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Segmental bone regeneration using a load-bearing biodegradable carrier of bone morphogenetic protein-2

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Abstract

Segmental defect regeneration has been a clinical challenge. Current tissue-engineering approach using porous biodegradable scaffolds to delivery osteogenic cells and growth factors demonstrated success in facilitating bone regeneration in these cases. However, due to the lack of mechanical property, the porous scaffolds were evaluated in non-load bearing area or were stabilized with stress-shielding devices (bone plate or external fixation). In this paper, we tested a scaffold that does not require a bone plate because it has sufficient biomechanical strength. The tube-shaped scaffolds were manufactured from poly(propylene) fumarate/tricalcium phosphate (PPF/TCP) composites. Dicalcium phosphate dehydrate (DCPD) were used as bone morphogenetic protein-2 (BMP-2) carrier. Twenty-two scaffolds were implanted in 5 mm segmental defects in rat femurs stabilized with K-wire for 6 and 15 weeks with and without 10 μg of rhBMP-2. Bridging of the segmental defect was evaluated first radiographically and was confirmed by histology and micro-computer tomography (μCT) imaging. The scaffolds in the BMP group maintained the bone length throughout the duration of the study and allow for bridging. The scaffolds in the control group failed to induce bridging and collapsed at 15 weeks. Peripheral computed tomography (pQCT) showed that BMP-2 does not increase the bone mineral density in the callus. Finally, the scaffold in BMP group was found to restore the mechanical property of the rat femur after 15 weeks. Our results demonstrated that the load-bearing BMP-2 scaffold can maintain bone length and allow successfully regeneration in segmental defects.

Keywords: Bone morphogenetic protein (BMP); Bone regeneration; Calcium phosphate cement; Bone tissue engineering; Free form fabrication

1. Introduction

Segmental bone defects resulting from trauma or pathology represent a common and significant clinical problem. Limb amputation was historically the principal treatment option for these defects as they typically do not heal spontaneously [1]. With advances in medicine and science, alternative treatment options have developed such as the use of bone-grafting techniques. Autologous bone grafts are preferred as they possess inherent osteoconductivity, osteogenicity and osteoinductivity. However, there is often limited supply of suitable bone for autologous grafting, and its collection is frequently associated with donor-site morbidity. An alternative is to use allogeneic bone grafts from donors or cadavers. These circumvent some of the limitations associated with harvesting autologous grafts, but allogeneic bone grafts lack osteogenicity, have limited osteoinductivity and present a risk of disease transmission. These limitations necessitate the pursuit of alternatives for the management of segmental bone defects, with the latest approach being to use tissue-engineering techniques.

Tissue engineering for bone typically involves coupling osteogenic cells and/or osteoinductive growth factors with osteoconductive scaffolds [2,3] In terms of osteoinductive growth factors, most research has focused on the use of the bone morphogenic proteins (BMPs) and, in particular,

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BMP-2 [1,4–8]. BMP-2 is a bone matrix protein that stimulates mesenchymal cell chemotaxis and proliferation, and promotes the differentiation of these cells into chondrocytes and osteoblasts [6,8]. These cellular effects bestow BMP-2 potent osteoinductive capabilities, which are primarily evident by the induction of new bone formation via a process of endochondral ossification when implanted at ectopic sites [9,10]. This osteoinductive action of BMP-2 is well established to be beneficial during the repair of fractures and segmental bone defects [1,5,7,8].

BMP-2 induces bone regeneration following injury and has been approved for limited clinical use in the form of recombinant human BMP-2 (rhBMP-2) [5]. However, rhBMP application has been limited by ongoing delivery issues. To facilitate retention of rhBMP-2 at the treatment site and reduce the effective dose, an appropriate carrier is required [9]. The preferred carrier consists of a scaffold that is both biocompatible and bioresorbable in order to limit tissue rejection and exposure to the scaffold material, respectively [11]. While numerous scaffolds have been manufactured that meet these requirements [12] many lack the ability to tolerate appreciable loads. This is of importance as segmental defects frequently occur in load-bearing bones. Scaffolds need to be able to tolerate loading so that patient morbidity is minimized during reparation and the structure of the engineered bone is optimized to the local mechanical environment. Few load-bearing scaffolds have been described in the literature, with many studies of tissue engineered bone regeneration with BMP-2 being conducted at non-load-bearing sites [13–16] or in defects stabilized with stress-shielding devices (bone plates or external fixation) [17–20].

In the current paper, we present a tissue-engineering strategy for bone regeneration using rhBMP-2 carried by a novel load-bearing biodegradable scaffold. Tube-shaped scaffolds were fabricated from a high strength biodegradable composite and calcium phosphate cement, and implanted into critical-sized defects in an established rodent model [21]. Defects and scaffolds were stabilized with a load-sharing device (intramedullary pin). The aim was to investigate the effect of our novel load-bearing scaffold carrying rhBMP-2 on segmental defect repair in the rat femur.

2. Materials and methods

2.1. Animals

Twenty-two adult male Long-Evans rats (weight = 450–550 g) were purchased from Charles River Laboratory (Wilmington, MA) and acclimatized for a minimum of 1 week prior to experimentation. Animals had ad libitum access to standard rat chow and water at all times, and all procedures were performed with prior approval of the Institutional Animal Care and Use Committee of Indiana University.

2.2. Scaffold manufacture

Polypropylene fumarate (PPF) with a molecular weight of 1750 g/mol and PI = 1.5 was obtained from Prof. Antonios Mikos (Rice University, Houston, TX). A thermal-curable PPF/tricalcium phosphate (TCP) suspension was prepared by mixing PPF, *N*-vinyl pyroolidinone (NVP), and TCP at a weight ratio of 1:0.75:0.66 [22]. Tube-shaped structures (outer diameter = 4 mm, inner diameter = 2 mm, height = 5 mm, with four side holes of 800 μm diameter) were created by the indirect casting technique developed by Chu et al. [23,24]. Briefly, a scaffold design was generated using commercial Computer-Aided-Design software and a negative model obtained by using Boolean computer operation. Wax casting-molds were fabricated on a 3-D Inkjet Printing Machine (T66, Solidscape Inc. NH) according to the model design. The PPF/TCP slurry was combined with 0.5% benzoyl peroxide (thermal initiator) and 10 μl of dimethyl *p*-toluidine (accelerator), and cast into the wax mold. Following polymerization, the wax mold was removed by acetone to reveal the scaffold. rhBMP-2 was aseptically added to half of the scaffolds prior to surgery by adding 10 μg of rhBMP-2 (Wyeth, Cambridge, MA) to porous dicalcium phosphate dihydrate (DCPD) cement previously packed into the side holes of the scaffold (BMP-2 group). In the remaining scaffolds, DCPD without rhBMP-2 was added to the side holes (control group).

2.3. Segmental defect induction and surgical implantation of the scaffolds

All animals underwent surgery to create a unilateral midshaft femur segmental defect into which either a rhBMP-containing scaffold (BMP group) or control scaffold (control group) was implanted. A non-scaffold control group was not used in this study since the non-healing nature of 5 mm segmental defects in the rat femur is well established [25,26]. Following a pre-operative subcutaneous dose of buprenorphine hydrochloride analgesia (0.05 mg/kg; Buprenex®—Reckitt Benckiser Pharmaceuticals Ltd., Inc., Richmond, VA), surgical anesthesia was achieved using a mixture of ketamine (60–80 mg/kg; Ketaset®—Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (7.5 mg/kg; Sedazine®—Fort Dodge Animal Health, Fort Dodge, IA) introduced intraperitoneally. The fur was clipped and cleaned using alternating chlorhexidine and 70% ethanol scrubs. Using a sterile technique, a 30-mm longitudinal incision was made over the lateral thigh, beginning just distal to the lateral knee joint and extending proximally. The intermuscular septum between the vastus lateralis and hamstring muscles was divided using blunt dissection to localize the femur. The lateral structures stabilizing the patella were divided and the patella manually dislocated medially. A 5 mm segment of the midshaft femur was removed following two parallel osteotomies under irrigation using a Dremel drill (Robert Bosch Tool Corporation, Mount Prospect, IL) with attached diamond-embedded wafer blade (Super Flex Diamond Disc, Miltex Inc, York, PA). To stabilize the fracture, a 1.25 mm diameter stainless steel K-wire (Synthes Inc, West Chester, PA) was inserted retrograde into the distal intramedullary canal, beginning in the knee between the femoral condyles. The wire was advanced to the segment defect and a scaffold centered over the tip. The wire passed through the central canal of the scaffold and was further advanced in a retrograde fashion into the proximal intramedullary canal and through the greater trochanter (Fig. 1). The distal tip of the wire was cut flush with the femoral condyles. After thorough irrigation, the patella was relocated and stabilized with an absorbable suture, and the muscle and skin layers closed and sutured.

2.4. Radiographic analysis

In vivo X-rays were taken of eight rats ($n = 4$/group) at 1, 3, 6, 12 and 15-weeks post-operatively using a portable X-ray machine (AMX-110, GE Corp, Waukesha, WI). The rats were anesthetized using isoflurane (Abbott Laboratories, North Chicago, IL) and placed prone on an X-ray film cassette 29 inches beneath the X-ray source. Exposure was at 60 kVp for 2.5 mAs. All films were evaluated in a blinded fashion by three independent evaluators using a three-point radiographic scoring system (0 = no callus formation; 1 = possible union across the gap; 2 = complete callus bridging across the gap).