 2006.

[23] F. Sarlilmaz, N. Orhan, E. Unsaldi, A. S. Durmus, and N. Colakoglu, “A polyethylene-high proportion hydroxyapatite implant and its investigation in vivo,” Acta of Bioengineering and Biomechanics, vol. 9, no. 2, pp. 9–16, 2007.

[24] W. Schneiders, A. Reinstorff, A. Biewener et al., “In vivo effects of modification of hydroxyapatite/collagen composites with and without chondroitin sulphate on bone remodeling in the sheep tibia,” Journal of Orthopaedic Research, vol. 27, no. 1, pp. 15–21, 2009.

[25] S. K. Nandi, B. Kundu, S. Datta, D. K. De, and D. Basu, “The repair of segmental bone defects with porous bioglass: an experimental study in goat,” Research in Veterinary Science, vol. 86, no. 1, pp. 162–173, 2009.

[26] M. B. Nair, H. Varma, S. J. Shenoy, and A. John, “Treatment of goat femur segmental defects with silica-coated hydroxyapatite—one year follow-up,” Tissue Engineering - Part A, vol. 16, no. 2, pp. 385–391, 2010.

[27] J. C. Reichert, M. E. Wullschleger, A. Cipitria et al., “Custom-made composite scaffolds for segmental defect repair in long bones,” International Orthopaedics, vol. 35, no. 8, pp. 1229–1236, 2011.

[28] B. Rentsch, R. Bernhardt, D. Scharmweb, W. Schneider, S. Rammelt, and C. Rentsch, “Embroidered and surface coated polycaprolactone-co-lactide scaffolds: a potential graft for bone tissue engineering,” Biomaterial, vol. 2, no. 3, pp. 158–165, 2012.

[29] J. M. Kim, J. S. Son, S. S. Kang, G. Kim, and S. H. Choi, “Bone regeneration of Hydroxyapatite/Alumina Bilayered Scaffold with 3mm Passage-Like Medullary Canal in Canine Tibia Model,” BioMed Research International, vol. 2015, Article ID 235108, 6 pages, 2015.

[30] J. Li, S.-I. Roohani-Esfahani, C. R. Dunstan et al., “Efficacy of novel synthetic bone substitutes in the reconstruction of large segmental bone defects in sheep tibiae,” Biomedical Materials (Bristol), vol. 11, no. 1 article, 015016, 2016.

[31] R. E. Grundel, M. W. Chapman, T. Yee, and D. C. Moore, “Autogenic bone marrow and porous biphasic calcium phosphate ceramic for segmental bone defects in the canine ulna,” Clinical Orthopaedics and Related Research, no. 266, pp. 244–258, 1999.

[32] K. D. Johnson, K. E. Frierson, T. S. Keller et al., “Porous ceramics as bone graft substitutes in long bone defects: a biomechanical, histological, and radiographic analysis,” Journal of Orthopaedic Research, vol. 14, no. 3, pp. 351–369, 1996.

[33] S. P. Bruder, K. H. Kraus, V. M. Goldberg, and S. Kadiyla, “The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects,” The Journal of Bone and Joint Surgery. American Volume, vol. 80, no. 7, pp. 985–996, 1998.

[34] E. Kon, A. Muragli, A. Corsi et al., “Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerates bone repair in critical-size defects of sheep long bones,” Journal of Biomedical Materials Research, vol. 49, no. 3, pp. 328–337, 2000.

[35] T. L. Arinzech, S. J. Peter, M. P. Archambault et al., “Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect,” The Journal of Bone and Joint Surgery. American Volume, vol. 85-A, no. 10, pp. 1927–1935, 2003.

[36] W. Bensaid, K. Oudina, V. Viateau et al., “De Novo reconstruction of functional bone by tissue engineering in the metatarsal sheep model,” Tissue Engineering, vol. 11, no. 5-6, pp. 814–824, 2005.

[37] M. Mastrogiacomo, A. Muraglia, V. Komlev et al., “Tissue engineering of bone: search for a better scaffold,” Orthodontics & Craniofacial Research, vol. 8, no. 4, pp. 277–284, 2005.

[38] V. Viateau, G. Guillemin, V. Bousson et al., “Long-bone critical-size defects treated with tissue-engineered grafts: a study on sheep,” Journal of Orthopaedic Research, vol. 25, no. 6, pp. 741–749, 2007.

[39] L. Zhu, W. Liu, L. Cui, and Y. Cao, “Tissue-engineered bone repair of goat-femur defects with osteogenically induced bone marrow stromal cells,” Tissue Engineering, vol. 12, no. 3, pp. 423–433, 2006.

[40] M. Mastrogiacomo, A. Papadimitropoulos, A. Cedola et al., “Engineering of bone using bone marrow stromal cells and a silicon-stabilized tricalcium phosphate bioceramic: evidence for a coupling between bone formation and scaffold resorption,” Biomaterials, vol. 28, no. 7, pp. 1376–1384, 2007.

[41] G. Liu, L. Zhao, W. Zhang, L. Cui, W. Liu, and Y. Cao, “Repair of goat tibial defects with bone marrow stromal cells and beta-tricalcium phosphate,” Journal of Materials Science: Materials in Medicine, vol. 19, no. 6, pp. 2367–2376, 2008.

[42] P. Giannoni, M. Mastrogiacomo, M. Alini et al., “Regeneration of large bone defects in sheep using bone marrow stromal cells,” Journal of Tissue Engineering and Regenerative Medicine, vol. 2, no. 5, pp. 253–262, 2008.

[43] M. B. Nair, H. K. Varma, K. V. Menon, S. J. Shenoy, and A. John, “Tissue regeneration and repair of goat segmental femur defect with bioactive triphasic ceramic-coated hydroxyapatite scaffold,” Journal of Biomedical Materials Research - Part A, vol. 91, no. 3, pp. 855–865, 2009.

[44] P. Niemeyer, T. S. Schönberger, J. Hahn et al., “Xenogenic transplantation of human mesenchymal stem cells in a critical...
size defect of the sheep tibia for bone regeneration,” Tissue Engineering - Part A, vol. 16, no. 1, pp. 33–43, 2010.

[45] L. Cai, Q. Wang, C. Gu et al., “Vascular and micro-environmental influences on MSC-coral hydroxyapatite construct-based bone tissue engineering,” Biomaterials, vol. 32, no. 33, pp. 8497–8505, 2011.

[46] M. Manassero, V. Viateau, M. Deschepper et al., “Bone regeneration in sheep using acropora coral, a natural resorbable scaffold, and autologous mesenchymal stem cells,” Tissue Engineering - Part A, vol. 19, no. 13-14, pp. 1554–1563, 2013.

[47] A. Berner, J. C. Reichert, M. A. Woodruff et al., “Autologous vs. allogenic mesenchymal progenitor cells for the reconstruction of critical-sized segmental tibial bone defects in aged sheep,” Acta Biomaterialia, vol. 9, no. 8, pp. 7874–7884, 2013.

[48] H. Fan, X. Zeng, X. Wang, R. Zhu, and G. Pei, “Efficacy of prevascularization for segmental bone defect repair using β-tricalcium phosphate scaffold in rhesus monkey,” Biomaterials, vol. 35, no. 26, pp. 7407–7415, 2014.

[49] D. Yoon, B. J. Kang, Y. Kim et al., “Effect of serum-derived albumin scaffold and canine adipose tissue-derived mesenchymal stem cells on osteogenesis in canine segmental bone defect model,” Journal of Veterinary Science, vol. 16, no. 4, pp. 397–404, 2015.

[50] A. Berner, J. Henkel, M. A. Woodruff et al., “Delayed minimally invasive injection of allogenic bone marrow stromal cell sheet regenerates large bone defects in an ovine preclinical animal model,” Stem Cells Translational Medicine, vol. 4, no. 5, pp. 503–512, 2015.

[51] T. Masaoka, T. Yoshii, M. Yuasa et al., “Bone defect regeneration by a combination of a β-tricalcium phosphate scaffold and bone marrow stromal cells in a non-human primate model,” Open Biomedical Engineering Journal, vol. 10, pp. 2–11, 2016.

[52] J. O. Smith, E. R. Tayton, F. Khan et al., “Large animal in vivo evaluation of a binary blend polymer scaffold for skeletal tissue-engineering strategies; translational issues,” Journal of Tissue Engineering and Regenerative Medicine, vol. 11, no. 4, pp. 1065–1076, 2017.

[53] C. A. Kirker-Head, T. N. Gerhart, S. H. Schelling, G. E. Hennig, E. Wang, and M. E. Holtrop, “Long-term healing of bone using recombinant human bone morphogenetic protein 2,” Clinical Orthopaedics and Related Research, no. 318, pp. 222–230, 1995.

[54] M. F. Sciadini, J. M. Dawson, K. D. Johnson, and M. F. Sciadini, “Evaluation of bovine-derived bone protein with a natural coral carrier as a bone-grafts substitute in a canine segmental defect model,” Journal of Orthopaedic Research, vol. 15, no. 6, pp. 844–857, 1997.

[55] T. J. Gao, T. S. Lindholm, B. Kommonen et al., “The use of a coral composite implant containing bone morphogenetic protein to repair a segmental tibial defect in sheep,” International Orthopaedics, vol. 21, no. 3, pp. 194–200, 1997.

[56] T. Tuuminen, T. Jämsä, J. Tuukkanen et al., “Native bovine bone morphogenetic protein improves the potential of biocoral to healsegmental canine ulnar defects,” International Orthopaedics, vol. 24, no. 5, pp. 289–294, 2000.

[57] Y. Hu, C. Zhang, S. Zhang, Z. Xiao, and J. Xu, “Development of a porous polylactic acid/hydroxyapatite/collagen scaffold as a BMP delivery system and its use in healing canine segmental bone defect,” Journal of Biomedical Materials Research, vol. 67, no. 2, pp. 591–598, 2003.

[58] S. D. Cook, S. L. Salkeld, and L. P. Patron, “Bone defect healing with an osteogenic protein-1 device combined with carboxymethylcellulose,” Journal of Biomedical Materials Research Part B: Applied Biomaterials, vol. 75, no. 1, pp. 137–145, 2005.

[59] O. Maissen, C. Eckhardt, S. Gogolewski et al., “Mechanical and radiological assessment of the influence of rHGF/β-3 on bone regeneration in a segmental defect in the ovine tibia: Pilot study,” Journal of Orthopaedic Research, vol. 24, no. 8, pp. 1670–1678, 2006.

[60] A. Cipitria, W. Wagermaier, P. Zaslansky et al., “BMP delivery complements the guiding effect of scaffold architecture without altering bone microstructure in critical-sized long bone defects: a multiscale analysis,” Acta Biomaterialia, vol. 23, pp. 282–294, 2015.

[61] H. Petite, V. Viateau, W. Bensaid et al., “Tissue-engineered bone regeneration,” Nature Biotechnology, vol. 18, no. 9, pp. 959–963, 2000.

[62] F. C. den Boer, B. W. Wippermann, T. J. Blockhuis, P. Patka, F. C. Bakker, and H. J. T. M. Haarman, “Healing of segmental bone defects with granular porous hydroxyapatite augmented with recombinant human osteogenic protein-1 or autologous bone marrow,” Journal of Orthopaedic Research, vol. 21, no. 3, pp. 521–528, 2003.

[63] G. Filardo, E. Kon, A. Tampieri et al., “New bio-ceramization processes applied to vegetable hierarchical structures for bone regeneration: an experimental model in sheep,” Tissue Engineering - Part A, vol. 20, no. 3-4, pp. 763–773, 2014.

[64] A. Berner, J. Henkel, M. A. Woodruff et al., “Scaffold-cell bone engineering in a validated preclinical animal model: Precursors vs differentiated cell source,” Journal of Tissue Engineering and Regenerative Medicine, 2015.

[65] M. B. Nair, H. K. Varma, K. V. Menon, S. J. Shenoy, and A. John, “Reconstruction of goat femur segmental defects using triphasic ceramic-coated hydroxyapatite in combination with autologous cells and platelet-rich plasma,” Acta Biomaterialia, vol. 5, no. 5, pp. 1742–1755, 2009.

[66] L. Zhu, D. Chuanchang, L. Wei, C. Yilin, and D. Jiasheng, “Enhanced healing of goat femur-defect using BMP7 gene-modified BMSCs and load-bearing tissue-engineered bone,” Journal of Orthopaedic Research, vol. 28, no. 3, pp. 412–418, 2010.

[67] J. C. Reichert, A. Cipitria, D. R. Epapi et al., “A tissue engineering solution for segmental defect regeneration in load-bearing long bones,” Science Translational Medicine, vol. 4, no. 141, article 141ra93, 2012.

[68] D.-Q. Li, M. Li, P.-L. Liu, Y.-K. Zhang, J.-X. Lu, and J.-M. Li, “Improved repair of bone defects with prevascularized tissue-engineered bones constructed in a perfusion bioreactor,” Orthopedics, vol. 37, no. 10, pp. 685–690, 2014.

[69] A. Ronca, V. Guarino, M. G. Raucci et al., “Large defect-tailored composite scaffolds for in vivo bone regeneration,” Journal of Biomaterials Applications, vol. 29, no. 5, pp. 715–727, 2014.

[70] K.-D. Werber, R. B. Brauer, W. Weiß, and K. Becker, “Osseous integration of bovine hydroxyapatite ceramic in metaphyseal bone defects of the distal radius,” Journal of Hand Surgery, vol. 25, no. 5, pp. 833–841, 2000.

[71] R. Quarto, M. Mastroiacomo, R. Cancetta et al., “Repair of large bone defects with the use of autologous bone marrow stromal cells,” New England Journal of Medicine, vol. 344, no. 5, pp. 385–386, 2001.
[72] E. Arai, H. Nakashima, S. Tsukushi et al., “Regenerating the fibula with beta-tricalcium phosphate minimizes morbidity after fibula resection,” Clinical Orthopaedics and Related Research, no. 431, pp. 233–237, 2005.

[73] M. Marcacci, E. Kon, V. Moukhachev et al., “Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study,” Tissue Engineering, vol. 13, no. 5, pp. 947–955, 2007.

[74] J. Stanovici, L. R. Le Nail, M. A. Brennan et al., “Bone regeneration strategies with bone marrow stromal cells in orthopaedic surgery,” Current Research in Translational Medicine, vol. 64, no. 2, pp. 83–90, 2016.

[75] L. Watson, S. J. Elliman, and C. M. Coleman, “From isolation to implantation: a concise review of mesenchymal stem cell therapy in bone fracture repair,” Stem Cell Research & Therapy, vol. 5, no. 2, article 51, 2014.

[76] G. M. Calori, E. Mazza, M. Colombo, and C. Ripamonti, “The use of bone-graft substitutes in large bone defects: any specific needs?” Injury, vol. 42, supplement 2, pp. S56–S63, 2011.

[77] I. Dumić-Cule, M. Pecina, M. Jelic et al., “Biological aspects of segmental bone defects management,” International Orthopaedics, vol. 39, no. 5, pp. 1005–1011, 2015.

[78] W. F. Enneking and D. A. Campanacci, “Retrieved human allografts: a clinicopathological study,” The Journal of Bone and Joint Surgery-American Volume, vol. 83-A, no. 7, pp. 971–986, 2001.

[79] H. T. Aro and A. J. Aho, “Clinical use of bone allografts,” Annals of Medicine, vol. 25, no. 4, pp. 403–412, 1993.

[80] B. H. Berrey Jr., C. F. Lord, M. C. Gehhardt, and H. J. Mankin, “Fractures of allografts. frequency, treatment, and end-results,” The Journal of Bone & Joint Surgery-American Volume, vol. 72, no. 6, pp. 825–833, 1990.

[81] M. Bohner, “Physical and chemical aspects of calcium phosphates used in spinal surgery,” European Spine Journal, vol. 10, 2, pp. S114–S121, 2001.

[82] G. S. Krishnakumar, A. Roffi, D. Reale, E. Kon, and G. Filardo, “Clinical application of bone morphogenetic proteins for bone healing: a systematic review,” International Orthopaedics, vol. 41, no. 6, pp. 1073–1083, 2017.

[83] E. Kon, G. Filardo, A. Roffi et al., ”Bone regeneration with mesenchymal stem cells,” Clinical Cases in Mineral and Bone Metabolism, vol. 9, no. 1, pp. 24–27, 2012.

[84] A. Rey-Rico and M. Cucchiarini, “Recent tissue engineering-based advances for effective rAAV-mediated gene transfer in the musculoskeletal system,” Bioengineered, vol. 7, no. 3, pp. 175–188, 2016.