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Human embryonic stem cell encapsulation in alginate microbeads in macroporous calcium phosphate cement for bone tissue engineering

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ABSTRACT

Human embryonic stem cells (hESC) are promising for use in regenerative medicine applications because of their strong proliferative ability and multilineage differentiation capability. To date there have been no reports on hESC seeding with calcium phosphate cement (CPC). The objective of this study was to investigate hESC-derived mesenchymal stem cell (hESCd-MSC) encapsulation in hydrogel microbeads in macroporous CPC for bone tissue engineering. hESC were cultured to form embryoid bodies (EB), and the MSC were then migrated out of the EB. hESCd-MSC had surface markers characteristic of MSC, with positive alkaline phosphatase (ALP) staining when cultured in osteogenic medium. hESCd-MSC were encapsulated in alginate at a density of 1 million cells ml^{-1}, with an average microbead size of 207 μm. CPC contained mannitol porogen to create a porosity of 64% and 218-μm macropores, with 20% absorbable fibers for additional porosity when the fibers degrade. hESCd-MSC encapsulated in microbeads in CPC had good viability from 1 to 21 days. ALP gene expression at 21 days was 25-fold that at 1 day. Osteocalcin (OC) at 21 days was two orders of magnitude of that at 1 day. ALP activity in colorimetric *p*-nitrophenyl phosphate assay at 21 days was fivefold that at 1 day. Mineral synthesis by the encapsulated hESCd-MSC at 21 days was sevenfold that at 1 day. Potential benefits of the CPC-stem cell paste include injectability, intimate adaptation to complex-shaped bone defects, ease in contouring to achieve esthetics in maxillofacial repairs, and in situ setting ability. In conclusion, hESCd-MSC were encapsulated in alginate microbeads in macroporous CPC, showing good cell viability, osteogenic differentiation and mineral synthesis for the first time. The hESCd-MSC-encapsulating macroporous CPC construct is promising for bone regeneration in a wide range of orthopedic and maxillofacial applications.

1. Introduction

In 2004, health-care costs plus lost wages for people in the US with musculoskeletal diseases reached ~US $849 billion, or 7.7% of national gross domestic product [1]. Skeletal disease, congenital malformation, trauma and post-cancer ablative surgery often require bone reconstruction, and the need is increasing dramatically as the population ages [2–6]. Tissue engineering approaches offer exciting promise to meet this need [7–12]. Extensive studies have resulted in significant progress in stem cell delivery via scaffolds, with great potential for tissue regeneration [13–16].

Scaffolds are important for bone regeneration, and injectable scaffolds can be used in minimally invasive procedures and fit intimately into complex-shaped defects [11,17–19]. Calcium phosphate scaffolds mimic bone minerals, provide a more natural substrate for cell attachment and expression of osteoblast phenotype, and can bond to bone to form a functional interface [14,20–24]. For a preformed bioceramic to fit into a bone cavity, the surgeon needs to machine the graft or carve the surgical site, leading to increases in bone loss, trauma and surgical time [17]. In contrast, calcium phosphate cements (CPC) are injectable and can self-harden in situ [19,25–29]. The first CPC was developed in 1986 [25] and approved in 1996 by the Food and Drug Administration (FDA) for repairing craniofacial defects [30]. Recent studies increased the macroporosity and mechanical strength of CPC, using porogens and absorbable fibers [31,32], and investigated stem cell seeding and osteogenic differentiation [33].

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