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Time-responsive osteogenic niche of stem cells: A sequentially triggered, dual-peptide loaded, alginate hybrid system for promoting cell activity and osteo-differentiation

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ABSTRACT

The efficacy of stem cell-based bone tissue engineering has been hampered by cell death and limited fate control. A smart cell culture system with the capability of sequentially delivering multiple factors in specific growth stages, like the mechanism of the natural extracellular matrix modulating tissue formation, is attractive for enhancing cell activity and controlling cell fate. Here, a bone forming peptide-1 (BFP-1)-laden mesoporous silica nanoparticles (pep@MSNs) incorporated adhesion peptide, containing the arginine-glycine-aspartic acid (RGD) domain, modified alginate hydrogel (RA) system (pep@MSNs-RA) was developed to promote the activity and stimulate osteo-differentiation of human mesenchymal stem cells (hMSCs) in sequence. The survivability and proliferation of hMSCs were enhanced in the adhesion peptide modified hydrogel. Next, BFP-1 released from pep@MSNs induced hMSCs osteo-differentiation after the proliferation stage. Moreover, BFP-1 near the cells was self-captured by the additional cell-peptide cross-linked networks formed by the ligands (RGD) binding to receptors on the cell surface, leading to long-term sustained osteo-stimulation of hMSCs. The results suggest that independent and sequential stimulation in proliferation and osteo-differentiation stages could synergistically enhance the survivability, expansion, and osteogenesis of hMSCs, as compared to stimulating alone or simultaneously. Overall, this study provided a new and valid strategy for stem cell expansion and osteo-differentiation in 2D or 3D culture systems, possessing potential applications in 3D bio-printing and tissue regeneration.

1. Introduction

Stem cell-based tissue engineering holds great clinical promise. Guiding stem cells to differentiate into a specific cell type is the most important step for successful cell therapy [1] because stem cells may otherwise differentiate into unwanted cells or tissues, including tumors [2,3]. Material-based matrixes and bio-factors are the two most important elements for guiding stem cell differentiation [3–5]. For bone tissue regeneration, biophysical and biochemical cues from extracellular matrix (ECM) such as stiffness, topography, and porosity, as well as binding functional groups have

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been reported to control stem cell differentiation [6–9]. Also, many bio-factors including β-glycerol phosphate, dexamethasone, and various growth factors (including bone morphogenetic proteins [BMPs]) have been developed [10–13]. However, most of these strategies typically rely on only a single differentiation factor to induce osteogenic differentiation (osteo-differentiation) and neglect to enhance the survival and proliferation of cells, which may partially mimic the native microenvironment and hamper tissue regeneration.

Importantly, cell survival and proliferation are important events preceding cell differentiation [14,15], and the osteo-differentiation is based on the extent of cell-cell contact [16]. The cells regard each other as their surrounding "microenvironment", and thus the biomechanical or biochemical cues from neighboring cells represent a way of stimulating osteo-differentiation [17–19]. Tang et al. found that the extent of osteo-differentiation was fairly linearly related to the extent of neighboring cells [16]. Moreover, cell therapy is based on a large number of cells, and therefore requires enough time for cell expansion before differentiation. In addition, increasing efforts have demonstrated that incorporated biological signals should be presented at the right time and right place [20]. Thus, the desired material-based matrix for cell therapy should hold the ability to bind, store, and deliver growth factors in the appropriate growth stage [21], which could provide a stimulatory environment for stem cell expansion and differentiation in sequence. Mooney et al. developed a dual delivery system for vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF)-BB from a structural polymer scaffold, resulting in the rapid formation of a mature vascular network [22]. Delivery of these molecules in tandem elicited synergistically enhanced vessel formation, whereas VEGF or PDGF-BB delivery alone resulted in fragile vascularity or no significant vessel formation [22]. For bone tissue engineering, some studies have attempted to achieve the goal by simultaneously bonding integrin binding ligands (such as vitronectin or laminin) and osteoinductive growth factors (including BMP-7) to promote osteo-differentiation of hMSCs or human embryonic stem cells [23–25]. To date, the most challenging aspect in these studies is sequentially presenting appropriate factors during specific growth stages. Thus, in this study, we explored a facile and versatile dual-peptide loaded alginate-based hydrogel system to deliver growth factors in specific stages, which is similar to the mechanism of the natural ECM where modulation of tissue formation occurs. Using this system, the activity and proliferation of stem cells was enhanced first, and then osteo-differentiation was markedly induced.

Alginate has been widely used in numerous biomedical applications including tissue regeneration, drug delivery and cell encapsulation [26,27], for its high biocompatibility, low toxicity and relatively low cost. Nevertheless, alginate is a flexible polymer that presents no cell adhesion ligands, resulting in a low cell survival rate. Typically, integrin binding ligands, such as RGD peptides, are coupled to alginate polymer chains to enhance cell viability and promote cell proliferation [28–30]. Bone morphogenetic proteins, including BMP-2, BMP-4, and BMP-7, are the most potent osteo-inductive growth factors [24,25]. A peptide derived from the immature region of BMP-7, which has higher osteogenesis activity than BMP-7 [31,32], named bone forming peptide-1 (BFP-1), was employed to induce the osteo-differentiation of hMSCs. In this system, BFP-1 should be stored in a reservoir and delivered in a controlled and sustained manner. Mesoporous silica nanoparticles (MSNs) with large surface areas, adjustable pore sizes and high biocompatibility, have been extensively studied as nanoscale drug delivery carriers [33–35]. Overall, BFP-1 was incorporated into MSNs to obtain the peptide-laden MSNs (pep@MSNs), and then the pep@MSNs were encapsulated into the RGD-treated alginate hydrogel (RA) to form pep@MSNs-RA.

In this niche, the activity of hMSCs is first promoted by the RGD peptide (proliferation factor). Thereafter, the BFP-1 (osteogenesis factor) released from the pep@MSNs induces hMSCs osteo-differentiation after cells expansion and the formation of cell-cell contacts. Moreover, Mooney et al. reported that the cell-polymer interactions would be formed by the ligands binding to receptors on the cell surface [36]. BFP-1 around the cells could be captured by the additional cell-peptide cross-linked networks, leading to long-term sustained osteo-stimulation of hMSCs (Fig. 1). In this study, we tested the hypothesis that independent and sequential stimulation in the proliferation and osteogenesis stages can synergistically enhance the survivability, expansion and osteo-differentiation of hMSCs, compared to stimulation alone or simultaneously. The time-responsive cell culture system provides a niche-like native ECM for stem cell survival and growth into mature bone tissue, with potential applications in tissue engineering and organoids culture.

2. Experimental section

2.1. Materials

Sodium alginate (SA) with high mannuronic acid content (G/M ≈ 0.64) and calcium sulfate powders were purchased from Sigma-Aldrich (St. Louis, USA). 2-(*N*-morpholino) ethanesulfonic acid (MES), *N*-hydroxy-sulfosuccinimide (sulfo-NHS), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). The RGD peptide (GGGGRGDASSP sequence), fluorescein isothiocyanate (FITC)-labeled RGD, BFP-1 (GQGFSYPYKAVFSTQ sequence), and the 6-carboxy tetramethyl rhodamine-labeled (TAMRA-labeled) BFP-1 were purchased from ChinaPeptides Co. Ltd. (Shanghai, China) and all the peptides were synthesized by a batch-wise fmoc-polyamide method to achieve greater than 98% purity. All other chemicals were of analytical reagent grade and used as received unless noted. All aqueous solutions were prepared with deionized water.

2.2. Fabrication of pep@MSNs

The preparation of MSNs and BFP-1 laden MSNs (pep@MSNs) was performed as previously described [33]. Briefly, 100 mg of the solid material was immersed in 10 mL of 10^{-4} mol L^{-1} BFP-1 (or TAMRA-labeled BFP-1) solution (in phosphate-buffered saline [PBS]) with stirring for 30 min. Subsequently, the BFP-1 (or TAMRA-labeled BFP-1) laden MSNs were washed with deionized water three times to remove excess non-adsorbed peptide, and dried at ambient temperature.

2.3. Preparation of alginate hydrogels

The adhesion peptide that contains the RGD sequence was utilized to promote hMSCs adhesion, spreading, and proliferation. RGD peptide was coupled to alginate polymers using published carbodiimide chemistry [28]. Briefly, EDC and sulfo-NHS were reacted with alginate solution in MES buffer to form a stable intermediate, and RGD was added to the solution and allowed to react overnight at room temperature. The concentration of peptides and polymer was 20 peptides per polymer chain, and the efficacy of peptides coupled to alginate was characterized using FITC-labeled RGD peptides. Following peptides treatment, alginate was dialyzed (3.5 kDa), sterile filtered (0.22 μm), and freeze-dried.