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Chondrogenesis of human bone marrow mesenchymal stem cells in 3-dimensional, photocrosslinked hydrogel constructs: Effect of cell seeding density and material stiffness

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ABSTRACT

Three-dimensional hydrogel constructs incorporated with live stem cells that support chondrogenic differentiation and maintenance offer a promising regenerative route towards addressing the limited self-repair capabilities of articular cartilage. In particular, hydrogel scaffolds that augment chondrogenesis and recapitulate the native physical properties of cartilage, such as compressive strength, can potentially be applied in point-of-care procedures. We report here the synthesis of two new materials, [poly-L-lactic acid/polyethylene glycol/poly-L-lactic acid] (PLLA-PEG 1000) and [poly-D,L-lactic acid/polyethylene glycol/poly-D,L-lactic acid] (PDLLA-PEG 1000), that are biodegradable, biocompatible (>80% viability post fabrication), and possess high, physiologically relevant mechanical strength (~1500 to 1800 kPa). This study examined the effects of physiologically relevant cell densities (4, 8, 20, and 50 × 10^6/mL) and hydrogel stiffnesses (~150 kPa to ~1500 kPa Young's moduli) on chondrogenesis of human bone marrow stem cells incorporated in hydrogel constructs fabricated with these materials and a previously characterized PDLLA-PEG 4000. Results showed that 20 × 10^6 cells/mL, under a static culture condition, was the most efficient cell seeding density for extracellular matrix (ECM) production on the basis of hydroxyproline and glycosaminoglycan content. Interestingly, material stiffness did not significantly affect chondrogenesis, but rather material concentration was correlated to chondrogenesis with increasing levels at lower concentrations based on ECM production, chondrogenic gene expression, and histological analysis. These findings establish optimal cell densities for chondrogenesis within three-dimensional cell-incorporated hydrogels, inform hydrogel material development for cartilage tissue engineering, and demonstrate the efficacy and potential utility of PDLLA-PEG 1000 for point-of-care treatment of cartilage defects.

Statement of Significance

Engineering cartilage with physiologically relevant mechanical properties for point-of-care applications represents a major challenge in orthopedics, given the generally low mechanical strengths of traditional hydrogels used in cartilage tissue engineering. In this study, we characterized a new material that possesses high mechanical strength similar to native cartilage, and determined the optimal cell density and scaffold stiffness to achieve the most efficient chondrogenic response from seeded human bone marrow stem cells. Results show robust chondrogenesis and strongly suggest the potential of this material to be applied clinically for point-of-care repair of cartilage defects.

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1. Introduction

Cartilage tissue possesses limited potential for self-repair, and damage resulting from various disease processes, aging, or trauma ultimately leads to the formation of a persistent cartilage defect and the onset of osteoarthritis (OA) [1]. OA is a degenerative joint disease that affects approximately 27 million people in the U.S. each year and it places a heavy burden on society at a cost of approximately $89.1 billion yearly [2,3]. Although the pathogenesis of OA is not fully understood, age and body habitus contribute heavily to the development of OA, and with an ever-aging population suffering from increasing rates of obesity, methods towards addressing and alleviating the burden of this debilitating disease are a necessity.

Traditional treatment options aimed at resolving the osteochondral defect in OA have provided limited success with each bearing their own inadequacies [4]. The use of an osteochondral allograft provides native tissue that can be used to fill the defect, but it has the potential for immune rejection, disease transmission, and infection. Microfracture stimulates endogenous repair by a small fracture injury to the bone, but it can result in the formation of mechanically inferior fibrocartilage instead of native hyaline cartilage, thereby requiring a second intervention. Total joint arthroplasty, which is usually reserved for severe cases of OA, increases mobility but eliminates the potential for biological joint repair and requires a major surgery, which may not be an option for many patients [5,6]. In addition, this repair has limited lifespan in young patients, thus necessitating multiple surgeries over a lifetime. Given the respective limitations of these techniques, new approaches towards treating OA are actively being pursued.

Recently, regenerative medicine strategies using autologous cells, biomaterial scaffolds, and growth factors have garnered significant interest as potential routes to repair the osteochondral defect [1,7]. One set of techniques, autologous chondrocyte implantation (ACI) and matrix-induced ACI (MACI), involve harvesting healthy chondrocytes from non-weight bearing regions of articular cartilage and expanding the chondrocytes *in vitro* for re-implantation with or without cell seeding onto a biomaterial extracellular matrix (ECM) [8,9]. While such techniques utilizing mature adult cells offer a viable regenerative approach, they are constrained by lengthy cell expansion times, the potential for de-differentiation of chondrocytes during the expansion period, and contamination [10]. Another promising avenue towards obtaining mature chondrocytes involves the use of adult mesenchymal stem cells (MSCs), which have the ability to differentiate into a variety of lineages, including chondrocytes [11]. Bone marrow derived stem cells (BMSCs) in particular are of great interest for they are one of the most extensively studied MSCs, and intra-articular injections of BMSCs have been reported to reduce osteoarthritic pain, improve joint mobility, and slow progressive osteoarthritic degeneration [12–14]. As such, regeneration in OA employing BMSCs is an attractive alternative to currently applied ACI procedures.

The ideal scaffold should mimic the mechanical properties of cartilage, degrade as cells secrete their own extracellular matrix (ECM), and provide an environment conducive to cell survival and maintenance of a chondrocyte lineage. Many biomaterials have been developed that allow for live cell incorporation, but none adequately fulfill all the requirements of an ideal scaffold [15–17]. Recently, we reported the use of a water soluble methacrylated polyethyleneglycol-poly-D,L-lactide (mPDLLA-PEG) biodegradable polymer for live cell scaffold fabrication that possessed high mechanical strength (~780 kPa) [18]. While this scaffold possessed physiologically relevant mechanical strength on fabrication, we found that after 4 weeks the strength of the cell-seeded scaffold had degraded drastically (~240 kPa). This finding implies that ECM deposition by the encapsulated cells failed to provide sufficient mechanical reinforcement to the scaffold. Augmenting this ability is thus necessary, for example by varying factors such as cell density and material properties, both of which may affect ECM production, deposition, and organization. Indeed, for cells incorporated in hyaluronic acid and alginate 3D scaffolds increasing levels of matrix organization and deposition were seen with increasing concentrations of initial cell seeding density up to approximately 20×10^6 cells/mL [19–22]. On the other hand, an important material property, stiffness, is also known to play a part in determining stem cell differentiation into different lineages on both 2D and 3D substrates [23–29]. For 2D surface-seeded chondrocytes, mechanically matching scaffolds allowed for retention of rounded chondrocyte morphology and higher ECM production than counterparts with lower stiffnesses [30]. However, this is contrasted by BMSC behavior in 3D hyaluronic acid hydrogels where higher crosslinking densities and moduli led to a decrease in ECM production [31,32]. Given these observations, optimization of cell concentration and material stiffness is likely to be critical for enhanced chondrogenesis in live cell incorporated scaffolds that possess physiologically relevant mechanical properties.

In this study, we report the development of two new biomaterials, PDLLA-PEG 1000 and PLLA-PEG 1000, which are low molecular weight versions of our previously reported material, PDLLA-PEG 4000 (the terminal number indicates the molecular weight of the PEG chain) for use in live cell 3D incorporation. These new polymers exhibit properties of biodegradability and biocompatibility similar to those of the previous PDLLA-PEG 4000, but they possess mechanical properties that are much higher due to increased crosslinking density. Using these 3D materials, for the first time we probe the cellular efficiency of ECM production with varying cell densities and the effects of modulating material stiffness on chondrogenesis on a physiologically relevant scale (~150 kPa to 1500 kPa Young's modulus) in static cultured human BMSC (hBMSC) incorporated hydrogels. Our results should inform cell seeding protocols and the development of mechanically appropriate scaffolds for point-of-care articular cartilage tissue engineering.

2. Materials and methods

All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.1. Human bone marrow stem cell isolation

hBMSCs were isolated from the femoral heads of patients undergoing total joint arthroplasty with IRB approval (University of Washington and University of Pittsburgh), and cultured and expanded as previously described [33]. Briefly, bone marrow was flushed out from the trabecular bone of the femoral neck and head using an 18-gauge needle and re-suspended in Dulbecco's Minimal Essential Medium (DMEM). The suspension was filtered through a 40 μm strainer and the flow-through was centrifuged at 300*g* for 5 min. After the supernatant was discarded, the cell pellets were re-suspended using growth medium (GM, α-MEM containing 10% fetal bovine serum (FBS, Invitrogen), 1× antibiotics-antimycotic (Ampicillin 100 units/mL, Streptomycin 100 μg/mL, Amphotericin B 250 ng/mL), and 1.5 ng/mL FGF-2 (RayBiotech, Norcross, GA)), and then plated into 150 cm^2 tissue culture flasks at a density of 20,000–40,000 nucleated cells/cm^2, and medium was changed every 3–4 days. Once 70%–80% confluence was reached, cells were passaged. All experiments were performed with passage 4 (P4) hBMSCs except the cell concentration experiments, which were performed with passage 3 (P3) hBMSCs. All cells used in this study