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ABSTRACT

Calcium phosphate cements (CPCs) have excellent biocompatibility and osteoconductivity for dental, craniofacial, and orthopedic applications. This article reviews recent developments in stem cell delivery *via* CPC for bone regeneration. This includes: (1) biofunctionalization of the CPC scaffold, (2) co-culturing of osteoblasts/endothelial cells and prevascularization of CPC, (3) seeding of CPC with different stem cell species, (4) human umbilical cord mesenchymal stem cell (hUCMSC) and bone marrow MSC (hBMSC) seeding on CPC for bone regeneration, and (5) human embryonic stem cell (hESC) and induced pluripotent stem cell (hiPSC) seeding with CPC for bone regeneration. Cells exhibited good attachment/proliferation in CPC scaffolds. Stem-cell-CPC constructs generated more new bone and blood vessels *in vivo* than did the CPC control without cells. hUCMSCs, hESC-MSCs, and hiPSC-MSCs in CPC generated new bone and blood vessels similar to those of hBMSCs; hence, they were viable cell sources for bone engineering. CPC with hESC-MSCs and hiPSC-MSCs generated new bone two- to three-fold that of the CPC control. Therefore, this article demonstrates that: (1) CPC scaffolds are suitable for delivering cells; (2) hUCMSCs, hESCs, and hiPSCs are promising alternatives to hBMSCs, which require invasive procedures to harvest with limited cell quantity; and (3) stem-cell-CPC constructs are highly promising for bone regeneration in dental, craniofacial, and orthopedic applications.

KEY WORDS: bone tissue engineering, dental and craniofacial, calcium phosphate scaffold, human embryonic stem cells, human induced pluripotent stem cells, animal studies.

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Stem Cells and Calcium Phosphate Cement Scaffolds for Bone Regeneration

INTRODUCTION

With an aging population, bone repair and regeneration become a substantial public-health need (Shi *et al.*, 2002; Mao *et al.*, 2006; Mikos *et al.*, 2006; Jiang *et al.*, 2009; Rios *et al.*, 2011; Rosa *et al.*, 2012). Approximately 600,000 bone graft procedures are performed each year in the United States, and about 2.2 million such procedures are performed worldwide annually (Marino and Ziran, 2010). In the majority of cases, either autografts or allografts are performed (Marino and Ziran, 2010). Although autografts are considered the gold standard, risks of donor site morbidity and limited availability restrict their applications. Allografts are impeded by potential infection and a high non-union rate with the host tissues. Therefore, tissue engineering with the combination of biomaterials and stem cells is being investigated as a promising alternative (Baksh *et al.*, 2007; Mao *et al.*, 2007; Abukawa *et al.*, 2009; Huang, 2010; Ye *et al.*, 2011; Luo *et al.*, 2012). Calcium phosphate (CaP) biomaterials are useful for bone repair due to their similarity to bone minerals (Leach *et al.*, 2006; Zhao *et al.*, 2009; Park *et al.*, 2014). Calcium phosphate cements (CPCs) are biocompatible, resorbable, osteoconductive, and injectable. The first CPC was developed in 1986 and consisted of a mixture of tetracalcium phosphate [TTCP, $\text{Ca}_4(\text{PO}_4)_2\text{O}$] and dicalcium phosphate anhydrous (DCPA, CaHPO_4) (Brown and Chow, 1986). Because of its excellent biocompatibility and ability to be replaced by new bone, CPC has been approved by the Food and Drug Administration (FDA) for the repair of craniofacial defects (Friedman *et al.*, 1998). Since then, other CPC formulations have also been investigated (Barralet *et al.*, 2002; Bohner *et al.*, 2005; Ruhe *et al.*, 2006; Bodde *et al.*, 2009; Ginebra *et al.*, 2010). Several CPCs have been commercialized and applied in various clinical situations, such as cranioplasty, bone augmentation, implant fixation, fracture, and vertebroplasty (Chow, 2000; Jansen *et al.*, 2005; Bohner, 2010; Ginebra *et al.*, 2012). Besides orthopedic applications, dental and craniofacial applications of CPC could include mandibular and maxillary ridge augmentation, major reconstructions of the maxilla or mandible after trauma or tumor resection, and supporting metal dental implants or augmentation of deficient implant sites.

The present review focuses on the combination of CPC scaffolds with stem cells for bone regeneration. A review such as this cannot cover the vast

number of meritorious publications on various formulations of CPCs and their *in vitro* properties and *in vivo* performance. The reader is referred to comprehensive review articles in this field (Chow, 2000; Bohner *et al.*, 2005; Jansen *et al.*, 2005; Xu *et al.*, 2008; Bohner, 2010; Ginebra *et al.*, 2010, 2012; Pérez *et al.*, 2013; Sugawara *et al.*, 2013). This article reviews recent work on stem cell seeding with CPC for bone tissue engineering, biofunctionalization of the CPC scaffold to enhance cell attachment and proliferation, the seeding of CPC with different stem cell species including human bone marrow mesenchymal stem cells (hBMSCs), human umbilical cord mesenchymal stem cells (hUCMSCs), human embryonic stem cells (hESCs), and human induced pluripotent stem cells (hiPSCs), and their *in vivo* bone regeneration behavior in animal models. These relatively new topics have not been addressed in previous reviews on CPC.

CPC BIOFUNCTIONALIZATION WITH ENHANCED CELL RESPONSE

The success of cell seeding not only depends on cell seeding techniques and scaffold structure, but also closely relates to the capability of scaffold material to favor cell attachment and proliferation. Human stem cell attachment to traditional CPCs was relatively poor because of the ion activities of the cement. The addition of bioactive signals such as proteins or peptides is frequently used to enhance cell affinity in various biomaterials. Cell membrane receptors can recognize and bind to these biochemical cues presented in CPC, which in turn can activate cellular signal pathways able to tune gene expression and control cell fate. Thein-Han *et al.* (2012) compared the effects of 5 types of biofunctional agents to mediate the interactions between hUCMSCs and CPC. The first type was Arg-Gly-Asp (RGD) peptide, a well-known integrin-recognition site to promote cell attachment, with advantages of simple synthesis, minimal cost, low immunogenic activity, relative stability, and tight control of conformation. The second type was fibronectin (Fn), a cell adhesion molecule and an extracellular matrix (ECM) protein. The third type is Fn-like engineered protein polymer (FEPP), a genetically engineered protein. The fourth type was Geltrex, a three-dimensional basement membrane ECM. The fifth type was human platelet concentrate, which contains many bioactive factors. Each agent was added to the liquid phase of CPC, then mixed with CPC powder and set to form a scaffold. hUCMSCs were seeded onto CPC scaffolds. Substantially enhanced cell attachment, proliferation, and osteogenic differentiation were achieved on biofunctionalized CPC compared with a CPC control. The CPC-RGD, CPC-Fn, and CPC-Platelets groups had the best cell response (Fig. 1) (Thein-Han *et al.*, 2012). In addition, the CPC-Platelets group had the best osteogenic differentiation (Thein-Han *et al.*, 2012). In other studies, collagen (Pérez and Ginebra, 2013), osteocalcin, and *O*-phospho-L-serine (Vater *et al.*, 2010) were incorporated into CPC. In addition, surface treatment techniques, such as plasma treatment, were used to immobilize bioactive molecules on CPC/polymer scaffolds for the benefits of increased wettability, roughness, and porosity to further boost cellular response (He *et al.*, 2013).

CO-CULTURING OSTEOBLASTS/ENDOTHELIAL CELLS TO PREVASCULARIZE CPC

The reconstruction of large skeletal defects is a major challenge because of inadequate vascularization. A slow or incomplete vascularization at the defect where the scaffold was implanted *in vivo* would lead to inadequate oxygen and nutrition supply and waste products removal, thus causing hypoxia and cell death. Therefore, angiogenesis in bone tissue constructs and the development of a functional microvasculature are vital to achieve successful therapeutic outcome in bone regeneration. Recently, a macroporous CPC was prevascularized *in vitro* by the co-culture of human umbilical vein endothelial cells (hUVECs) and human osteoblasts (hOBs) (Thien-Han and Xu 2013; Chen *et al.*, 2014). For fabrication of the scaffold, chitosan was grafted with RGD, and the chitosan-RGD liquid was mixed with CPC powder to biofunctionalize the CPC. hUVECs and hOBs were co-cultured on macroporous CPC-RGD, and on CPC control without RGD, for up to 42 days. Immunostaining with endothelial marker showed that the quantity of microcapillary-like structures on CPC increased with time (Figs. 2A, 2B). At 42 days, the cumulative vessel length for the CPC-RGD scaffold was 1.69-fold that of the CPC control (Fig. 2C). Scanning electron microscopy (SEM) examination confirmed the morphology of self-assembled microcapillary-like structures on the CPC scaffold (Fig. 2D) (Chen *et al.*, 2014). These results demonstrated the promise of generating a prevascularized network on a macroporous CPC scaffold for potential enhancement of *in vivo* vascularization and bone regeneration.

SEEDING OF CPC WITH DIFFERENT STEM CELL SPECIES

hBMSCs are known for their ability to differentiate into different mesenchymal cell types, including osteoblasts, chondrocytes, and adipocytes. hBMSCs are considered the gold-standard cell source for bone tissue engineering research (Baksh *et al.*, 2007; Mao *et al.*, 2007). hBMSCs attached well to surface-modified CPCs (Vater *et al.*, 2010; He *et al.*, 2013). They exhibited an elongated, spindle-shaped morphology with active filopodial protrusions to adhere to CPC (Vater *et al.*, 2010; He *et al.*, 2013). hBMSCs populated and permeated the inside of porous CPCs, and higher porosity yielded better cell response (He *et al.*, 2013). Cells seeded on various CPCs successfully underwent osteogenic differentiation (Vater *et al.*, 2010; He *et al.*, 2013). However, hBMSCs generally have several disadvantages, including the need for an invasive procedure to harvest, a relatively small MSC amount from each procedure, and loss of potency in hBMSCs from seniors and patients with diseases and disorders. Therefore, other sources of stem cells need to be explored for tissue engineering applications.

hUCMSCs are harvested from umbilical cords and represent an inexpensive and non-invasive cell source. hUCMSCs have robust proliferation and self-renewal capability, and a high plasticity and developmental flexibility owing to their origin in neonatal tissues (Bailey *et al.*, 2007; Baksh *et al.*, 2007; Xu *et al.*,

Figure 1. Biofunctionalization of calcium phosphate cement (CPC) with human umbilical cord mesenchymal stem cell (hUCMSC) seeding. (A-F) Live/dead staining images of hUCMSCs on biofunctionalized CPCs. Incorporation of biofunctional agents into CPC increased cell attachment and proliferation. (G) Live cell density vs. culture time and biofunctional agents in CPC (mean $\pm$ SD; $n = 5$). (Adapted from Thein-Han *et al.*, 2012, with permission.)

2010; Zhao *et al.*, 2010). When seeded on the RGD-grafted macroporous CPC, hUCMSCs demonstrated good cell viability, proliferation, osteogenic differentiation, and mineral synthesis similar to that of hBMSCs (Chen *et al.*, 2013a). These studies indicated that hUCMSCs are a potent alternative to the gold-standard hBMSCs. Several factors were found to affect the cellular response of hUCMSCs to CPCs: (1) *Surface chemistry*. Besides surface modification by the aforementioned bioactive molecules, the addition of collagen fibers into CPC also enhanced hUCMSC attachment, osteogenic differentiation, and bone mineral synthesis (Thein-Han and Xu, 2011). (2) *Surface area*. The incorporation of submicron electrospun poly(D,L-lactide-co-glycolide) (PLGA) fibers increased the surface area and

Figure 2. Prevascularization of CPC. (A,B) Fluorescence images of hUVECs + hOBs co-cultured on macroporous CPC control and CPC-RGD scaffold at 42 days. hUVECs were identified by immunostaining with endothelial marker PECAM1 in green on cell membrane, and the nuclei were counterstained with DAPI in blue. hOBs were depicted by nuclei counterstaining with DAPI in blue but without green stain on cell membrane. Microcapillary-like structures increased with culture time and with the addition of RGD in CPC. (C) Cumulative length of microcapillary-like structures on CPC scaffolds (mean $\pm$ SD; $n = 5$). Bars with dissimilar letters indicate significantly different values ($p < .05$). (D) Representative SEM image of microcapillary-like structure on CPC-RGD scaffold at 14 days. (Adapted from Chen *et al.*, 2014, with permission.) This figure is available in color online at <http://jdr.sagepub.com>.

enhanced hUCMSC proliferation, osteogenic differentiation, and mineralization (Bao *et al.*, 2011). (3) *Cell seeding density*. Varying the cell seeding density is another strategy to optimize the cell-biomaterial microenvironments and osteogenic signal expression *via* different paracrine signal distances. The seeding of hUCMSCs onto the CPC scaffold at approximately 2,654 cells/mm² yielded a high percentage of live cells, fast proliferation, and high bone marker expression and mineralization (Zhou *et al.*, 2011). Besides hBMSCs and hUCMSCs, stem cells derived from dental tissues, such as dental pulp stem cells (DPSCs) and deciduous teeth stem cells (DTSCs) with the property to form a calcified matrix, also showed promising results for the regeneration of mineralized tissues in combination with CPCs (Xia *et al.*, 2013).

hUCMSC AND hBMSC SEEDING ON CPC FOR BONE REGENERATION *in vivo*

Strategies for the acceleration of bone regeneration *via* CPC include the addition of bioactive agents such as osteocalcin, *O*-phospho-L-serine (Vater *et al.*, 2010), and bioactive glass (Yu *et al.*, 2013). Another strategy for the enhancement of bone regeneration is to combine stem cells with CPC (Zeng *et al.*, 2012; Zou *et al.*, 2012; Chen *et al.*, 2013a). With critical-sized cranial defects in rats (Kaigler *et al.*, 2006; Leach *et al.*, 2006), when hUCMSCs and hBMSCs were seeded onto a CPC scaffold and implanted into the defects, new bone formation increased with time (Figs. 3A-3F) (Chen *et al.*, 2013a). New bone started from the defect peripherals at the interface between the implant and native bone. Then, more new bone was gradually generated along CPC surfaces and inside the CPC scaffold (Chen *et al.*, 2013a). Compared with CPC control without cells, 88% and 57% increases in new bone were achieved when hBMSCs and hUCMSCs were seeded with CPC, respectively (Fig. 3G) (Chen *et al.*, 2013a). At a higher magnification (Fig. 4), the new bone appeared to be in the maturing process at 24 wk, manifested by the appearance of osteoid with osteocytes and blood vessels, and with newly formed bone being lined by osteoblasts. CPC resorption could be noticed in defects, with osteoclast-like multinuclear giant cells present at the internal surfaces of the macropores in CPC (Fig. 4B) (Chen *et al.*, 2013a). In summary, the least new bone was seen in the CPC control group, with more new bone formed in the hUCMSC and hBMSC groups than in the control group ($p < .05$), and there was no statistically significant difference between the hUCMSC and hBMSC groups ($p > .1$) (Chen *et al.*, 2013a).

In a rabbit maxillary sinus floor elevation model, seeding of CPC with hBMSCs generated greater new bone area than in a model without cells (Zeng *et al.*, 2012). These results suggest that the transplanted stem cells indeed participated in and contributed to osteogenesis. Initially, the seeded cells directly deposited bone matrix minerals in the scaffold. Then, *via* cell-mediated paracrine stimulation, the recruited native cells from the host tissues, including but not limited to osteoblast progenitors, endothelial cells, and osteoclasts, took over the responsibility of subsequent bone formation and remodeling (Wang *et al.*, 2014). Studies showed that the secretion of bioactive molecules by the seeded cells enabled the cell-CPC constructs to have bone-forming ability even in ectopic sites of nude mice, thus changing the osteoconductive CPC to become osteoinductive (Wang XJ *et al.*, 2011). In addition, prominent angiogenesis was observed in cell-seeded CPCs *in vivo*. Chen *et al.* (2013a) reported that the seeding of hBMSCs and hUCMSCs with CPC resulted in 20% and 15% increases in blood vessel density, respectively. Furthermore, the addition of stem cells was reported to accelerate the degradation of CPC and could thus help balance the catabolism and anabolism of bone remodeling (Zeng *et al.*, 2012; Wang *et al.*, 2014). In a canine peri-implant bone defect model, the application of the BMSC/BMP-2/bFGF/CPC construct demonstrated the highest level of new bone apposition, followed by BMSC/BMP-2/CPC and BMSC/bFGF/CPC, which were still higher than BMSC/CPC, while CPC without cells had the least new bone (Wang L *et al.*, 2011).

Figure 3. Bone regeneration in critical-sized cranial defects in nude rats with 3 groups (CPC control without cells; CPC with hUCMSCs; CPC with hBMSCs). Representative H&E-stained histological images at: (A-C) 4 wk and (D-F) 24 wk. Arrows indicate examples of new bone formation. (G) Scaffolds containing hUCMSCs and hBMSCs exhibited greater bone regeneration than CPC controls without cells (mean $\pm$ SD; $n = 5$). There was no statistically significant difference between the hUCMSC and hBMSC groups at each time. Bars with dissimilar letters indicate statistically significantly different values ($p < .05$). (Adapted from Chen *et al.*, 2013a, with permission.)

USE OF hESCs WITH CPC FOR BONE TISSUE ENGINEERING

Besides the use of hBMSCs and hUCMSCs, hESCs are an exciting cell source for regenerative medicine applications (Hwang

Figure 4. High-magnification images showing typical details in cranial defects in rats: (A) from the solid-line rectangle of Fig. 3F, and (B) from the dotted-line rectangle of Fig. 3F. (A) Osteoblasts with a spindle morphology were found around the new bone. Osteocytes were found inside the new bone. (B) Osteoclast-like multinuclear giant cells (encircled by blue lines) surrounded the CPC surface in the resorption lacunae. New blood vessels are indicated by arrows. (Adapted from Chen *et al.*, 2013a, with permission.) This figure is available in color online at <http://jdr.sagepub.com>.

et al., 2008; Arpornmaeklong *et al.*, 2009; Smith *et al.*, 2010). hESCs are relatively homogenous, possess a high self-renewal activity, have nearly unlimited proliferation capability, and can be differentiated into all cell types in the body. A recent study investigated the use of hESCs with CPC for osteogenic differentiation (Chen *et al.*, 2013b). hESCs were cultured to form embryoid bodies (EBs), and then, in further culture, MSCs were migrated out of the EBs. Flow cytometry evaluation indicated that the hESC-derived MSCs (hESC-MSCs) expressed the surface antigen profile typical of MSCs and were negative for typical hematopoietic and endothelial cell markers (Chen *et al.*, 2013b). When seeded onto CPC scaffolds, hESC-MSCs were differentiated into the osteogenic lineage, expressing high levels of osteogenic markers including alkaline phosphatase, osteocalcin, collagen I, and Runx2 (Chen *et al.*, 2013b).

In an animal study, hESC-MSCs were seeded, for the first time, onto CPC for bone regeneration in critical-sized cranial defects in rats. Four groups were tested: (1) CPC control without cells; (2) CPC with hESC-derived MSCs ("CPC+hESC-MSC"); (3) CPC with hESC-MSCs and 30% human platelet concentrate (hPC) ("CPC+hESC-MSC+30%hPC"); and (4) CPC+hESC-MSC+50%hPC. The hESC-MSCs on CPC had successful osteogenic differentiation, and new bone was generated *in vivo* (Fig. 5A). Calcified new bone area fraction was plotted in Fig. 5B, and new blood vessel density was plotted in Fig. 5C (mean $\pm$ SD; $n = 6$). Seeding hESC-MSCs onto the CPC scaffold generated more new bone than in the CPC control ($p < .05$). The incorporation of hPCs into the CPC-hESC-MSC construct further increased new bone generation ($p < .05$). The groups containing hESC-MSCs and hPCs had new bone about three-fold, and blood vessel density 1.6-fold, those of the CPC control.

USE OF hiPSCs WITH CPC FOR BONE TISSUE ENGINEERING

hiPSCs represent a major breakthrough in stem cell research and provide an invaluable resource for regenerative medicine (Takahashi and Yamanaka, 2006; Nakagawa *et al.*, 2008; Amabile and Meissner, 2009; Ye *et al.*, 2011; Villa-Diaz *et al.*, 2012). hiPSCs provide a promising method for obtaining patient-specific stem cells for tissue engineering. hiPSCs are believed to be very similar to hESCs in many respects, including the expression of certain stem cell genes and proteins, doubling time, chromatin methylation patterns, embryoid body formation, teratoma formation, viable chimera formation, potency, and differentiability (Takahashi and Yamanaka, 2006; Nakagawa *et al.*, 2008). Furthermore, hiPSCs possess nearly unlimited potential to proliferate and differentiate into all derivatives of the 3 primary germ layers (ectoderm, endoderm, and mesoderm) (Amabile and Meissner, 2009). In addition, hiPSCs can be easily and autogeneically accessible, thus avoiding both the ethical and immunological concerns related to hESCs. In a recent study, hiPSCs were cultured to derive MSCs (hiPSC-MSCs), after which hiPSC-MSCs underwent bone morphogenic protein 2 (BMP2) gene modification and were seeded onto CPC for the first time (Liu *et al.*, 2013). hiPSC-MSCs were infected with green fluorescence protein (GFP-hiPSC-MSCs) or BMP2 lentivirus (BMP2-hiPSC-MSCs). BMP2-hiPSC-MSCs expressed much higher BMP2 levels than hiPSC-MSCs. After 14-day culture in osteogenic medium, the ALP activity of BMP2-hiPSC-MSCs on CPC was 1.8 times that of the hiPSC-MSC control, indicating that the BMP2 gene transduction of hiPSC-MSCs enhanced the osteogenic differentiation. Osteocalcin gene expression of BMP2-hiPSC-MSCs on CPC was 2.3 times that of the hiPSC-MSC control. Bone matrix mineralization by BMP2-hiPSC-MSCs on CPC was 1.8 times that of the hiPSC-MSC control at 21 days (Liu *et al.*, 2013). These *in vitro* results indicated that CPCs seeded with hiPSC-MSCs are promising for bone tissue engineering.

In vivo bone regeneration of hiPSC-MSC-seeded CPC constructs was investigated, for the first time, in a critical-sized cranial defect model in rats. Four groups were tested:

hiPSC-MSC-seeded CPC, hUCMSC-seeded CPC, hBMSC-seeded CPC, and CPC control without cells. Histological H&E staining showed that the hiPSC-MSC and hUCMSC groups had nearly complete osseous bridging of the critical-sized defect, especially on the dura side at 12 wk, where the new bone had an organized pattern and mature bone morphology. The new bone area fraction for the hiPSC-MSC group was $30.4 \pm 5.8\%$, 2.8-fold the $11.0 \pm 6.3\%$ for the CPC control ($p < .01$) (Fig. 6A). No significant differences were detected among the 3 groups with stem cells ($p > .05$). New blood vessels were present in the new bone and the fibrous tissue filling the defects. New blood vessel densities of the hiPSC-MSC, hUCMSC, and hBMSC groups were significantly higher than that of the CPC control ($p < .05$). New blood vessel density of the hiPSC-MSC group was 1.7-fold that of the CPC control. Therefore, iPSC-MSCs appeared to have excellent osteogenic and angiogenic capabilities similar to those of hBMSCs and hUCMSCs. These results show that hiPSCs are promising for bone regeneration applications and that CPC is a biocompatible and resorbable scaffold suitable for delivering stem cells for craniofacial and orthopedic repairs.

CONCLUSIONS

CPC scaffolds have high potential in a wide range of dental, craniofacial, and orthopedic applications due to their excellent biocompatibility, osteoconductivity, and resorbability. The present article reviewed new developments in stem cell seeding and delivery with CPC scaffolds, biofunctionalization of CPC to enhance cell attachment and proliferation, the seeding of CPC with different stem cell species including hBMSCs, hUCMSCs, hESCs, and hiPSCs, and their *in vivo* properties. While hBMSCs are considered the gold standard for stem cell-based bone regeneration research, their drawbacks include the need for an invasive procedure to harvest hBMSCs and the limited cell quantity. hUCMSCs, hESCs, and hiPSCs have been demonstrated to be potent alternative cell sources to hBMSCs for bone regeneration. When seeded onto CPC scaffolds, these cells exhibited good attachment, proliferation, and osteogenic differentiation. When implanted into cranial defects in rats, stem cell-seeded CPC constructs generated much greater

Figure 5. Bone regeneration via hESC-MSC seeding in CPC in cranial defects in rats. Four groups were tested: (1) CPC control without cells; (2) CPC with hESC-derived MSCs ("CPC+hESC-MSC"); (3) CPC with hESC-MSCs and 30% human platelet concentrate (hPC) ("CPC+hESC-MSC+30%hPC"); and (4) CPC+hESC-MSC+50%hPC. (A) H&E image showing that new bone grew in the interior of the CPC scaffold and was maturing as indicated by osteocytes and blood vessels for the CPC+hESC-MSC+50%hPC group. Similar features were observed in other groups. (B) Calcified new bone area fraction at 12 wk (mean $\pm$ SD; $n = 6$). (C) New blood vessel density. The groups seeded with hESC-MSCs and hPCs had calcified new bone approximately three-fold, and blood vessel density 1.6-fold, those of the CPC control.

amounts of new bone and blood vessels compared with the CPC control without cells. Seeding of CPC with hUCMSCs, hESC-MSCs, and hiPSC-MSCs generated new bone and blood vessels similar to those of hBMSCs, indicating that these cells are promising viable cell sources for bone tissue engineering. For example, CPC constructs seeded with hESC-MSCs and hiPSC-MSCs generated new bone amounts two- to three-fold that of the CPC control. Therefore, the seeding of CPC scaffolds with stem cells is highly promising for the enhancement of bone regeneration in dental, craniofacial, and orthopedic applications.

Figure 6. Bone regeneration via stem cell seeding in CPC in cranial defects in rats. Four groups were tested: CPC with hiPSC-MSCs; CPC with hUCMSCs; CPC with hBMSCs; and CPC control without cells. **(A)** New bone area fraction at 12 wk (mean $\pm$ SD; $n = 6$). **(B)** New blood vessel density at 12 wk (mean $\pm$ SD; $n = 6$). The hiPSC-MSC group had new bone nearly three-fold, and blood vessel density 1.7-fold, those of the CPC control.

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