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Dual growth factor delivery from bilayered, biodegradable hydrogel composites for spatially-guided osteochondral tissue repair

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ABSTRACT

The present work investigated the use of biodegradable hydrogel composite scaffolds, based on the macromer oligo(poly(ethylene glycol) fumarate) (OPF), to deliver growth factors for the repair of osteochondral tissue in a rabbit model. In particular, bilayered OPF composites were used to mimic the structural layers of the osteochondral unit, and insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein-2 (BMP-2) were loaded into gelatin microparticles and embedded within the OPF hydrogel matrix in a spatially controlled manner. Three different scaffold formulations were implanted in a medial femoral condyle osteochondral defect: 1) IGF-1 in the chondral layer, 2) BMP-2 in the subchondral layer, and 3) IGF-1 and BMP-2 in their respective separate layers. The quantity and quality of osteochondral repair was evaluated at 6 and 12 weeks with histological scoring and micro-computed tomography (micro-CT). While histological scoring results at 6 weeks showed no differences between experimental groups, micro-CT analysis revealed that the delivery of BMP-2 alone increased the number of bony trabecular islets formed, an indication of early bone formation, over that of IGF-1 delivery alone. At 12 weeks post-implantation, minimal differences were detected between the three groups for cartilage repair. However, the dual delivery of IGF-1 and BMP-2 had a higher proportion of subchondral bone repair, greater bone growth at the defect margins, and lower bone specific surface than the single delivery of IGF-1. These results suggest that the delivery of BMP-2 enhances subchondral bone formation and that, while the dual delivery of IGF-1 and BMP-2 in separate layers does not improve cartilage repair under the conditions studied, they may synergistically enhance the degree of subchondral bone formation. Overall, bilayered OPF hydrogel composites demonstrate potential as spatially-guided, multiple growth factor release vehicles for osteochondral tissue repair.

1. Introduction

The osteochondral unit is a multiphasic tissue comprised of two main tissue types: the articulating cartilage responsible for nearly frictionless movement and shock-absorbance, and the subchondral bone indispensible for underlying mechanical support [1–3]. Within the knee, the health of cartilage tissue has been linked to the maintenance of the natural biomechanics of the subchondral bone, and given the contact between cartilage and bone, total joint homeostasis will ultimately rely on the functional restoration of both tissues [4–7]. As a result, tissue engineering strategies to regenerate the osteochondral unit have evolved over the years from monolithic structures to gradient/bilayered scaffolds as a means of controlling treatment to repair the cartilage–bone interface or to target each tissue separately [8–10]. In addition to selecting an appropriate scaffold design, successful regeneration of osteochondral tissue is greatly influenced by the microenvironment in which cells and tissues grow [11]. A number of soluble cues have been implicated in the osteochondral wound healing environment [10], and the goal of eliciting the desired phenotypic response from co-delivered and/or host progenitor cells *in vivo* remains a difficult

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challenge. Due to the numerous signaling pathways involved in osteochondral repair, the delivery of multiple growth factors to improve therapeutic potency is an area of great interest, particularly when both cartilage and bone tissues are considered [12].

The present work seeks to repair osteochondral tissue through the dual delivery of growth factors from acellular, bilayered hydrogel composites. These hydrogel composites are fabricated from a synthetic macromer, oligo(poly(ethylene glycol) fumarate) (OPF), which is synthesized by the esterification of poly(ethylene glycol) (PEG) and fumaryl chloride. The resulting macromer can be crosslinked via the double bonds of the fumarate group to form a hydrogel as well as degrade through hydrolysis of the ester groups [13,14]. Gelatin microparticles (GMPs) are also embedded within the crosslinked hydrogel matrix to act as both an enzymatically digestible porogen and a delivery vehicle for growth factors [15–17]. These OPF-based hydrogel composites have been used in a number of *in vitro* and *in vivo* studies as growth factor delivery vehicles, and allow for comparisons between growth factor types and delivery methods for cartilage and osteochondral tissue repair applications [15–20]. In an effort to mimic the osteochondral unit, these hydrogels are laminated to form two distinct layers as a means of tailoring scaffold composition and drug loading to the cartilage and bone areas of the defect [18,21,22]. With this design, the current study incorporates a chondroinductive factor and an osteoinductive factor into the bilayered hydrogels in separate layers to facilitate simultaneous cartilage and subchondral tissue repair within an osteochondral defect.

The strategy of spatially incorporated biologics for simultaneous *in vivo* cartilage and bone repair is a growing area of research: Chen et al. delivered plasmids encoding for transforming growth factor-β1 (TGF-β1) and bone morphogenetic protein-2 (BMP-2) in separate layers of a chitosan-gelatin scaffold [23], Re'em et al. spatially presented TGF-β1 and BMP-4 in an alginate-sulfate hydrogel [24], and Mohan et al. and Dormer et al. created sintered poly(D,L-lactic-co-glycolic acid) microsphere scaffolds with reverse gradients of TGF-β1 and BMP-2 [25,26]. However, an optimal combination of chondrogenic and osteogenic growth factors has yet to be achieved for osteochondral regeneration.

As a result, the main hypothesis of this study is that insulin-like growth factor-1 (IGF-1) delivered from the chondral layer and BMP-2 delivered from the subchondral layer of bilayered OPF hydrogel composites can act synergistically to promote osteochondral tissue repair over the delivery of either growth factor alone. Additionally, it is hypothesized that earlier subchondral bone repair, stimulated by the release of BMP-2, will affect the degree of cartilage repair. IGF-1 is the main anabolic growth factor in articular cartilage and plays a key role in homeostasis by balancing synthesis and break down of proteoglycans by chondrocytes [27]. Additionally, previous studies have demonstrated similar or improved cartilage repair with the single delivery of IGF-1 over the dual delivery of IGF-1 with TGF-β1 or TGF-β3 from bilayered OPF composites in a medial femoral condyle osteochondral defect model [19,20]. BMP-2 is a potent osteogenic growth factor for inducing *de novo* bone formation in ectopic and orthotopic sites and has been shown to improve subchondral bone formation when delivered to osteochondral defects [28–34]. Additionally, BMP-2 is expressed throughout the entire chondrogenic process of mesenchymal stem cells (MSCs), from condensation to proliferation, differentiation, maturation, and calcification [10]. With the ability to induce the growth of both cartilage and bone tissue, several research groups have delivered BMP-2 from monolithic scaffolds to osteochondral defects and have improved cartilage repair over blank scaffolds [32,34,35]. However, the current study isolates the incorporation of BMP-2 to the subchondral layer to maximize its osteoinductive potential and reduce its direct morphogenetic effects on progenitor cell chondrogenesis in the chondral layer.

To test the two hypotheses aforementioned, the current study 1) investigates the repair of cartilage and subchondral bone tissue in an osteochondral defect facilitated by bilayered hydrogel composites delivering IGF-1 and/or BMP-2 incorporated in a spatially controlled manner, 2) evaluates the degree of subchondral bone repair with micro-computed tomography (micro-CT) and osteochondral tissue repair through histological scoring, and 3) compares the extent of cartilage and subchondral bone repair at 6 week and 12 week time points.

2. Materials and methods

2.1. Oligo(poly(ethylene glycol) fumarate) synthesis

OPF was synthesized from PEG (Sigma–Aldrich, St. Louis, MO) with a nominal molecular weight (MW) of 35,000 according to methods previously developed in our laboratory [13,14]. Gel permeation chromatography was used to characterize the OPF macromer to give a number average molecular weight of 50,400 ± 900 Da and a weight average molecular weight of 127,400 ± 3200 Da. Prior to the fabrication of hydrogel implants, OPF was sterilized for 12 h by exposure to ethylene oxide (EO) according to established protocols [20,36].

2.2. Gelatin microparticle fabrication and growth factor loading

Using established methods, GMPs were fabricated using acidic gelatin (Nitta Gelatin, Osaka, Japan) with an isoelectric point (IEP) of 5.0 and crosslinked in 40 mM glutaraldehyde (Sigma–Aldrich, St. Louis, MO), followed by glycine quenching of unreacted glutaraldehyde [16,37]. Following lyophilization, GMPs of 50–100 μm in diameter were obtained through sieving and EO sterilized for 12 h prior to GF loading. GMPs were then partially swelled with either phosphate buffered saline (PBS) or a GF solution at a ratio of 5 μL solution to 1 mg dried GMP for 15 h at 4 °C before hydrogel encapsulation according to previously described methods [15,17]. In particular, 225 μL of PBS, IGF-1 solution, or BMP-2 solution was combined with 45 mg of GMPs. Recombinant human IGF-1 (Peprotech, Rocky Hill, NJ) was reconstituted with PBS to a GF loading concentration of 7.95 μg per mL solution and loaded into GMPs to give a total of 5.6 ng IGF-1 per hydrogel construct. Recombinant human BMP-2 (Peprotech, Rocky Hill, NJ) was reconstituted with a mixture of ddH_2O and PBS to a GF loading concentration of 712 μg per mL solution to give a total of 1 μg BMP-2 per hydrogel construct.

2.3. Bilayered hydrogel composite fabrication

Bilayered hydrogel composites were fabricated via a two-step crosslinking procedure similar to methods previously described [19,20]. Using a 2 mm × 2 mm cylindrical teflon mold, the subchondral layer was first partially crosslinked, then the chondral layer was crosslinked on top of the subchondral layer. Specifically, to prepare the OPF precursor solution, 113 mg of OPF and 37 mg of PEGDA (3400 Da MW, Laysan Bio, Arab, AL) were dissolved in 353 μL of PBS and set at room temperature for 45 min to eliminate air bubbles. 46.8 μL each of the thermal radical initiators, 0.3 M ammonium persulfate (APS, Sigma–Aldrich, St. Louis, MO) and 0.3 M N,N,N',N'-tetramethylethylenediamine (TEMED, Sigma–Aldrich, St. Louis, MO), were then mixed into the polymer solution. The addition of APS and TEMED at the concentrations used are expected to have minimal toxic effects on the surrounding tissue post-implantation, particularly in consideration of previous studies showing high viability of encapsulated cells in OPF-based hydrogels following the fabrication process [22,38,39]. Lastly, 45 mg of GMPs swollen with either 225 μL of PBS or a GF solution were added to the solution and mixed carefully to avoid the formation of bubbles. The polymer-GMP mixture was then injected into the bottom two-thirds of the teflon mold for the subchondral layer and partially crosslinked at 37 °C for 5 min. A similar polymer-GMP mixture was then prepared for the chondral layer and injected on the subchondral layer and crosslinked at 37 °C for 25 min. Table 1 highlights the three groups used in the study with BMP-2 delivered from the subchondral layer and IGF-1 delivered from the chondral layer of the bilayered hydrogel composites. Prior to implantation in an osteochondral defect, hydrogels were transferred with a drop of sterile PBS to prevent the hydrogel composites from

Table 1
Experimental groups investigated in this study. Growth factors were loaded into gelatin microparticles prior to encapsulation within bilayered OPF hydrogels.

Experimental groups	IGF-1	BMP-2	Both
Chondral layer (top 1 mm)	IGF-1	Blank	IGF-1
Subchondral layer (bottom 2 mm)	Blank	BMP-2	BMP-2
6 Week repetitions	12	10	12
12 Week repetitions	12	11	12