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Digital micromirror device projection printing system for meniscus tissue engineering

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ABSTRACT

Meniscus degeneration due to age or injury can lead to osteoarthritis. Although promising, current cell-based approaches show limited success. Here we present three-dimensional methacrylated gelatin (GelMA) scaffolds patterned via projection stereolithography to emulate the circumferential alignment of cells in native meniscus tissue. Cultured human avascular zone meniscus cells from normal meniscus were seeded on the scaffolds. Cell viability was monitored, and new tissue formation was assessed by gene expression analysis and histology after 2 weeks in serum-free culture with transforming growth factor β1 (10 ng ml^{-1}). Light, confocal and scanning electron microscopy were used to observe cell–GelMA interactions. Tensile mechanical testing was performed on unseeded, fresh scaffolds and 2-week-old cell-seeded and unseeded scaffolds. 2-week-old cell–GelMA constructs were implanted into surgically created meniscus defects in an explant organ culture model. No cytotoxic effects were observed 3 weeks after implantation, and cells grew and aligned to the patterned GelMA strands. Gene expression profiles and histology indicated promotion of a fibrocartilage-like meniscus phenotype, and scaffold integration with repair tissue was observed in the explant model. We show that micropatterned GelMA scaffolds are non-toxic, produce organized cellular alignment, and promote meniscus-like tissue formation. Prefabrication of GelMA scaffolds with architectures mimicking the meniscus collagen bundle organization shows promise for meniscal repair. Furthermore, the technique presented may be scaled up to repair larger defects.

1. Introduction

The meniscus has a role in stabilizing the knee joint and functions as a shock absorber which protects articular cartilage during walking and sporting activities. A meniscal tear is the most frequently recorded orthopedic diagnosis, and partial or total meniscectomy remains the most common orthopedic procedure [1]. The annual incidence of meniscal injuries in the USA is estimated to be between 600,000 and 850,000, with 90% resulting in meniscal surgery. The vast majority of these procedures involve partial, sub-total or total meniscectomy [2–4]. Untreated damaged or degenerate meniscus can lead to the development of osteoarthritis (OA). OA is the main cause of disability in the USA, affecting over 27,000,000 people [5]. Despite substantial developments in surgical techniques, instrumentation, and orthopedic devices, long-term clinical outcomes are unsatisfactory.

Partial meniscectomy, in which only the torn and damaged portions of the menisci are removed [6], is now the treatment of choice for meniscal tears that cannot be repaired. Partial meniscectomy effectively relieves the acute symptoms, such as pain, swelling, and locking of the knee. However, partial meniscectomy fails to prevent the onset of severe OA, which occurs on average 14 years after the original procedure [7,8].

Surgical attempts to repair the torn tissues are ineffective in the avascular zone and are associated with a re-rupture rate of 30% even in the vascular zone [4]. Furthermore, repairs deemed successful in the short term do not mitigate long-term degenerative changes and the onset of OA. The sequelae of meniscal injury and the clinical outcomes of meniscectomy or repair are significantly worse in patients over the age of 40 [9]. Despite major advances in surgical techniques and biomedical device development, a meta-analysis of 42 clinical studies found no difference in the incidence of radiographic OA after meniscal repair compared with partial or total meniscectomy [8].

To address this medical challenge an attempt to repair meniscus tears was the first logical step taken by many researchers. Biomaterials used to culture meniscus cells or stem cells for meniscus

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repair include bioresorbable collagen matrices [10], which have been implanted in human patients with varied outcomes [11–13]. Fibrin alone or fibrin with added growth factors has been used to heal horizontal tears in human patients [14]. A recent report describes human meniscus cells seeded on a resorbable combination of polyglycolic acid (PGA) and hyaluronic acid in fibrin [15]. Polyurethane scaffolds possess sufficient mechanical properties with optimal interconnective macro-porosity to facilitate cell in-growth and differentiation [16]. Vicryl mesh scaffolds have also been used to repair bucket handle lesions in porcine meniscus [17]. A combination of human bone marrow-derived stem cells (hMSC) and a collagen scaffold was used in ovine meniscus explants showing promising integration [18]. Better integration was seen when the open/spongy scaffold structure was adjacent to the tissue. Devitalized meniscus has also been used to take advantage of the existing tissue architecture [19,20]. Although using the natural tissue may seem more promising for meniscus tears, a mature tissue scaffold without cells may not be ideal, and the availability of mensiscal tissue is also an issue.

A cell-seeded supportive scaffold system that emulates the structure and possesses the mechanical properties of native meniscus may aid in integrating and stabilizing the repair site and promote seamless repair. In the short term such an implanted cell-seeded scaffold should permit the cells to proliferate locally, migrate into the interface between the scaffold and native tissue, and secrete matrix components that integrate the scaffold.

Building structures mimicking native tissues can be accomplished using a number of nano- and micro-fabrication techniques, including melt molding, porogen leaching, gas foaming, phase separation, lamination, and fiber-based techniques [21–26]. More recently rapid prototyping techniques have been applied to biomaterial scaffold fabrication to refine the spatial complexity with which complex three-dimensional (3-D) physiological architectures can be replicated in vitro using laser ablation, microfluidics and 3-D printing [21,27–29]. In particular, projection stereolithography (PSL) or digital micromirror device (DMD) microfabrication, which uses an array of digitally controlled micromirrors to fabricate 3-D scaffolds layer by layer via a reflective photomask, is a promising technique. Because photomasks can be easily changed on demand the PSL approach is attractive due to its relative speed and flexibility compared with other photopatterning techniques [27,30–34]. This technique allows the rapid assembly of cell-responsive hydrogels that feature highly specified complex 3-D geometries with micrometer resolution.

In this study we demonstrate the feasibility of combining cell therapy, photocrosslinkable hydrogels, and digital micromirror device projection stereolithography (DMD PSL) microfabrication to produce graft tissue for implantation and integration into a meniscus tear. We fabricated scaffolds using a hydrolyzed form of collagen type I, the major structural component of the meniscal tissue, to provide structure and function similar to the surrounding native meniscus tissue. We confirmed cell compatibility (viability), observed cell interactions with the patterned scaffold (attachment and organization), characterized the mechanical properties of the scaffold before and after cell seeding, demonstrated new human meniscus tissue formation, and explored the potential for cell-seeded GelMA scaffolds to integrate with native meniscus tissue in an ex vivo human meniscus defect.

2. Methods

2.1. Tissue procurement

Normal human meniscus (medial and lateral) was obtained from tissue banks (approved by Scripps institutional review board), from six donors (age range 18–61 years, mean age 37.2 ± 17.5, one female, five males). A previously reported macroscopic and histologic grading system was used to select normal menisci [35].

2.2. Cell isolation and monolayer culture

Meniscus tissue was cut to isolate the avascular (inner two thirds) and vascular (outer one third) regions. The separated tissues were subjected to collagenase digestion as previously described [36], except that digestion over 5–6 h. The digested tissues were filtered through 100 μm cell strainers (BD Biosciences, San Jose, CA) and seeded in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) (Mediatech Inc., Manassas, VA) supplemented with 10% calf serum (Omega Scientific Inc., Tarzana, CA) and penicillin/streptomycin/gentamycin (Invitrogen, Carlsbad, CA).

2.3. Synthesis of methacrylated gelatin incorporating $CaCO_3$ particles (GelMA)

Gelatin methacrylate was prepared as described previously [37]. Briefly, porcine skin gelatin (Sigma-Aldrich, St Louis, MO) was dissolved at 10% w/v in phosphate-buffered saline (PBS) (Gibco/Life Technologies, Grand Island, NY) at 60 °C and stirred for 1 h. Methacrylic anhydride (Sigma–Aldrich) was added at a rate of 0.5 ml min^{-1} at 50 °C to achieve a final concentration of 7.5 vol.% and allowed to react for 2 h. The product was dialyzed against dH_2O at 40 °C for 1 week using dialysis tubing (molecular weight cut-off 12–14 kDa, Spectrum Laboratories). Finally, the solution was filtered (0.2 μm), frozen overnight (−80 °C), and lyophilized for 1 week. The final product was stored at −80 °C until further preparation.

To aid mechanical stability during fabrication $CaCO_3$ particles were incorporated into the final GelMA macromer solution. First, a 3% w/v macromer solution was prepared by adding GelMA to pre-warmed PBS (60 °C) and stirring until fully dissolved. An equal volume of 1.65 M $CaCl_2$ in PBS was added to the GelMA solution until thoroughly mixed. 1.65 M Na_2CO_3 in PBS (volume equivalent to the $CaCl_2$ solution) was added dropwise, and the mixture was stirred at 40 °C for 24 h. After allowing the mixture to settle, excess supernatant was removed to produce a 1.5% w/v GelMA concentration. Additional GelMA was then added to reach a 15% w/v concentration. Photoinitiator Irgacure 2959 (1% w/v, Ciba/BASF, Florham Park, NJ), UV absorber 2-hydroxy-4-methoxy-benzphenone-5-sulfonic acid (0.1% w/v), and UV quencher TEMPO (0.01% w/v, Sigma–Aldrich) were added sequentially until fully dissolved.

To determine the extent of methacrylate conversion (i.e. the degree of modification of ε-amine groups on lysines in gelatin), the 2,4,6-trinitrobenzenesulfonic acid solution (TNBS) assay as described by Habeeb was used [38]. The percentage amine substitution was calculated using the formula:

$$\text{Percentage substitution} = [1 - (\text{absorbance of the methacrylated protein} - \text{absorbance of the blank})/(\text{absorbance of the native protein} - \text{absorbance of the blank})] \times 100\%.$$

2.4. Digital micromirror device (DMD) projection stereolithography system

GelMA scaffolds were fabricated using a modified version of a DMD projection stereolithography (PSL) system described elsewhere [33]. The fabrication platform (Fig. 1A) comprises a DMD system (1920 × 1080 Discovery 4000, Texas Instruments), a servo-controlled stage (CMA-25-CCCL & ESP300, Newport), a UV light source (200 W, S2000, EXFO), a UV grade projection lens (Edmond Optics), and a replaceable glass coverslip window

ID	Title	Pages
518	Digital micromirror device projection printing system for meniscus tissue engineering	9