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A self-setting iPSMSC-alginate-calcium phosphate paste for bone tissue engineering

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ABSTRACT

Objectives. Calcium phosphate cements (CPCs) are promising for dental and craniofacial repairs. The objectives of this study were to: (1) develop an injectable cell delivery system based on encapsulation of induced pluripotent stem cell-derived mesenchymal stem cells (iPSMSCs) in microbeads; (2) develop a novel tissue engineered construct by dispersing iPSMSC-microbeads in CPC to investigate bone regeneration in an animal model for the first time.

Methods. iPSMSCs were pre-osteoinduced for 2 weeks (OS-iPSMSCs), or transduced with bone morphogenetic protein-2 (BMP2-iPSMSCs). Cells were encapsulated in fast-degradable alginate microbeads. Microbeads were mixed with CPC paste and filled into cranial defects in nude rats. Four groups were tested: (1) CPC-microbeads without cells (CPC control); (2) CPC-microbeads-iPSMSCs (CPC-iPSMSCs); (3) CPC-microbeads-OS-iPSMSCs (CPC-OS-iPSMSCs); (4) CPC-microbeads-BMP2-iPSMSCs (CPC-BMP2-iPSMSCs).

Results. Cells maintained good viability inside microbeads after injection. The microbeads were able to release the cells which had more than 10-fold increase in live cell density from 1 to 14 days. The cells exhibited up-regulation of osteogenic markers and deposition of minerals. *In vivo*, new bone area fraction (mean ± SD; $n = 5$) for CPC-iPSMSCs group was (22.5 ± 7.6)%. New bone area fractions were (38.9 ± 18.4)% and (44.7 ± 22.8)% for CPC-OS-iPSMSCs group and CPC-BMP2-iPSMSCs group, respectively, 2–3 times the (15.6 ± 11.2)%

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in CPC control at 12 weeks ($p < 0.05$). Cell-CPC constructs accelerated scaffold resorption, with CPC-BMP2-iPSMSCs having remaining scaffold material that was 7-fold less than CPC control.

Significance. Novel injectable CPC-microbead-cell constructs promoted bone regeneration, with OS-iPSMSCs and BMP2-iPSMSCs having 2–3 fold the new bone of CPC control. Cell delivery accelerated scaffold resorption, with CPC-BMP2-iPSMSC having remaining scaffold material that was 7-fold less than CPC control. Therefore, CPC-microbead-iPSMSC is a promising injectable material for orthopedic, dental and craniofacial bone regenerations.

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1. Introduction

The generation of induced pluripotent stem cells (iPSCs) is an exciting discovery in the field of cell-based therapy [1]. Through expression of a small combination of transcription factors, somatic cells can be converted into an embryonic state, thus, exhibiting tremendous possibilities in treatment of various diseases. IPSCs represent an enormous source of patient-specific stem cells derived from plentiful and easily accessible tissues like skin, hair and fat, *etc.* Development of virus-free and vector-free reprogramming technologies reduces the chance of virally-induced tumor formation, thus provides optimism for clinical applications of iPSCs [2,3]. When transplanted back into the patients, iPSCs need to be induced into high-quality progenitor cells like mesenchymal stem cells (MSCs), or fully-differentiated homogenous mature cells to circumvent the risk of teratoma formation caused by undifferentiated cells contaminating the final products [4,5]. It has been reported that iPSC-derived MSCs (iPSMSCs) exhibited a higher proliferative capability than bone marrow MSCs (BMSCs) [6], and are less tumorigenic than undifferentiated iPSCs and BMSCs [7–9]. Thus in the field of bone tissue engineering, iPSMSCs escalate the hope especially for patients with compromised health conditions whose autologous BMSCs are no longer vibrant for tissue repair and regeneration [10].

Calcium phosphate cements are promising bone substitutes with excellent bioactivity, biocompatibility and osteoconductivity [11–14]. These materials stand out as injectable bone cements owing to their self-setting and *in situ*-hardening capabilities [15–17]. One such cement consisted of tetracalcium phosphate [TTCP: $Ca_4(PO_4)_2O$] and dicalcium phosphate (DCPA: $CaHPO_4$), and was referred to as CPC [17]. The CPC powder can be mixed with an aqueous liquid to form a paste that can be injected or sculpted during surgery to conform to the defects in hard tissues. CPC was approved in 1996 by the Food and Drug Administration for repairing craniofacial defects in humans, thus becoming the first CPC available for clinical use [18].

Our previous studies enhanced the mechanical, physical and biological properties of CPC through the introduction of absorbable fibers [19], chitosan [20], mannitol porogen [21], gas-foaming agents [22], alginate microbeads [23], and biofunctionalization [24]. These approaches improved the CPC's mechanical strength, setting time, degradability, macroporosity, cell attachment, and delivery of cells and growth factors. Potential dental and craniofacial applications of an improved CPC include periodontal bone lesion repair, socket preservation, maxillary sinus floor elevation, augmentation of deficient implant sites, ridge augmentation, as well as other dental and orthopedic applications [17,18].

Our recent study showed that osteoinduced iPSMSCs seeded on pre-formed CPC scaffolds in rat cranial defects had comparable *in vivo* bone regeneration capability to BMSCs and umbilical cord MSCs (UCMSCs), but significantly higher than CPC control [25]. No teratoma was found during a 12 weeks observation [25]. Although it was promising in supporting iPSMSCs' role in prompting bone regeneration efficiency of CPCs, cells were only loaded on one side of the scaffolds. This type of static cell seeding method has limitations of low seeding efficiency and minimal cell penetration into scaffold, leading to non-uniform distribution of cells and subsequently compromised regeneration *in vivo* [26]. To address these problems, in the present study, alginate microbeads were used as cell delivery vehicles to protect the encapsulated cells during CPC paste mixing, injection and setting reactions. The CPC-microbead-constructs can be readily injected or placed into bone defects with minimal invasion and intimate adaptation to complex defect shapes [27]. Alginate has been selected because it is non-cytotoxic and can form an ionically cross-linked network under mild conditions producing no detrimental effects to cells [28]. To promote alginate degradation and subsequent cell release, fast-degradable alginate-fibrin microbeads were fabricated following a previous study [29]. Furthermore, to enhance osteogenicity, iPSMSCs were either pre-osteoinduced for 2 weeks (OS-iPSMSCs), or transduced with bone morphogenic protein-2 (BMP2) gene (BMP2-iPSMSCs).

The aims of this study were to: (1) develop a novel injectable cell delivery system based on iPSMSC encapsulation in alginate microbeads and investigate cell viability, proliferation and osteogenic differentiation; and (2) develop a novel tissue engineered construct by dispersing iPSMSC-microbeads in CPC and investigate bone regeneration *in vivo*. The following hypotheses were tested: (1) microencapsulation and injection would not harm the encapsulated iPSMSCs; (2) iPSMSCs released from fast-degradable microbeads could proliferate and differentiate into osteogenic lineage; (3) cell-encapsulating-microbeads can induce osteogenic differentiation of co-cultured BMSCs; and (4) pre-osteogenic differentiation and BMP2 transduction would promote bone regeneration of iPSMSCs in CPC constructs *in vivo*.

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