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Research Paper

A collagen membrane containing osteogenic protein-1 facilitates bone regeneration in a rat mandibular bone defect

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ABSTRACT

Objectives: Osteogenic protein-1 (OP-1) has shown osteoinductive activities and is useful for clinical treatments, including bone regeneration. Regenerative procedures using a bioabsorbable collagen membrane (BCM) are well established in periodontal and implant dentistry. We evaluated the subsequent effects of the BCM in combination with OP-1 on bone regeneration in a rat mandibular circular critical-sized bone defect *in vivo*.

Design: We used 8 rats that received surgery in both sides of the mandible, and created the total 16 defects which were divided into 4 groups: Group 1; no treatment, as a control, Group 2; BCM alone, Group 3; BCM containing low dose 0.5 μg of OP-1 (L-OP-1), and Group 4; BCM containing high dose 2.0 μg of OP-1 (H-OP-1). Newly formed bone was evaluated by micro computed tomography (micro-CT) and histological analyses at 8 weeks postoperatively. In quantitative and qualitative micro-CT analyses of the volume of new bone formation, bone density, and percentage of new bone area was evaluated.

Results: BCM with rhOP-1 significantly increased and accelerated bone volume, bone mineral density, and percentage of new bone area compared to control and BCM alone at 8 weeks after surgery; these enhancements in bone regeneration in the OP-1-treated groups were dose-dependent.

Conclusions: OP-1 delivered with a BCM may have effective osteoinductive potency and be a good combination for bone regeneration. The use of such a combination device for osteogenesis may result in safer and more predictable bone regenerative outcomes in the future.

1. Introduction

Bioresorbable collagen membranes (BCMs) have been used widely to treat guided bone regeneration (GBR) and guided tissue regeneration (GTR) affectively for periodontal regeneration and improving bone defects for appropriate dental implant placement (Sheikh et al., 2017; Stoecklin-Wasmer et al., 2013). Various synthetic and natural bioresorbable barriers have been prepared and studied *in vitro* and *in vivo* (Bozkurt et al., 2014; Cui et al., 2014; Hunter & Ma, 2013; Sculean, Nikolidakis, & Schwartz, 2008). Collagen type I is one of the commonly used natural materials, whereas polyglycolic acid is a frequently used synthetic membrane that has the potential to be handled in periodontal therapy (Berahim, Moharamzadeh, Rawlinson, & Jowett, 2011). Such materials must provide biocompatibility, tissue integration, cell occlusivity, space-making ability, and clinical ease of use (Gottlow, 1993). According to previous reports, in connection with the use of BCMs in clinical cases for dentistry and in various animal studies, favorable results have been achieved (Locci et al., 1997; Mattson, Gallagher, & Jabro, 1999; Oh, Meraw, Lee, Giannobile, & Wang, 2003). This is because collagen is reportedly superior to other materials, as it

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plays an active role in coagulum formation, is chemotactic for periodontal ligament fibroblasts and gingival fibroblasts, and is a major constituent of periodontal connective tissue (Behring, Junker, Walboomers, Chessnut, & Jansen, 2008; Piao et al., 2014). Also, collagen is particularly well-suited as a barrier membrane because it is a normal component of the extracellular matrix (Behring et al., 2008) and serves as a fibrillar scaffold for early vascular and tissue ingrowth (Patino, Neiders, Andreana, Noble, & Cohen, 2002).

A synergistic approach to membrane techniques is the use of growth factors that promote the regeneration of selective tissues. Of particular relevance is the use of bone morphogenic proteins (BMPs) that comprise the largest TGF-β subfamily and belong to a group of non-collagen proteins (Mikulski & Urist, 1977). They are responsible for several biological activities involved in tissue morphogenesis, regeneration, healing, and cell differentiation processes (Al-Salleeh, Beatty, Reinhardt, Petro, & Crouch, 2008; Koch, Becker, Terheyden, Capsius, & Wagner, 2010; Leknes et al., 2008). In particular, they are known for their ability to induce osteogenesis, and are also involved in embryonic development and fracture healing (Kroczek et al., 2010; Zhong et al., 2011).

Recombinant human BMP-2 and BMP-7 have been approved for clinical use in the regeneration of bone in fracture healing and spine fusion (Grauer et al., 2004; Laursen et al., 1999). BMP-7, also known as osteogenic protein-1 (OP-1), has shown multiple biological activities in different cell types and strong anabolic activity in both bone and cartilage *in vitro* (Chubinskaya & Kuettner, 2003). Previous studies showed that the effects of OP-1 on cell proliferation depend on cell types and culture conditions. Moreover, it has been shown that OP-1 stimulated both alkaline phosphatase (ALP) activity and osteocalcin production by osteoblast-like MC3T3-E1 cells, in a dose-dependent manner (Asahina, Sampath, & Hauschka, 1996), and that OP-1 not only stimulated the maturation of committed osteoblast progenitors but also induced the commitment of undifferentiated non-osteogenic cells into osteoblasts (Tou, Quibria, & Alexander, 2003).

Many *in vivo* studies have used OP-1 with biocompatible and/or biodegradable polymers, collagenous carriers and scaffolds, and nanoparticles on the basis that its osteoinductive potential can drive enhanced bone defect regeneration (Kidder, Chen, Schmidt, & Lew, 2009; Lee et al., 2013). In clinical dentistry, hydroxyapatite (HA)-coated rhOP-1 accelerated the healing of fresh dental extraction defects and encouraged the osseointegration of dental implants with good initial stability. Application of rhOP-1 produced a larger amount, density, and degree of bone remodeling *versus* untreated defect sites (Cook, Salkeld, & Rueger, 1995). The use of rhOP-1 combined with a collagen carrier of bovine origin has the potential to induce bone formation in the human maxillary sinus after sinus floor elevation procedure (Groenveld et al., 1999).

Recently, the ability of a BCM to successfully deliver sustained release of platelet-derived growth factor (PDGF), and subsequent osteogenic effects of the released factor on rat mandibular defects *in vivo*, were reported (Yamano, Lin, Dai, Fabella, & Moursi, 2011). Furthermore, it was demonstrated that a BCM containing growth/differentiation factor 5, which regulates cell growth and differentiation in both embryonic and adult tissues and plays a role in skeletal development, accelerated bone regeneration significantly *in vivo*, compared with a BCM containing PDGF (Yamano et al., 2014). We also reported the subsequent osteogenic effects of the BCM carrying released factor, stromal-derived factor-1, on rat mandibular bone defects *in vivo* (Takayama et al., 2017).

In an attempt to further improve clinical outcomes, such as healing speed, quantity, and quality of newly formed bone, and to search for new candidate growth factors for an effective method of bone regeneration, we devised a new regeneration device that is a combination of a BCM containing OP-1 (BCM/OP-1) for bone regeneration. However, the exact properties by which alveolar osteoblasts react to barrier membranes as well as the effects following addition of growth factors to the membranes are still poorly understood. No data are available on the application of BCM/OP-1 for circular critically sized bone defects *in vivo*. Accordingly, the main purpose of this study was to evaluate the effects of BCM/OP-1 on bone healing in a rat mandibular bone defect model *in vivo*.

2. Materials and methods

2.1. Preparation of BCMs containing OP-1

A commercially available BCM, BioMend (cross-linked bovine type I collagen; Calcitek, Carlsbad, CA, USA), was prepared for *in vitro* or *in vivo* studies, in sizes of 5 mm × 7.5 mm and 5 mm × 15 mm, respectively. Recombinant human OP-1 (rhOP-1) solution, carrier-free (R & D Systems, Minneapolis, MN, USA), was applied to the BCMs. OP-1 was impregnated in the BCMs just before the experiment. The amount of rhOP-1 applied to each BCM for each experiment was as follows: (1) enzyme-linked immunosorbent assay (ELISA): 0.5 or 2.0 μg of rhOP-1, (2) MTT assay: 50, 100, or 200 ng of rhOP-1, (3) *in vivo* study: 0.5 μg (low concentration, L) or 2.0 μg (high concentration, H) of rhOP-1.

2.2. ELISA for release kinetics of rhOP-1 from BCMs

In vitro release kinetics of OP-1 from the BCM were determined over the course of 2 weeks. *In vitro* release of OP-1 from the BCM was examined at 37 °C in 2 mL of PBS. At pre-determined intervals, the release medium was withdrawn and renewed with fresh medium each time. Thus, samples of 2 mL PBS containing OP-1 at 1, 3, 5, 10, and 14 days were collected and replaced with an equal amount of fresh PBS. Samples were stored at −20 °C until testing. All collected samples were centrifuged and filtered to remove free-floating impurities and analyzed quantitatively using a Human OP-1 ELISA kit (R & D Systems, Minneapolis, MN, USA) at the end of the experiment (Wang et al., 2016). Data show the results of a single experiment (n = 3) that was conducted twice.

2.3. Cell culture

Murine calvaria osteoblasts MC3T3-E1 were obtained from Riken Bio Resource Center (Tsukuba, Japan) and cultured in α-minimal essential medium (α-MEM; GIBCO BRL, Rockville, MD, USA) supplemented with 10% (v/v) fetal bovine serum HyClone Laboratories, Logan, UT, USA) and 1% (v/v) penicillin-streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.4. The effect of BCM/OP-1 on the cell viability of MC3T3-E1 cells

Cell viability was determined *via* reduction of 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) by NAD-dependent dehydrogenase activity to form a colored reaction product. MC3T3-E1 cells (5×10^3 cells/well) in 80 μL of α-MEM supplemented 10% FBS were seeded in a clear bottom 96-well tissue culture plates and incubated overnight. Briefly, 15 μL MTT regent (Cell Viability Assay Kit; BioAssay Systems, Hayward, CA, USA) were added into each well and the plates were incubated for 4 h at 37 °C. After solutions were removed, dimethyl sulfoxide (100 μL/well) was added to dissolve formazan products, and the plates were shaken for 1 h at room temperature. The absorbance of each well was recorded on a microplate spectrophotometer at 570 nm to index cell viability. The percentage of cell viability was calculated by comparing the appropriate optimal density to the control cells.

2.5. Animals

In total, eight 10-week-old male Fischer 344jcl rats, weighing