Review Article

Calcium Phosphate Scaffolds Combined with Bone Morphogenetic Proteins or Mesenchymal Stem Cells in Bone Tissue Engineering

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Abstract

Objective: The purpose of this study was to review the current status of calcium phosphate (CaP) scaffolds combined with bone morphogenetic proteins (BMPs) or mesenchymal stem cells (MSCs) in the field of bone tissue engineering (BTE).

Date Sources: Data cited in this review were obtained primarily from PubMed and Medline in publications from 1979 to 2014, with highly regarded older publications also included. The terms BTE, CaP, BMPs, and MSC were used for the literature search.

Study Selection: Reviews focused on relevant aspects and original articles reporting *in vitro* and/or *in vivo* results concerning the efficiency of CaP/BMPs or CaP/MSCs composites were retrieved, reviewed, analyzed, and summarized.

Results: An ideal BTE product contains three elements: Scaffold, growth factors, and stem cells. CaP-based scaffolds are popular because of their outstanding biocompatibility, bioactivity, and osteoconductivity. However, they lack stiffness and osteoinductivity. To solve this problem, composite scaffolds of CaP with BMPs have been developed. New bone formation by CaP/BMP composites can reach levels similar to those of autografts. CaP scaffolds are compatible with MSCs and CaP/MSC composites exhibit excellent osteogenesis and stiffness. In addition, a CaP/MSC/BMP scaffold can repair bone defects more effectively than an autograft.

Conclusions: Novel BTE products possess remarkable osteoconduction and osteoinduction capacities, and exhibit balanced degradation with osteogenesis. Further work should yield safe, viable, and efficient materials for the repair of bone lesions.

Key words: Bone Morphogenetic Proteins; Calcium Phosphate; Mesenchymal Stem Cells; Tissue Engineering

Introduction

Bone tissue is capable of complete regeneration without scarring. This property has enabled the development of bone tissue engineering (BTE), which has been widely explored since its inception by Langer and Vacanti.[1,2]

There are three primary elements required for BTE: A scaffold, growth factors, and stem cells.[3] Each plays an important role in the utility of the final composite, and current efforts are focused on developing combinations of these elements that provide optimum performance.

This review provides an overview of the current state of BTE with a focus on bone morphogenetic proteins (BMPs), calcium phosphate (CaP) scaffolds, and mesenchymal stem cells (MSCs).

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Present Situation of Bone Grafts and Bone Tissue Engineering

One potential application of BTE is the production of grafts to heal bone defects in nonunion fractures, for which ordinary open reduction and internal fixation is inadequate or inappropriate.[4] In such cases, autogenous or allogenous bone grafts are widely used. Bone grafting is commonly performed, with autografts currently considered the gold standard for many procedures–including spinal fusion–because of its osteoinductive and osteoconductive characteristics.[5,6] However, allografts and synthetic grafts are also commonly used. Each of these approaches to graft production has benefits and drawbacks. Autografts are associated with the deficiency of limited resource, deep infection, chronic pain, and donor site morbidity.[6,7] Allografts have lower osteogenic capacity and carry risks of pathogen transmission and immunological rejection,[8-10]

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yet synthetic grafts are incapable of being remodeled.[4] These limitations indicate a clear need for novel strategies for graft production.

Scaffolds for Bone Tissue Engineering

The acrylic material polymethylmethacrylate (PMMA) is currently one of the most widely used scaffold materials in BTE. PMMA cement is frequently used in orthopedic procedures, including percutaneous vertebroplasty and percutaneous kyphoplasty, in many joint replacements, and for arthroplasty.[11-17] PMMA is also a good carrier vehicle for antibiotics and can facilitate sustained release at the site of infection.[16,18,19] However, PMMA is nonosteoinductive and nonosteoconductive, and the monomer is toxic and may initiate allergic reactions.[20] Additionally, the exothermic nature of the material may injure surrounding tissues and vessels during inappropriate application.

To overcome the limitations of traditional scaffolds, the ideal novel scaffold should be biocompatible, robust, osteoinductive, and osteoconductive, and should support cell attachment, proliferation, and differentiation.[21] Additionally, the scaffold should be biodegradable and be resorbed at a rate comparable with that of tissue regeneration to avoid a second surgery to remove the implant.[22]

Advantages of calcium phosphate scaffolds

Calcium phosphate-based scaffolds are typically constructed using either hydroxyapatite (HA) or biphasic CaP (BCP; a composite of HA and β-tricalcium phosphate). Synthetic polymers such as polylactic acid and polyglycolic acid, and natural polymers such as collagen, glycosaminoglycan, and fibrin are also widely used.[3]

Calcium phosphate is regarded as an excellent candidate for novel scaffold material because of its outstanding biocompatibility, bioactivity, and osteoconductivity.[23] Additionally, its degradation products can participate in biomineralization by redepositing on carbonate HA (CHA).[24] The biocompatibility of CaP is attributed to its structural similarity to bone CHA.[21,25] Hirasawa *et al*. applied CaP cement (CPC) to lumbar interbody fusions to achieve a fusion rate of 94%, which was similar to that for the use of iliac crest bone grafts (93%) or local bone grafts (95%).[26] This result indirectly demonstrates the significant potential for CaP in BTE applications.

Another beneficial feature of CaP is its injectability, which is similar to that of PMMA. CPC injectability can be further enhanced by increasing the liquid-to-powder ratio, rapid injection after mixing and using a paste made of round particles.[27,28] Zhang *et al*. determined that the best way to improve injectability of CaP is to use a viscous solution in the liquid phase.[29]

Disadvantages of calcium phosphate scaffolds

The most serious shortcoming of CaP is its porosity, which renders it brittle, weak and suitable for use only in nonload-bearing bone repairs.[30,31] One study reported that the compressive strength of a HA scaffold was 30.2 ± 6.0 MPa,[32] which is higher than that of cancellous bone (4–12 MPa) but far less than that of cortical bone (130–180 MPa).[33] Other parameters such as crystallinity and grain size can also influence the flexural and tensile strength of CaP scaffolds.[25] In order to maintain the hardness as well as relatively high porosity and large pore size, which contribute to bioactivity and osteoconductivity, several new hybrid CaP-polymer composites have been developed, including poly (lactic-co-glycolic acid) (PLGA)/CPC,[34] CPC-fibrin glue,[35] and CPC-chitosan[36] and so on.

Calcium phosphate scaffolds also lack osteoinductive activity.[37] However, BMPs have significant osteogenic properties,[38] and their combination with CaP can result in a scaffold with greater osteoinductive capacity.

Porosity

Scaffold porosity is crucial for appropriate wound repair, as cells and growth factors must have access to much of the scaffold surface area. Interconnected pores are essential to facilitate the invasion, growth, and nutrition of cells,[3] and the diffusion of waste from the inner core.[39] However, larger pore size and porosity compromise compressive strength and hardness.[40]

Small pore size and lower porosity facilitate osteoblast cell proliferation and osteogenic differentiation *in vitro*, while higher porosity enhances cell recruitment and vascularization *in vivo*.[41,42] Therefore, an appropriate pore size and porosity must be reached for specific situations. Karageorgiou and Kaplan reported that the minimum pore size to enable cell migration and transport is 100 μm.[41]

Combination Scaffolds of Bone Morphogenetic Proteins and Calcium Phosphate

Bone morphogenetic proteins

Bone morphogenetic proteins are expressed in the epithelium of the limb bud where they play crucial roles in the proliferation and differentiation of underlying mesodermal progenitor cells.[43] BMPs can upregulate growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor, and insulin-like growth factor-1 (IGF-1), and BMP-2 and -7 have been used in clinical applications.[44-46] BMP-2 expression is induced during early MSC recruitment and is sustained throughout chondrogenic and osteogenic differentiation to the stage of woven bone formation.[47] Meanwhile, BMP-7 is similarly upregulated during the early stages of intramembranous and endochondral ossification.[43]

Basic studies of calcium phosphate scaffolds combined with bone morphogenetic proteins

Bone morphogenetic proteins can be combined with injectable CPCs (ICPCs), and such osteoinductive composites are expected to be widely used in minimally invasive surgery in the future.

The release kinetics of BMP-2 loaded onto CPC/PLGA, incorporated into the liquid phase of CPC (CPC/liquid), and BMP-2 adsorbed to the surface of CPC (CPC/surface) have been evaluated.[48] CPC/PLGA and CPC/liquid shared a similar release profile, which was feasible for ICPC and bone regeneration at orthotopic locations, whereas CPC/surface had a significant burst release of BMP-2 that facilitated osteoinduction and extopic bone formation.

Following scaffold material injection, microporosity and BMP-2 positively influence bone regeneration in different but complementary ways. Polak *et al*.[49] found that microporosity increased the bone volume fraction and facilitated a near-perfect uniform distribution of bone within the scaffold. BMP-2 enhanced surface area rather than the bone volume fraction. This study showed that a BMP-microporous-BCP scaffold facilitated a healing speed four times faster than a BMP-nonmicroporous-BCP scaffold and five times faster than the no-BMP scaffold.

The osteogenic capacity of the composite can be further enhanced by addition of further elements. Zhang *et al*. found that silicon ions could stimulate the synergistic action of rhBMP-2 and calcium silicate (CaS) to facilitate osteogenic differentiation and osteoinductivity.[50] They manufactured an rhBMP-2-loaded CaS/CPC scaffold that promoted greater osteogenic efficacy *in vivo* compared with CPC/rhBMP-2.

Different uses of calcium phosphate cement/bone morphogenetic protein composites

Qian *et al*. used an ICPC and fibrin sealant (FS) rhBMP-2 composite for vertebroplasty in New Zealand rabbits.[51] The ICPC/FS/rhBMP-2 scaffold possessed an increased osteogenic capacity and a faster FS absorption rate than the ICPC/FS group. ICPC/FS/rhBMP-2 scaffold degradation synchronized with the new bone formation, and the scaffold material integrated closely with adjacent bones. Both the anti-compression and anti-torsion ability of bone repaired with ICPC/FS/rhBMP-2 scaffold increased with time. This study also suggested that no bone grew into the material gap, and no bone replacement occurred with the use of PMMA scaffolds.

Similarly, Gu *et al*. evaluated an injectable silk fibroin (SF)-enhanced CPC loaded with rhBMP-2 in ovine lumbar interbody fusion and found that both the amount of new bone formation and the stiffness of fusions in the CPC/SF/rhBMP-2 group were higher than those of the CPC/SF group, which was similar to autografts at 12 months.[5] However, compared with the CPC/SF group, the ceramic residue volume in the CPC/SF/rhBMP-2 group was lower. The fusion rate of the CPC/SF/rhBMP-2 group (56% at 6 months and 78% at 12 months) was markedly higher than that of the CPC/SF group (0% at 6 months and 11% at 12 months), and reached the same level as autografts (78%) at 12 months.

Li *et al*. used a rhBMP-2/CPC composite to treat osteoporosis *in vitro* and showed that the push-out test value of the rhBMP-2/CPC group was 5.9 ± 1.3 MPa at 140 days while that of the untreated group was 3.1 ± 0.9 MPa.[52] In addition, the mineralization rate of new bone was 3.99 ± 0.62 μm/day versus 1.95 ± 0.16 μm/day at 45 days. These results indicate that the composite could accelerate bone healing in osteoporosis. They also found that the addition of gelatin microspheres could further facilitate the release of rhBMP-2.

These studies demonstrate that CPC/BMPs composites possess significant potential for widespread clinical application in the future.

The Combination of Mesenchymal Stem Cells and Calcium Phosphate Scaffolds

Mesenchymal stem cells

Properties of mesenchymal stem cells

Sufficient numbers of MSCs for grafting can be readily harvested from patients, and their application does not induce immune-mediated rejection. Additionally, MSCs have a high proliferative capacity, and their osteogenic potency is greater than that of total bone marrow (TBM).[53-55] In addition, MSCs regulate bone remodeling by balancing the osteoblast-osteoclast ratio.

Bone marrow MSCs (bMSCs) can be identified by: Their ability to adhere to plastic during culture; their expression of the surface antigens CD29, CD73, CD90, and CD105, with the concurrent absence of CD19, CD34, CD45, CD79a, and HLA-II; and their ability to differentiate into osteogenic, adipogenic, and chondrogenic lineages under appropriate conditions.[56]

Secretion of signaling molecules by mesenchymal stem cells

During fracture healing, the matrix surrounding the defect site can secrete multiple signaling molecules, including transforming growth factor-β (TGF-β), IGF-1, PDGF, interleukin-1 (IL-1), and IL-6.[57-60] These molecules can recruit MSCs and their progeny, and further stimulate their proliferation, differentiation, and maturation. Interestingly, some of these molecules–including BMPs–can also be secreted by MSCs themselves in addition to their release by the matrix.[60]

Inflammation is a very important stage in the healing process. However, the mechanisms by which inflammation influences MSCs are poorly understood. Sundelacruz and Kaplan reported that the release of tumor necrosis factor-α (TNF-α), PDGF, IL-1, and IL-6 from inflammatory cells can affect MSC migration and proliferation.[21] In contrast, Forostyak *et al*. found that an anti-inflammatory effect is beneficial for MSC function, with anti-inflammatory TGF-β1 present at a higher level than other anti-inflammatory chemokines/cytokines such as TNF-α, IL-1 β, and IL-6.[61]

Varieties of mesenchymal stem cell source for bone tissue engineering application

Mesenchymal stem cells can be isolated from organs and tissues including adult bone marrow,[62] fetal bone marrow,[63] the umbilical cord,[64] umbilical cord blood,[65] periosteum,[66] and adipose.[67] However, there is no consensus as to which

source is optimal. Forostyak *et al*. found adipose-derived MSCs to be the most promising,[61] while Zhang *et al*. concluded that human fetal MSCs were the best source.[53]

Calcium phosphate scaffolds combined with mesenchymal stem cells

Influence in cells and scaffolds

The primary problem concerning the combination of MSCs with CaP scaffolds is the viability of cells within the scaffold. To enhance the survival of MSCs, cells are often encapsulated within alginate microbeads.[68-71] These microbeads can protect cells during transplantation, and rapidly degrade to release cells after grafting.[72]

Tang *et al*. reported that the percentage of live stem cells encapsulated within microbeads in CPC between days 1 and 21 was 85% and 95%, suggesting that encapsulated stem cells were viable within CPC.[73] Weir and Xu enhanced the CPC physical properties by adding chitosan lactate and reinforcing fibers and found that the live-cell density of MSCs in CPC and CPC-chitosan-fiber scaffolds was similar to that of microbeads alone.[74] However, the density of cells in all three groups at day 7 was significantly lower than that at day 1, with little difference between groups at days 14 and 21. This decrease in density was attributed to the continued swelling of the alginate beads during cell culture. In contrast, a similar study from Chen *et al*. reported that the live-cell density of all groups exhibited an upward trend with time.[75] In their study, fibronectin and arginine-glycine-aspartic acid (RGD) were combined with CPC. These two agents are known to biofunctionalized scaffolds and promote cell adhesion. The live-cell density on scaffolds made from CPC + 0.1% RGD was approximately four times that of the CPC control.

The addition of stem cells does not appear to compromise the physical characteristics of the CaP scaffold, either. Zhao *et al*. found that even though addition of microbeads slightly increased the injection force required compared with scaffolds of CPC alone, this was still a relatively low force level.[70] In addition, when the cement was further combined with chitosan, the injection force could actually be lower than that for CPC alone. This study also reported that the paste mixing and injection processes did not harm the encapsulated MSCs.

Attachment of cells

Cells can efficiently attach to CaP scaffolds within a satisfactory period. Zhao *et al*. reported that while cell attachment was below 300 cells/mm^2 at day 1, nearly 700 cells/mm^2 were attached by day 4.[76] This lower level of attachment at day 1 was attributed primarily to MSC proliferation. Additionally, there was no significant difference between the attachment of MSCs to CPC or polymer scaffolds.

Osteogenic differentiation of cells

During osteogenesis, MSCs express alkaline phosphatase (ALP) and osteocalcin (OC), which are well-defined markers of osteogenic differentiation.[77-80] Tang *et al*. reported that ALP and OC gene expression were increased 10–100-fold and ALP activity was increased 5-fold by day 21 compared with day 1.[73] These data demonstrated that MSCs encapsulated within CPC scaffolds differentiated down the osteogenic lineage and synthesized bone minerals.

Bao *et al*. enhanced CPC with electrospun submicron fibers and detected elevated ALP and OC expression in MSCs on CPC with fibers.[81] Schumacher *et al*. used a novel strontium (II)-modified CPC and also detected elevated ALP expression in MSCs.[77] Zhao *et al*. found that the percentage of mineral area synthesized by encapsulated MSCs increased from 3% at day 7–12% at day 21, demonstrating that MSCs in CPC-chitosan-fiber scaffold can efficiently undergo osteogenic differentiation and synthesize bone minerals.[82] These findings indicate that appropriate materials combined with CaP can facilitate osteogenesis by MSCs.

Animal model experiments

Wang *et al*. studied a bMSC-CPC composite in lumbar fusion in rhesus monkeys. Fusions in the bMSC-CPC group were significantly stiffer than those in the cell-free ceramic group with regard to bending and torsion, but weaker than the autograft group.[83] Conversely, bMSC-CPC fusions developed an osseous union while cell-free ceramic fusions only developed a fibrous union. However, the graft site may experience an inflammatory reaction resulting from cell damage around the implanted biphasic bioceramic following the release of microparticles.[84]

When comparing bone regeneration in the dog mandible directed by BCP and natural bovine bone mineral loaded MSCs, Jafarian *et al*. found that the osteogenic capacity of BCP scaffolds was notably higher than that of natural bone mineral group, which indicated that BCP loaded with MSCs provided better conditions for bone regeneration.[85]

Chen *et al*. found that human umbilical cord MSCs (hUCMSCs) and human bone marrow MSCs (hBMSCs) seeded on CaP for bone regeneration in rat cranial defects induced a similar bone mineral density, new bone amount, and vessel density in regenerated bone tissue.[86] Given that hBMSCs require an invasive procedure to harvest and will lose their potency with diseases, while hUCMSCs can be harvested for a low cost, are effectively inexhaustible, and have a high plasticity and developmental capability, hUCMSCs are considered more suitable for osteogenic applications.

When comparing bone regeneration by MSCs with that by TBM in association with a CaP scaffold in irradiated hind limbs of rats, Espitalier *et al*. found that the TBM group possessed higher bone ingrowth.[87] This suggested that the BCP-TBM composite induced increased vascularization of the irradiated bone.

Cooperation of bone morphogenetic proteins and mesenchymal stem cells on calcium phosphate scaffolds

Overman *et al*. incubated stem cells with BMP-2 prior to loading them on CaP scaffolds and found that cell attachment

was unaffected, while gene expression of collagen-1, and the osteogenic markers core binding factor alpha 1, osteonectin, and OC was stimulated.[88] Their subsequent study identified increased expression of many bone formation-associated factors including IL-2, BMP-7, IGF-1 in the BMP-2-treatment group.[89] These findings indicate that the composite possessed a long-lasting modulating effect on bone formation.

Zhao *et al*. directly loaded rhBMP-2 and hUCMSCs on an injectable CaP-chitosan fibrous scaffold.[90] The stem cells encapsulated within the cement maintained their viability, while the release of rhBMP-2 was also satisfactory, and lead to successful osteogenic differentiation. The composite cement demonstrated excellent mineralization, ALP activity, and gene expression (OC, ALP, osterix, collagen and ALP protein synthesis) compared with the control group, indicating that BMPs and MSCs could cooperate to promote efficient osteogenic differentiation.

Kai *et al*. used a lumbar fusion model in rabbits to assess the impact of BMP-2 on a bMSC/CPC scaffold.[91] All individuals treated with bMSCs/CPC or bMSCs/CPC/BMP-2 underwent fusion while fusion occurred in only 50% and 67% of individuals treated with CPC alone or autograft, respectively. Importantly, disc height losses in the bMSCs/CPC and bMSCs/CPC/BMP-2 groups were also minimal. Flexion, extension, bending, torsion, and bone formation were similar for bMSC/CPC with and without BMP-2. However, fusion size and stiffness was significantly enhanced when using bMSCs/CPC/BMP-2. This is likely because BMP-2 can induce MSCs to differentiate, integrate with bone cells, and enter a resting stage in which a complete ossicle with a cortex of lamellar bone and marrow cavity develops.[92]

Conclusions

Bone tissue engineering holds great potential for the repair of bone defects. However, this technology remains immature and composite scaffolds have not been widely used in clinical applications. Autografts remain the current gold standard treatment, and PMMA is still one of the mostly widely used cement. There are several crucial issues to address before clinical use of BTE products, including the ethical concerns surrounding MSC use,[53] the optimal size and density of pores and the most ideal way to enhance CaP scaffolding, and the limitations of manufacturing technology in fabricating ideal biocompatible materials with cell-permissive internal architectures.[22] Additionally, the current literature reports varied concentrations of BMP-2 were required for adequate bone formation depending on the model used.[92,93] Therefore, the optimal dose and mode of delivery for BMP-2 in humans remains uncertain. Additionally, the current understanding of the mechanisms and methods whereby MSCs can be differentiated towards mature cells is rudimentary.[94,95] The behavior of MSCs within the pathological environment following transplantation also requires further study, as does the optimal time frame and the most efficient route for delivery. An initial release of angiogenic growth factor can induce new blood vessels at an early stage of bone healing while later stage release of BMP-2 and IGF-1 can induce the osteogenic properties.[96] Thus, the sequential delivery of biomolecules may also play an important role in regulating the natural bone remodeling process. Finally, ideal bone scaffolds will be stiff enough and will enable commitment to the desired mature cell-type *in vitro*, with complete and rapid integration *in vivo*.[97]

Nevertheless, many studies have reported that composite CaP scaffold with BMPs or MSCs can develop cortical bone and produce results superior to those of autografts. Therefore, autografts should be replaced with off-the-shelf products in the foreseeable future.

References

1. Kneser U, Schaefer DJ, Polykandriotis E, Horch RE. Tissue engineering of bone: The reconstructive surgeon's point of view. J Cell Mol Med 2006;10:7-19.
2. Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920-6.
3. Murphy CM, O'Brien FJ, Little DG, Schindeler A. Cell-scaffold interactions in the bone tissue engineering triad. Eur Cell Mater 2013;26:120-32.
4. Salgado AJ, Coutinho OP, Reis RL. Bone tissue engineering: State of the art and future trends. Macromol Biosci 2004;4:743-65.
5. Gu Y, Chen L, Yang HL, Luo ZP, Tang TS. Evaluation of an injectable silk fibroin enhanced calcium phosphate cement loaded with human recombinant bone morphogenetic protein-2 in ovine lumbar interbody fusion. J Biomed Mater Res A 2011;97:177-85.
6. Banwart JC, Asher MA, Hassanein RS. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine (Phila Pa 1976). 1995;20:1055-60.
7. Goulet JA, Senunas LE, DeSilva GL, Greenfield ML. Autogenous iliac crest bone graft. Complications and functional assessment. Clin Orthop Relat Res 1997;339:76-81.
8. Parikh SN. Bone graft substitutes: Past, present, future. J Postgrad Med 2002;48:142-8.
9. Grauer JN, Beiner JM, Kwon B, Vaccaro AR. The evolution of allograft bone for spinal applications. Orthopedics 2005;28:573-7.
10. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. J Am Acad Orthop Surg 2005;13:77-86.
11. Ryu KS, Park CK. The prognostic factors influencing on the therapeutic effect of percutaneous vertebroplasty in treating osteoporotic vertebral compression fractures. J Korean Neurosurg Soc 2009;45:16-23.
12. Yan D, Duan L, Li J, Soo C, Zhu H, Zhang Z. Comparative study of percutaneous vertebroplasty and kyphoplasty in the treatment of osteoporotic vertebral compression fractures. Arch Orthop Trauma Surg 2011;131:645-50.
13. Lieberman IH, Togawa D, Kayanja MM. Vertebroplasty and kyphoplasty: Filler materials. Spine J 2005;5 6 Suppl: 305S-16.
14. Griza S, Ueki MM, Souza DH, Cervieri A, Strohaecker TR. Thermally induced strains and total shrinkage of the polymethyl-methacrylate cement in simplified models of total hip arthroplasty. J Mech Behav Biomed Mater 2013;18:29-36.
15. Griffin JW, Guillot SJ, Redick JA, Browne JA. Removed antibiotic-impregnated cement spacers in two-stage revision joint arthroplasty do not show biofilm formation *in vivo*. J Arthroplasty 2012;27:1796-9.
16. Ueng SW, Hsieh PH, Shih HN, Chan YS, Lee MS, Chang Y. Antibacterial activity of joint fluid in cemented total-knee arthroplasty: An *in vivo* comparative study of polymethylmethacrylate with and without antibiotic loading. Antimicrob Agents Chemother 2012;56:5541-6.
17. Ni GX, Lu WW, Chiu PK, Wang Y, Li ZY, Zhang YG, *et al.*

Mechanical properties of femoral cortical bone following cemented hip replacement. J Orthop Res 2007;25:1408-14.
18. Fontanesi G, Giancecchi F, Ruini D, Rotini R. Use of acrylic cement with an antibiotic in prosthetic surgery of the hip. Chir Organi Mov 1982;68:287-95.
19. Magnan B, Bondi M, Maluta T, Samaila E, Schirru L, Dall'Oca C. Acrylic bone cement: Current concept review. Musculoskelet Surg 2013;97:93-100.
20. Uzun IH, Tatar A, Hacimuftuoglu A, Saruhan F, Bayindir F. *In vitro* evaluation of long-term cytotoxic response of injection-molded polyamide and polymethyle metacrylate denture base materials on primary fibroblast cell culture. Acta Odontol Scand 2013;71:1267-72.
21. Sundelacruz S, Kaplan DL. Stem cell-and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. Semin Cell Dev Biol 2009;20:646-55.
22. Haugh MG, Murphy CM, McKiernan RC, Altenbuchner C, O'Brien FJ. Crosslinking and mechanical properties significantly influence cell attachment, proliferation, and migration within collagen glycosaminoglycan scaffolds. Tissue Eng Part A 2011;17:1201-8.
23. Ambard AJ, Mueninghoff L. Calcium phosphate cement: Review of mechanical and biological properties. J Prosthodont 2006;15:321-8.
24. Wang L, Nancollas GH. Pathways to biomineralization and biodemineralization of calcium phosphates: The thermodynamic and kinetic controls. Dalton Trans 2009;15:2665-72.
25. Billström GH, Blom AW, Larsson S, Beswick AD. Application of scaffolds for bone regeneration strategies: Current trends and future directions. Injury 2013;44 Suppl 1:S28-33.
26. Hirasawa M, Mure H, Toi H, Nagahiro S. Surgical results of lumbar interbody fusion using calcium phosphate cement. Neurol Med Chir (Tokyo) 2014;54:722-6.
27. Khairoun I, Boltong MG, Driessens FC, Planell JA. Some factors controlling the injectability of calcium phosphate bone cements. J Mater Sci Mater Med 1998;9:425-8.
28. Ishikawa K, Matsuya S, Nakagawa M, Udoh K, Suzuki K. Basic properties of apatite cement containing spherical tetracalcium phosphate made with plasma melting method. J Mater Sci Mater Med 2004;15:13-7.
29. Zhang J, Liu W, Schnitzler V, Tancret F, Bouler JM. Calcium phosphate cements for bone substitution: Chemistry, handling and mechanical properties. Acta Biomater 2014;10:1035-49.
30. Friedman CD, Costantino PD, Takagi S, Chow LC. BoneSource hydroxyapatite cement: A novel biomaterial for craniofacial skeletal tissue engineering and reconstruction. J Biomed Mater Res 1998;43:428-32.
31. Bose S, Tarafder S. Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: A review. Acta Biomater 2012;8:1401-21.
32. Oliveira JM, Silva SS, Malafaya PB, Rodrigues MT, Kotobuki N, Hirose M, *et al.* Macroporous hydroxyapatite scaffolds for bone tissue engineering applications: Physicochemical characterization and assessment of rat bone marrow stromal cell viability. J Biomed Mater Res A 2009;91:175-86.
33. Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomater 2011;7:16-30.
34. He F, Li J, Ye J. Improvement of cell response of the poly (lactic-co-glycolic acid)/calcium phosphate cement composite scaffold with unidirectional pore structure by the surface immobilization of collagen via plasma treatment. Colloids Surf B Biointerfaces 2013;103:209-16.
35. Dong J, Cui G, Bi L, Li J, Lei W. The mechanical and biological studies of calcium phosphate cement-fibrin glue for bone reconstruction of rabbit femoral defects. Int J Nanomedicine 2013;8:1317-24.
36. Moreau JL, Xu HH. Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate-chitosan composite scaffold. Biomaterials 2009;30:2675-82.
37. Boden SD. The ABCs of BMPs. Orthop Nurs 2005;24:49-52.
38. Urist MR, Mikulski A, Lietze A. Solubilized and insolubilized bone morphogenetic protein. Proc Natl Acad Sci U S A 1979;76:1828-32.
39. Mandal BB, Kundu SC. Non-mulberry silk gland fibroin protein 3-D scaffold for enhanced differentiation of human mesenchymal stem cells into osteocytes. Acta Biomater 2009;5:2579-90.
40. Xu HH, Quinn JB. Calcium phosphate cement containing resorbable fibers for short-term reinforcement and macroporosity. Biomaterials 2002;23:193-202.
41. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26:5474-91.
42. Roy TD, Simon JL, Ricci JL, Rekow ED, Thompson VP, Parsons JR. Performance of degradable composite bone repair products made via three-dimensional fabrication techniques. J Biomed Mater Res A 2003;66:283-91.
43. Sakou T. Bone morphogenetic proteins: From basic studies to clinical approaches. Bone 1998;22:591-603.
44. Yeh LC, Lee JC. Osteogenic protein-1 increases gene expression of vascular endothelial growth factor in primary cultures of fetal rat calvaria cells. Mol Cell Endocrinol 1999;153:113-24.
45. Yeh LC, Unda R, Lee JC. Osteogenic protein-1 differentially regulates the mRNA expression of bone morphogenetic proteins and their receptors in primary cultures of osteoblasts. J Cell Physiol 2000;185:87-97.
46. Veeravagu A, Cole TS, Jiang B, Ratliff JK, Gidwani RA. The use of bone morphogenetic protein in thoracolumbar spine procedures: Analysis of the MarketScan longitudinal database. Spine J 2014;14:2929-37.
47. Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res 2002;17:513-20.
48. Van de Watering FC, Molkenboer-Kuenen JD, Boerman OC, van den Beucken JJ, Jansen JA. Differential loading methods for BMP-2 within injectable calcium phosphate cement. J Control Release 2012;164:283-90.
49. Polak SJ, Levengood SK, Wheeler MB, Maki AJ, Clark SG, Johnson AJ. Analysis of the roles of microporosity and BMP-2 on multiple measures of bone regeneration and healing in calcium phosphate scaffolds. Acta Biomater 2011;7:1760-71.
50. Zhang J, Zhou H, Yang K, Yuan Y, Liu C. RhBMP-2-loaded calcium silicate/calcium phosphate cement scaffold with hierarchically porous structure for enhanced bone tissue regeneration. Biomaterials 2013;34:9381-92.
51. Qian G, Dong Y, Yang W, Wang M. Injectable calcium phosphate cement and fibrin sealant recombined human bone morphogenetic protein-2 composite in vertebroplasty: An animal study. Bosn J Basic Med Sci 2012;12:231-5.
52. Li M, Liu X, Liu X, Ge B. Calcium phosphate cement with BMP-2-loaded gelatin microspheres enhances bone healing in osteoporosis: A pilot study. Clin Orthop Relat Res 2010;468:1978-85.
53. Zhang ZY, Teoh SH, Hui JH, Fisk NM, Choolani M, Chan JK. The potential of human fetal mesenchymal stem cells for off-the-shelf bone tissue engineering application. Biomaterials 2012;33:2656-72.
54. Abdallah BM, Kassem M. Human mesenchymal stem cells: From basic biology to clinical applications. Gene Ther 2008;15:109-16.
55. Pioletti DP, Montjovent MO, Zambelli PY, Applegate L. Bone tissue engineering using foetal cell therapy. Swiss Med Wkly 2006;136:557-60.
56. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
57. Gantenbein-Ritter B, Benneker LM, Alini M, Grad S. Differential response of human bone marrow stromal cells to either TGF-ß(1) or rhGDF-5. Eur Spine J 2011;20:962-71.
58. Fan VH, Tamama K, Au A, Littrell R, Richardson LB, Wright JW, *et al.* Tethered epidermal growth factor provides a survival advantage to mesenchymal stem cells. Stem Cells 2007;25:1241-51.
59. Reddi AH. Bone morphogenetic proteins: From basic science to clinical applications. J Bone Joint Surg Am 2001;83-A Suppl 1:S1-6.
60. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury 2005;36:1392-404.
61. Forostyak S, Jendelova P, Sykova E. The role of mesenchymal stromal cells in spinal cord injury, regenerative medicine and possible clinical applications. Biochimie 2013;95:2257-70.
62. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001;98:2396-402.
63. Zhang ZY, Teoh SH, Chong MS, Schantz JT, Fisk NM, Choolani MA, *et al.* Superior osteogenic capacity for bone tissue engineering of fetal

compared with perinatal and adult mesenchymal stem cells. Stem Cells 2009;27:126-37.
64. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: A source of mesenchymal progenitors. Stem Cells 2005;23:220-9.
65. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004;103:1669-75.
66. Nakahara H, Dennis JE, Bruder SP, Haynesworth SE, Lennon DP, Caplan AI. *In vitro* differentiation of bone and hypertrophic cartilage from periosteal-derived cells. Exp Cell Res 1991;195:492-503.
67. Nicpon J, Marycz K, Grzesiak J. Therapeutic effect of adipose-derived mesenchymal stem cell injection in horses suffering from bone spavin. Pol J Vet Sci 2013;16:753-4.
68. Trouche E, Girod Fullana S, Mias C, Ceccaldi C, Tortosa F, Seguelas MH, *et al.* Evaluation of alginate microspheres for mesenchymal stem cell engraftment on solid organ. Cell Transplant 2010;19:1623-33.
69. Gryshkov O, Pogozhykh D, Zernetsch H, Hofmann N, Mueller T, Glasmacher B. Process engineering of high voltage alginate encapsulation of mesenchymal stem cells. Mater Sci Eng C Mater Biol Appl 2014;36:77-83.
70. Zhao L, Weir MD, Xu HH. An injectable calcium phosphate-alginate hydrogel-umbilical cord mesenchymal stem cell paste for bone tissue engineering. Biomaterials 2010;31:6502-10.
71. Weir MD, Xu HH, Simon CG Jr. Strong calcium phosphate cement-chitosan-mesh construct containing cell-encapsulating hydrogel beads for bone tissue engineering. J Biomed Mater Res A 2006;77:487-96.
72. Liu J, Zhou H, Weir MD, Xu HH, Chen Q, Trotman CA. Fast-degradable microbeads encapsulating human umbilical cord stem cells in alginate for muscle tissue engineering. Tissue Eng Part A 2012;18:2303-14.
73. Tang M, Chen W, Weir MD, Thein-Han W, Xu HH. Human embryonic stem cell encapsulation in alginate microbeads in macroporous calcium phosphate cement for bone tissue engineering. Acta Biomater 2012;8:3436-45.
74. Weir MD, Xu HH. Human bone marrow stem cell-encapsulating calcium phosphate scaffolds for bone repair. Acta Biomater 2010;6:4118-26.
75. Chen W, Zhou H, Weir MD, Bao C, Xu HH. Umbilical cord stem cells released from alginate-fibrin microbeads inside macroporous and biofunctionalized calcium phosphate cement for bone regeneration. Acta Biomater 2012;8:2297-306.
76. Zhao L, Burguera EF, Xu HH, Amin N, Ryou H, Arola DD. Fatigue and human umbilical cord stem cell seeding characteristics of calcium phosphate-chitosan-biodegradable fiber scaffolds. Biomaterials 2010;31:840-7.
77. Schumacher M, Lode A, Helth A, Gelinsky M. A novel strontium (II)-modified calcium phosphate bone cement stimulates human-bone-marrow-derived mesenchymal stem cell proliferation and osteogenic differentiation *in vitro*. Acta Biomater 2013;9:9547-57.
78. Benoit DS, Nuttelman CR, Collins SD, Anseth KS. Synthesis and characterization of a fluvastatin-releasing hydrogel delivery system to modulate hMSC differentiation and function for bone regeneration. Biomaterials 2006;27:6102-10.
79. Reilly GC, Radin S, Chen AT, Ducheyne P. Differential alkaline phosphatase responses of rat and human bone marrow derived mesenchymal stem cells to 45S5 bioactive glass. Biomaterials 2007;28:4091-7.
80. Karp JM, Ferreira LS, Khademhosseini A, Kwon AH, Yeh J, Langer RS. Cultivation of human embryonic stem cells without the embryoid body step enhances osteogenesis *in vitro*. Stem Cells 2006;24:835-43.
81. Bao C, Chen W, Weir MD, Thein-Han W, Xu HH. Effects of electrospun submicron fibers in calcium phosphate cement scaffold on mechanical properties and osteogenic differentiation of umbilical cord stem cells. Acta Biomater 2011;7:4037-44.
82. Zhao L, Weir MD, Xu HH. Human umbilical cord stem cell encapsulation in calcium phosphate scaffolds for bone engineering. Biomaterials 2010;31:3848-57.
83. Wang T, Dang G, Guo Z, Yang M. Evaluation of autologous bone marrow mesenchymal stem cell-calcium phosphate ceramic composite for lumbar fusion in rhesus monkey interbody fusion model. Tissue Eng 2005;11:1159-67.
84. Lee LT, Kwan PC, Chen YF, Wong YK. Comparison of the effectiveness of autologous fibrin glue and macroporous biphasic calcium phosphate as carriers in the osteogenesis process with or without mesenchymal stem cells. J Chin Med Assoc 2008;71:66-73.
85. Jafarian M, Eslaminejad MB, Khojasteh A, Mashhadi Abbas F, Dehghan MM, Hassanizadeh R, *et al*. Marrow-derived mesenchymal stem cells-directed bone regeneration in the dog mandible: A comparison between biphasic calcium phosphate and natural bone mineral. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:e14-24.
86. Chen W, Liu J, Manuchehrabadi N, Weir MD, Zhu Z, Xu HH. Umbilical cord and bone marrow mesenchymal stem cell seeding on macroporous calcium phosphate for bone regeneration in rat cranial defects. Biomaterials 2013;34:9917-25.
87. Espitalier F, Vinatier C, Lerouxel E, Guicheux J, Pilet P, Moreau F, *et al.* A comparison between bone reconstruction following the use of mesenchymal stem cells and total bone marrow in association with calcium phosphate scaffold in irradiated bone. Biomaterials 2009;30:763-9.
88. Overman JR, Farré-Guasch E, Helder MN, ten Bruggenkate CM, Schulten EA, Klein-Nulend J. Short (15 minutes) bone morphogenetic protein-2 treatment stimulates osteogenic differentiation of human adipose stem cells seeded on calcium phosphate scaffolds *in vitro*. Tissue Eng Part A 2013;19:571-81.
89. Overman JR, Helder MN, ten Bruggenkate CM, Schulten EA, Klein-Nulend J, Bakker AD. Growth factor gene expression profiles of bone morphogenetic protein-2-treated human adipose stem cells seeded on calcium phosphate scaffolds *in vitro*. Biochimie 2013;95:2304-13.
90. Zhao L, Tang M, Weir MD, Detamore MS, Xu HH. Osteogenic media and rhBMP-2-induced differentiation of umbilical cord mesenchymal stem cells encapsulated in alginate microbeads and integrated in an injectable calcium phosphate-chitosan fibrous scaffold. Tissue Eng Part A 2011;17:969-79.
91. Kai T, Shao-qing G, Geng-ting D. *In vivo* evaluation of bone marrow stromal-derived osteoblasts-porous calcium phosphate ceramic composites as bone graft substitute for lumbar intervertebral spinal fusion. Spine (Phila Pa 1976) 2003;28:1653-8.
92. Takahashi T, Tominaga T, Watabe N, Yokobori AT Jr, Sasada H, Yoshimoto T. Use of porous hydroxyapatite graft containing recombinant human bone morphogenetic protein-2 for cervical fusion in a caprine model. J Neurosurg 1999;90 2 Suppl: 224-30.
93. Boden SD, Schimandle JH, Hutton WC. 1995 Volvo Award in basic sciences. The use of an osteoinductive growth factor for lumbar spinal fusion. Part II: Study of dose, carrier, and species. Spine (Phila Pa 1976) 1995;20:2633-44.
94. Subramony SD, Su A, Yeager K, Lu HH. Combined effects of chemical priming and mechanical stimulation on mesenchymal stem cell differentiation on nanofiber scaffolds. J Biomech 2014;47:2189-96.
95. Wobus AM. Potential of embryonic stem cells. Mol Aspects Med 2001;22:149-64.
96. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol 2012;30:546-54.
97. Cordonnier T, Sohier J, Rosset P, Layrolle P. Biomimetic materials for bone tissue engineering – State of the art and future trends. Adv Eng Mater 2011;13:B135-50.

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