Biotribology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biotribology

journal homepage: http://www.elsevier.com/locate/biotri

Mesenchymal Stem Cells Enhance Lubrication of Engineered Meniscus Through Lubricin Localization in Collagen Gels

Edward D. Bonnevie, Ph.D. [a], Mary Clare McCorry, M.S. [b], Lawrence J. Bonassar, Ph.D. [a,b,*]

[a] Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA
[b] Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA

ARTICLE INFO

Article history:
Received 16 August 2016
Received in revised form 4 October 2016
Accepted 7 November 2016
Available online xxxx

Keywords:
Boundary friction
Fibrochondrocytes
Tissue engineering

ABSTRACT

This study evaluated the role of cell source in the boundary lubrication of engineered meniscus tissue. To accomplish this, both primary meniscal fibrochondrocytes (FCC) and bone marrow-derived mesenchymal stem cells (MSC) were obtained from neonatal bovine, seeded in high density collagen gels (20 mg/mL collagen with 25×10^6 total cells/mL) at various MSC:FCC ratios, and cultured for two weeks. After culture, the boundary friction coefficient, mechanical properties, surface roughness, and lubricin localization were all evaluated for engineered constructs. A strong correlation between MSC content and boundary friction coefficient was found ($R^2 = 0.948$). Aggregate modulus, permeability, and surface roughness revealed insignificant trends with MSC content; however, lubricin localization was highly correlated with increasing MSC content ($R^2 = 0.902$). Similarly, boundary friction coefficient had no significant trends with modulus, permeability, or roughness, but lubricin localization was significantly correlated with the boundary friction coefficient ($R^2 = 0.800$). Collectively, these data revealed a structure-function relationship in meniscus tissue engineering that is dictated by cell source. Specifically, the connection between MSC content, lubricin localization, and boundary friction coefficient reveal a method through which tuning the lubricating properties of engineered tissue is possible.

1. Introduction

The meniscus is an essential tissue within the knee joint, important for shock absorption, joint conformity, and lubrication [1]. Recently, meniscal surgeries numbered close to 1 million per year in the United States [2] and this number is rising [3]. The common surgical practice to treat meniscal tears is either partial meniscectomy or full meniscectomy followed by allograft transplantation [4]. For allograft transplantation, the high prevalence of meniscal injuries results in a shortage of suitable allograft tissue, and even in cases where transplant tissue is available there is still the possibility of infection or host rejection. For these reasons, tissue engineering meniscus replacements has emerged as a promising solution.

The past decade has seen important advancements in meniscus tissue engineering, but there are still areas where engineered menisci perform inferior to native tissue. The use of aligned, fibrous scaffolds has emerged as a tool to recapitulate the load bearing properties of native meniscus in terms of both elastic moduli and degree of anisotropy (i.e., stiffer in the circumferential direction than the radial direction) [5–7]. Further, the emergence of 3D fabrication methods provides a tool to anatomically mimic healthy menisci to begin to restore joint conformity [8,9]. While these advancements have brought meniscus tissue engineering closer to being clinically relevant, a comparatively under studied area has been lubrication.

Lubrication in meniscus tissue engineering is an important factor not only to ensure an engineered implant will be preserved in vivo, but also to ensure the contacting adjacent cartilage is left relatively unperturbed [10]. Native meniscus naturally provides low friction similar to that of native cartilage [11,12], which is dictated by both interstitial fluid pressurization [13] and boundary lubrication [14,15]. The contribution of interstitial fluid pressurization is dependent on physical factors such as porosity, elastic modulus, permeability and tension-compression anisotropy [13]. The contribution of boundary lubrication may be dictated by factors such as surface chemistry, roughness [16], and the adsorption of lubricating molecules, such as lubricin [14,17–19]. In this study, we focus specifically on boundary lubrication, which is an important factor in cartilage homeostasis as increased boundary friction can result in cartilage degeneration [18] and chondrocyte apoptosis [20]. For meniscus tissue engineering, friction is an important factor as the boundary friction coefficients of possible scaffold materials are often over twice as high as native meniscus (polyurethane: $\mu \sim 0.7$ [10], alginate: $\mu \sim 0.5$ [19], collagen: $\mu \sim 0.4$ [15], native meniscus: $\mu \sim 0.2$ [15]).

* Corresponding author at: 149 Weill Hall, Cornell University, Ithaca, NY 14850, USA.
E-mail address: lb244@cornell.edu (L.J. Bonassar).

http://dx.doi.org/10.1016/j.biotri.2016.11.001
2352-5738/

In musculoskeletal tissue engineering in general, tuning the lubrication of implants has been studied based on a variety of factors. For cartilage tissue engineering, physical stimulation in the form of shear deformations attenuates lubricin production and localization, but this has not been studied for meniscus tissue engineering [21,22]. Further, we have recently shown that growth factor stimulation can promote the localization of lubricin at the construct surface and consequently reduce boundary friction in meniscus tissue engineering [15]. Finally, for cartilage tissue engineering, cell source plays a role in lubricin content as selectively seeding superficial chondrocytes enhances lubricin content [23], but cell source has not been studied for lubrication in meniscus tissue engineering.

Within meniscus tissue engineering, primary meniscal fibrochondrocytes (FCCs) [24,25], mesenchymal stem cells (MSCs) [19], or co-culture of populations of the two [26–28] have been used to seed constructs. Use of FCCs may be limited due to either availability or expansion potential, and use of stem cells may be limited due to inclination towards hypertrophy after differentiation [27]. Consequently, co-culture has emerged as a method to balance these limitations [27,28]. In this study we evaluate the role of cell source in meniscus tissue engineering regarding boundary lubrication and determine which physical and biochemical factors are most predictive of effective boundary lubrication.

2. Methods

2.1. Cell Isolation

Cell isolation was based on previously reported methods using tissue from neonatal bovine [28,29]. Briefly, MSCs were extracted by incubating cubes from the trabecular bone of the femoral head in media supplemented with 300 U/mL heparin. This solution was centrifuged at 300*g* followed by washing and plating of the pellet. After 48 h of incubation, the unattached cell population was removed. Trilineage differentiation assays were performed to confirm multipotency of the remaining cell population for chondrogenic, osteogenic, and adipogenic differentiation as previously described and presented [28]. MSCs were expanded in 2D in low glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 U/mL streptomycin, 2 mM L-glutamine, and 1 ng/mL basic fibroblast growth factor. MSCs were seeded at passage 4. FCCs were isolated from medial and lateral menisci overnight in 0.3% collagenase (Worthington Biochemical Corporation, Lakewood, NJ) in DMEM with 100 μg/mL penicillin and 100 μg/mL streptomycin. Following previously reported purification methods [28], cell-media mixtures were created at MSC:FCC ratios of 0:100, 25:75, 50:50, 75:25, and 100:0.

2.2. Construct Generation

As previously described, type I collagen gels were used as a construct material in this study [15,25,28]. Collagen type I was extracted from Sprague-Dawley rat tails (Pel-Freez Biologicals, Rogers, AZ) and reconstituted in 0.1% acetic acid at 30 mg/mL as previously described [15,25,28]. Briefly, 30 mg/mL collagen solution was mixed with a working solution of 1 N NaOH, 10× phosphate-buffered saline (PBS), and 1× PBS to return the collagen solution to a neutral 7.0 pH and 300 mOsm to initiate gelation. Cell-media suspensions were homogeneously mixed with the collagen solutions to form a collagen-cell-media solution at 20 mg/mL collagen seeded at a total density of 25×10^6 cells/mL. This solution was gelled between two glass plates to create 2 mm thick sheets, and then allowed to gel for 30 min at 37 °C. From each 2 mm thick gel, 8 mm diameter samples were obtained using biopsy punches. Samples were cultured at 37 °C and 5% CO_2 in media containing DMEM, 10% FBS, 100 μg/mL penicillin, 100 μg/mL streptomycin, 0.1 mM non-essential amino acids, 50 μg/mL ascorbate, and 0.4 mM L-proline. Culture media was refreshed every 3–4 days. Samples were cultured for 1 or 2 weeks for friction studies and for 2 weeks for all other analyses.

2.3. Boundary Friction Analysis

Boundary friction coefficients were measured on a custom-built tribometer as previously described in multiple publications [10,15,19,30]. Briefly, samples were mounted onto a cylindrical post and attached to a biaxial, strain gauged load cell. Samples were compressed to 30–50% axial strain against polished glass and allowed to equilibrate before sliding (20–30 min relaxation). Samples were then slid ±1 cm at 0.1 mm/s for 3 cycles. These conditions are known to produce boundary mode lubrication in both native [31] and engineered tissue [15,19]. The friction coefficient was calculated as the shear force divided by the normal force and averaged for the forward and reverse sliding directions over the 3 cycles.

2.4. Histology

For histological analysis, samples were fixed in 10% buffered formalin, dehydrated in progressively stronger ethanol, embedded into paraffin blocks, sectioned, and mounted on slides. Immunohistochemistry was conducted as previously described [15] to analyze localization of lubricin (Abcam, Cambridge, MA, USA; Ab28484) at the construct surface as well as type II collagen (Abcam; Ab34710) and fibronectin (ICN/Cappel), which are extracellular matrix constituents that are known to have a high affinity to lubricin [32]. Briefly, antigen retrieval was conducted using citric acid at 90 °C. Slides were then washed twice for 5 min in TRIS buffered saline with 0.5% TWEEN-20 (pH 7.4), incubated for 30 min in 0.01% hyaluronidase, 30 min in 3% hydrogen peroxide, and 60 min in a blocking solution containing normal serum, bovine serum albumin, Triton X-100, and TWEEN-20. Primary antibodies were applied overnight at 4 °C. Secondary antibodies (Vectastain ABC, Vector) were applied for 30 min followed by 30 min in an avidin-biotin complex (Vectastain ABC, Vector). Staining was conducted with a peroxidase substrate (ImmPACT DAB, Vector). Images of stained sections were taken with a SPOT RT camera (Diagnostic Instruments, Sterling Heights, MI) attached to a Nikon Eclipse TE2000-S microscope (Nikon Instruments, Melville, NY). To minimize data variation due to staining, samples from all groups were stained at the same time with the same reagents. Quantitative image analysis for lubricin localization was carried out on 3 stained slides for each construct group and conducted using ImageJ. Images at 200× that each contained the surface of the construct were thresholded and converted to binary images in ImageJ, and the percentage of pixels staining for lubricin were calculated for each image.

2.5. Mechanical Analysis

Three to four samples per experimental group were trimmed to 4 mm diameter and mounted using an Enduratec ElectroForce 3200 System (Bose, Eden Prairie, MN) in a confined compression set up. Briefly, 10 steps of 5% strain were applied in stress relaxation. Data were fit to a poroelastic model using a custom MATLAB program to determine aggregate modulus and hydraulic permeability [33].

2.6. Surface Analysis

The surface roughness of both constructs and native meniscus were measured using a scanning white light interferometer (ADE Phase Shift MicroXAM Optical interferometric profiler). Four samples for each tissue engineered group and 9 of native meniscus were examined by analyzing the height distribution in a 209 μm × 179 μm window. The root mean squared roughness (S_q) was calculated for each sample and reported.

Please cite this article as: E.D. Bonnevie, et al., Mesenchymal Stem Cells Enhance Lubrication of Engineered Meniscus Through Lubricin Localization in Collagen Gels, (2016), http://dx.doi.org/10.1016/j.biotri.2016.11.001