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Targeted delivery system for juxtacrine signaling growth factor based on rhBMP-2-mediated carrier-protein conjugation

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Abstract

We propose a model of artificial juxtacrine signaling for the controlled release of recombinant human bone morphogenetic protein-2 (rhBMP-2) suitable for guided bone regeneration. A porous three-dimensional scaffold of poly-(lactide-co-glycolide) was fabricated by means of gel molding and particulate leaching. Collagen immobilization onto the scaffold surface was produced by performing photo-induced graft polymerization of acrylic acid, and rhBMP-2 was tethered to the collagenous surface by covalent conjugation. On pharmacokinetic analysis, in vitro enzyme-linked immunosorbent and alkaline phosphatase assays revealed sustained, slow release of rhBMP-2 over 28 days, with a cumulative release of one third of the initial load diffusing out of the scaffold. Conjugation of rhBMP-2 inhibited the free lateral diffusion and internalization of the activated complex of rhBMP-2 and the bone morphogenetic protein receptor. Osteoprogenitor cells were used as bone precursors to determine the expression of biosignaling growth factor in regulating cell proliferation and differentiation. To identify the phenotype of cells seeded on the rhBMP-2-conjugated scaffold, cellular activity was evaluated with scanning electron microscopy and with viability, histological, and immunohistochemical testing. The rhBMP-2-conjugated scaffold prolonged stimulation of intracellular signal proteins in cells. Enhancement of cell growth and differentiation was considered a consequence of juxtacrine signaling transduction. Animal studies of rhBMP-2-containing filling implants showed evidence of resorption and de novo bone formation. The present study revealed the potential of biomimetic constructs with co-immobilized adhesion and growth factors to induce osteoinduction and osteogenesis. Such constructs may be useful as synthetic bone-graft materials in orthopaedic tissue engineering.

Keywords: Conjugation; Growth factor; Juxtacrine; Osteoprogenitor; Tissue engineering

Introduction

Tissue-engineering approaches for osteoinductive bone-graft extenders rely on the stimulation of biosignaling proteins, such as cytokines or growth factors, to induce host–cell chemotaxis, proliferation, differentiation, and new-tissue formation at the site of bone deficiencies. Although many intercellular signals function as soluble proteins, certain growth factors act as ligands for cell receptors in membrane-bound forms, and their binding triggers an intracellular cascade of biochemical reactions that change cell behavior or function. Therefore, various protein-delivery systems have been developed in the field of tissue engineering [1,8,48]. Hubbell et al. has pursued schemes of growth factor delivery from biopolymeric cell ingrowth matrices that prevent diffusion of growth factor from the matrix but permits its sustained release under the control of matrix-degrading enzymes locally activated by cells [52,61,62].

Polymeric scaffolds serve a central role in the field of tissue engineering by directing cellular processes based on the structural and biochemical properties of the scaffold. The ability of scaffold to regulate cell behavior should be designed by mimicking the native extra cellular matrix and require control over surface chemistry and microstructure. To overcome this drawback of the synthetic materials, many different biologically functional molecules may be either physical adsorption or covalent attachment for surface engineering. These surfaces can be designed to present specific cell adhesion sequences at

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controlled densities [11], or can be functionalized with growth factor [41,51,62]. Importantly, the scaffold must provide the appropriate signals to direct cellular cellular processes that lead to tissue formation.

Previous investigators have generally believed that growth factors in solution stimulate cells only as diffusible proteins with autocrine, paracrine, or endocrine mechanisms to transduce signals for proliferation and differentiation. Massagué coined the term *juxtacrine signaling* to indicate the process of cellular communication in which both biosignaling molecules and receptors are anchored in the cell membrane, where they can deliver intercellular signals while supporting adhesive interactions between cells [43]. New stimulation mode, juxtacrine or matricrine stimulation has been reported to regulate cell functions. This mode is based on the discovery of membrane-anchored growth factor proteins, such as heparin-binding epidermal growth factor, tumor necrosis factor, and transforming growth factor-β [44]. In addition, some researchers have found that immobilized growth factors work by means of a juxtacrine or matricrine mechanism [18,29,30,33]. Ito et al. demonstrated that artificial juxtacrine stimulation by insulin immobilized on a solid matrix enhanced cell growth and showed mitogenic effects [28]. To visualize the effect of immobilized growth factors, micropatterns of growth factors have been immobilized on matrices [26]. Subsequently, several biosignal molecules were immobilized on various matrices and their biological activities were reported. It was shown that insulin and epidermal growth factor stimulated cell growth even after immobilization [5,25]. In other words, this type of stimulation by non-diffusional growth factors enabled us to regulate tissue formation with artificial biomaterials.

Bone morphogenetic protein (BMP)-induced signal transduction is an important positive regulator of osteoblastic growth and differentiation [7,31,58]. In experimental models, recombinant human BMP (rhBMP) and other members of the transforming growth factor-β superfamily possessed potent bone-forming activity [35,53,60]. Delivered in a suitable matrix, rhBMP-2 can potentially repair local skeletal defects by inducing new bone formation from undifferentiated pluripotent stem cells in host tissue [56]. Polymeric scaffolds fabricated by using various methods are suitable materials for bone repair because they act as temporary substrates for anchorage-dependent osteoblasts [24,39,42]. Collagen is natural candidates for the delivery of BMP since the major protein component of bone is type I collagen. Collagen has been used as a carrier for rhBMP-2 and BMP-7 in experimental systems [6,50]. For example, inactivated and demineralized bone matrix, mostly type I collagen, was used to deliver BMP-2-transfected bone-marrow cells [37,38]. Bonadio and Goldstein have reported success with collagen and DNA plasmids encoding human PTH1-34 and mouse BMP-4 [2,3,10]. Collagen sponges currently are being evaluated in both preclinical and preliminary clinical studies for rhBMP-2 delivery. Preliminary clinical results of rhBMP-2 delivered with absorbable collagen sponges for maxillary sinus floor augmentation have been presented [4]. Recently, biologically active rhBMP-2 has also been immobilized on succinylated type I atelocollagen, studies of alkaline phosphatase activity confirmed the effectiveness of rhBMP-2 immobilized on succinylated atelocollagen in augmenting cellular activity [12].

A biodegradable and porous framework that can localize and protect the payload, release that payload in a predictable and temporally controlled fashion, and deliver the signaling protein to the target is essential to express activity and to control the spatial configuration of the signaling-induced bone mass [16,17,32,34,49,55]. To this end, we designed a bioactive complex by developing a poly-(lactide-co-glycolide) (PLGA) matrix to which collagen was immobilized by grafting it with poly-(acrylic acid) and to which rhBMP-2 was tethered by covalent conjugation, as previously reported [36,46,54,59]. This architecture provided a delivery system offering prolonged retention, along with relevant growth-factor induction of signaling pathways in bone regeneration (Fig. 1). Such bioactive surfaces can influence cells and tissues by chemotactic as well as juxtacrine mechanisms. The purpose of this study was to examine the ex vivo potential of artificial juxtacrine-signaling therapy to provide alternate control of cell function. Osteoprogenitor cells (OPCs), which are pluripotent progenitor cells, were used as bone precursors to determine the expression of biosignaling growth factor in regulating cell proliferation and differentiation. Acellular scaffolds were also implanted into animals in vivo to allow cells to migrate onto the surface of the material and form new tissue.

Materials and methods

Fabrication of the scaffold and surface modification

A porous three-dimensional matrix was fabricated by mixing PLGA copolymer (75:25, inherent viscosity of 0.86 dl/g; Purac Biochem, Gorinchem,

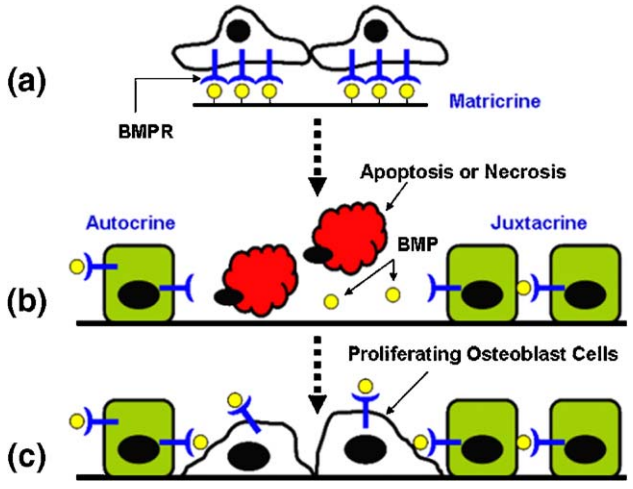

Fig. 1. Schematic diagram of stimulation modes of cell growth-factor proteins. (a) Proposed model of matricrine growth factor contribution to bone regeneration. BMP receptor (BMPR) and matrix-anchored growth factors are localized to the lateral membrane to maintain biological activity and direct differentiation without receptor internalization. (b) Injury of the cell layer by scratching it breaks the juxtacrine association between the ligand and the receptor in the disrupted area so soluble growth factors can diffuse and act on surrounding cells in the paracrine manner. (c) When the injured area is filled with matrix-anchored growth factors, signals are transmitted to neighboring cells in the juxtacrine manner to induce cell proliferation and migration.