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Osteochondral defect repair using bilayered hydrogels encapsulating both chondrogenically and osteogenically pre-differentiated mesenchymal stem cells in a rabbit model

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SUMMARY

Objective: To investigate the ability of cell-laden bilayered hydrogels encapsulating chondrogenically and osteogenically (OS) pre-differentiated mesenchymal stem cells (MSCs) to effect osteochondral defect repair in a rabbit model. By varying the period of chondrogenic pre-differentiation from 7 (CG7) to 14 days (CG14), the effect of chondrogenic differentiation stage on osteochondral tissue repair was also investigated.

Methods: Rabbit MSCs were subjected to either chondrogenic or osteogenic pre-differentiation, encapsulated within respective chondral/subchondral layers of a bilayered hydrogel construct, and then implanted into femoral condyle osteochondral defects. Rabbits were randomized into one of four groups (MSC/MSC, MSC/OS, CG7/OS, and CG14/OS; chondral/subchondral) and received two similar constructs bilaterally. Defects were evaluated after 12 weeks.

Results: All groups exhibited similar overall neo-tissue filling. The delivery of OS cells when compared to undifferentiated MSCs in the subchondral construct layer resulted in improvements in neo-cartilage thickness and regularity. However, the addition of CG cells in the chondral layer, with OS cells in the subchondral layer, did not augment tissue repair as influenced by the latter when compared to the control. Instead, CG7/OS implants resulted in more irregular neo-tissue surfaces when compared to MSC/OS implants. Notably, the delivery of CG7 cells, when compared to CG14 cells, with OS cells stimulated morphologically superior cartilage repair. However, neither osteogenic nor chondrogenic pre-differentiation affected detectable changes in subchondral tissue repair.

Conclusions: Cartilage regeneration in osteochondral defects can be enhanced by MSCs that are chondrogenically and osteogenically pre-differentiated prior to implantation. Longer chondrogenic pre-differentiation periods, however, lead to diminished cartilage repair.

Introduction

Articular cartilage is a well-studied flexible connective tissue that facilitates the tribological interaction of bones in major diarthrodial joints throughout the body. Despite its seemingly simple structure, the avascular nature of cartilage compromises its endogenous capacity for repair, leading to a high incidence of unresolved cartilage-related injuries[1,2]. Given the lack of a surgical

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cure, such cartilage-related injuries still present a substantial economic burden on society[3]. However, significant research advances over the years have allowed for the sophistication of conventional reparative clinical procedures such as marrow stimulation techniques, leading to measurable improvement in patient outcomes[4–7]. Yet, these techniques are contraindicated for larger critical sized lesions, where cell-based regenerative procedures including autologous chondrocyte transplantation (ACT) or matrix-assisted ACT often prove more effective[3,8]. Nevertheless, modern generation ACT techniques are still unable to obviate significant clinical hurdles involving joint arthrofibrosis, limited donor chondrocyte availability and donor site morbidity.

Articular chondrocytes, being the constituent cell type of native articular cartilage, still represent the standard cell source considered for cell-based cartilage therapies. However, given limited donor supply, mesenchymal stem cells (MSCs) are becoming increasingly coveted as an alternative cell source. Their phenotypic plasticity and renewability make MSCs ideal candidates for the development of new therapies for osteochondral tissue repair. Indeed, the delivery of undifferentiated MSCs to osteochondral defect sites generally confers some therapeutic value[9–16]. However, inconsistencies in efficacy challenge such undirected approaches as viable clinical treatment options[17–20], and suggest that ideal conditions for unlocking the full healing potential of MSCs still remain largely unknown.

Emerging cell-based strategies for osteochondral tissue regeneration are increasingly recognizing the importance of cellular differentiation state and its influence on treatment outcomes. In particular, it was recently shown that MSCs chondrogenically pre-differentiated with transforming growth factor-β3 (TGF-β3) for 14 days outperformed undifferentiated MSCs and even articular chondrocytes when implanted with a collagen scaffold into an ovine chronic defect model[21]. However, other efforts aiming to leverage the curative properties of chondrogenically pre-differentiated MSCs failed to elicit improved cartilage tissue repair over undifferentiated MSCs[22,23], indicating that the strategy for pre-differentiation still requires extensive optimization. Toward this effort, our laboratory recently evaluated the chondrogenic and osteogenic capacity of bilayered cell-laden constructs developed using MSCs that have undergone various degrees of pre-differentiation[24]. It was found that MSCs subjected to shorter chondrogenic pre-differentiation periods, when co-cultured with osteogenically pre-differentiated cells, exhibited greater chondrogenic potential as indicated by higher glycosaminoglycan (GAG)-to-collagen synthetic ratios *in vitro*[24].

Using a similar oligo(poly(ethylene glycol) fumarate) (OPF)-based hydrogel system[25,26], the present study investigates the ability of these cell-laden constructs to effect osteochondral tissue regeneration *in vivo*. Accordingly, we hypothesized that the delivery of osteogenically pre-differentiated cells in a spatially controlled manner within the subchondral layer of a single bilayered construct would elicit improved histological tissue repair when compared to scaffolds containing only undifferentiated MSCs. It is also hypothesized that the prior chondrogenic pre-differentiation of MSCs encapsulated within the chondral layer with OS cells in the subchondral layer would further enhance osteochondral repair depending on the degree of chondrogenic pre-differentiation. Specifically, the objectives of the study were (1) to measure the effect of osteogenic pre-differentiation of cells in the subchondral layer, (2) to evaluate the additional effects of chondrogenic pre-differentiation of cells in the chondral layer, and (3) to assess the influence of chondrogenic pre-differentiation duration of the cells in the chondral layer on key histological markers of osteochondral tissue repair *in vivo* using a rabbit defect model.

Materials and methods

Experimental design

As outlined in Table I, four experimental groups were designed to address the objectives of this study. Briefly, undifferentiated MSCs or MSCs chondrogenically pre-differentiated for 7 (CG7) or 14 days (CG14) were encapsulated with osteogenically pre-differentiated MSCs (OS cells) within respective chondral and subchondral hydrogel layers of the bilayered hydrogel system. The MSC/MSC group was utilized as the experimental control.

OPF synthesis and characterization

OPF macromers were synthesized using poly(ethylene glycol) (PEG) with a nominal molecular weight of 35,000 g/mol (Sigma–Aldrich, St. Louis, MO) following previously established methods[25]. The synthesized OPF was characterized using gel permeation chromatography and stored at −20°C under N_2(g) until use. Prior to use, the polymer was sterilized by ethylene oxide (EO) exposure for 12 h following established procedures[27].

Gelatin microparticle (GMP) fabrication

GMPs were fabricated using acidic gelatin of a 5.0 isoelectric point (Nitta Gelatin INC., Osaka, Japan) following well-established methods[28]. Prior to hydrogel encapsulation, sterilized GMPs of 50–100 μm in diameter were swollen in phosphate buffered saline (PBS) (55 μL of PBS per 11 mg of dried GMPs) to achieve swelling according to previously established procedures[29]. GMPs were incorporated into hydrogels to provide moieties for cell–material interactions and to aid hydrogel degradation[30].

Rabbit marrow MSC isolation and culture

All experimental and surgical protocols for this study were reviewed and approved by the Rice University and The University of Texas Health Science Center Institutional Animal Care and Use Committees (IACUC), and performed according to the National Institutes of Health animal care and use guidelines. Rabbit bone marrow-derived MSCs were harvested from the tibiae of six 6-month old New Zealand white rabbits as previously described[30]. The isolated bone marrow was cultured in general medium (GM) containing low glucose Dulbecco's modified Eagle's medium (LG-DMEM), 10% v/v fetal bovine serum (FBS), and 1% v/v penicillin/streptomycin/fungizone (PSF) for 2 weeks. The rabbit marrow-derived MSCs were then pooled to minimize interanimal variation and cryopreserved until use as previously described[24].

Pre-differentiation of MSCs

Cryopreserved cells were thawed and expanded in monolayer (3,500 cells/cm^2) in GM before pre-differentiation. The various cell populations used in this study were derived according to a recent study from our laboratory[24]. Accordingly, in order to generate cell populations at varying stages of chondrogenic pre-differentiation, MSCs were first expanded for 2 weeks and then subjected to either 7 (CG7) or 14 (CG14) days of pre-differentiation in serum-free chondrogenic media containing LG-DMEM, ITS + Premix (6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 μg/mL selenious acid, 5.35 μg/mL linoleic acid, 1.25 μg/mL bovine serum albumin) (BD Biosciences, San Jose, CA), 50 mg/L ascorbic acid, 10^{-7} M dexamethasone, 10 ng/mL TGF-β3 (PeproTech, Rocky Hill, NJ), and 1% v/v PSF. It was shown previously that 7 and 14 days of chondrogenic pre-differentiation using this method led to the