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Sequential delivery of BMP-2 and IGF-1 using a chitosan gel with gelatin microspheres enhances early osteoblastic differentiation

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ABSTRACT

The purpose of this study was to develop and characterize a chitosan gel/gelatin microsphere (MSs) dual delivery system for sequential release of bone morphogenetic protein-2 (BMP-2) and insulin-like growth factor-1 (IGF-1) to enhance osteoblast differentiation in vitro. We made and characterized the delivery system based on its degree of cross-linking, degradation, and release kinetics. We also evaluated the cytotoxicity of the delivery system and the effect of growth factors on cell response using pre-osteoblast W-20-17 mouse bone marrow stromal cells. IGF-1 was first loaded into MSs, and then the IGF-1-containing MSs were encapsulated into the chitosan gel which contained BMP-2. Cross-linking of gelatin with glyoxal via Schiff bases significantly increased thermal stability and decreased the solubility of the MSs, leading to a significant decrease in the initial release of IGF-1. Encapsulation of the MSs into the chitosan gel generated polyelectrolyte complexes by intermolecular interactions, which further affected the release kinetics of IGF-1. This combinational delivery system provided an initial release of BMP-2 followed by a slow and sustained release of IGF-1. Significantly greater alkaline phosphatase activity was found in W-20-17 cells treated with the sequential delivery system compared with other treatments ($P < 0.05$) after a week of culture.

1. Introduction

Therapeutic biomacromolecules such as RGD-like peptides and growth factors have been used to enhance the regeneration of damaged tissues by stimulating cellular activities such as cell migration, proliferation, and differentiation [1–6]. However, they have short biological half-lives in physiological conditions due to rapid degradation and deactivation by enzymes and other chemical and physical reactions [1–4]. In addition, the course of wound healing and tissue regeneration is complicated by the interactions of multiple factors [4,7–10]. Local delivery carriers have been developed for the controlled, sustained release of these active proteins [11–14]. Nevertheless, there is a great need for drug delivery systems that allow for improved release kinetics of multiple growth factors in order to enhance their therapeutic efficacy [3,8,11–17].

Recent studies have shown that the combined delivery of bone morphogenetic protein-2 (BMP-2) and insulin-like growth factor-1 (IGF-1) enhances wound healing and tissue regeneration compared with single growth factor delivery [3,5,11,14,18]. BMP-2 is an FDA-approved growth factor, which plays an important role in the expression of osteogenic markers such as alkaline phosphatase (ALP) and osteocalcin. It is used clinically to help induce osteogenesis [3,5,15,16,19]. IGF-1 is a mitogenic factor affecting the growth of adult cells as well as supporting the growth and differentiation of embryonic cells [17,18,20–24]. It has been used to stimulate osteoblast growth and proliferation, resulting in enhanced osseointegration at the local site [10,17].

Raiche et al. used two layers of glutaraldehyde cross-linked gelatin coatings with different concentrations of growth factors to control the release kinetics of BMP-2 and IGF-1 [14,18]. They found that the sequential release of BMP-2 and IGF-1 resulted in the earliest, most robust elevation of ALP activity of both mouse pluripotent C3H and rat bone marrow stromal cells and that the simultaneous release of BMP-2 and IGF-1 did not promote ALP activity compared with BMP-2 alone. They suggested that treatment with BMP-2 upregulated the expression of the IGF-I receptor, enabling IGF-I to further enhance cell responses [14]. Similarly, Chen et al. reported that the combined delivery of BMP-2 and IGF-1 resulted in the greatest ALP activity of periodontal ligament fibroblasts

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