Journal of Biomechanics 48 (2015) 4333–4338

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Short communication

Comparing the mechanical properties of the porcine knee meniscus when hydrated in saline versus synovial fluid

Emily H. Lakes [a,b], Courtney L. Kline [a], Peter S. McFetridge [a,b], Kyle D. Allen [a,b,*]

[a] J Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, United States
[b] Institute for Cell & Tissue Science and Engineering, University of Florida, Gainesville, FL, United States

ARTICLE INFO

Article history:
Accepted 31 October 2015

Keywords:
Meniscus
Synovial fluid
Mechanical testing
Phosphate buffered saline

ABSTRACT

As research progresses to find a suitable knee meniscus replacement, accurate in vitro testing becomes critical for feasibility and comparison studies of mechanical integrity. Within the knee, the meniscus is bathed in synovial fluid, yet the most common hydration fluid in laboratory testing is phosphate buffered saline (PBS). PBS is a relatively simple salt solution, while synovial fluid is a complex non-Newtonian fluid with multiple lubricating factors. As such, PBS may interact with meniscal tissue differently than synovial fluid, and thus, the hydration fluid may be an important factor in obtaining accurate results during in vitro testing. To evaluate these effects, medial porcine menisci were used to evaluate tissue mechanics in tension ($n=11$) and compression ($n=15$). In all tests, two samples from the same meniscus were taken, where one sample was hydrated in PBS and the other was hydrated in synovial fluid. Statistical analysis revealed no significant differences between the mean mechanical properties of samples tested in PBS compared to synovial fluid; however, compressive testing revealed the variability between samples was significantly reduced if samples were tested in synovial fluid. For example, the compressive Young's Modulus was 12.69 ± 7.49 MPa in PBS versus 12.34 ± 4.27 MPa in synovial fluid. These results indicate testing meniscal tissue in PBS will largely not affect the mean value of the mechanical properties, but performing compression testing in synovial fluid may provide more consistent results between samples and assist in reducing sample numbers in some experiments.

1. Introduction

Like most tissues, the knee meniscus is viscoelastic and displays different behavior when dehydrated compared to hydrated (Nicolle and Palierne, 2010). Thus, to fully understand meniscus mechanics, both solid and fluid constituents within the tissue should be considered. Within the knee, the menisci are hydrated by synovial fluid, which serves as a source of nutrition and lubrication (Tamer, 2013). Yet, previous research on meniscus mechanics often uses phosphate-buffered saline (PBS) for tissue hydration (Chia and Hull, 2008; Lechner et al., 2000; Proctor et al., 1989; Maes and Haut Donahue, 2006; LeRoux and Setton, 2002; Sweigart and Athanasiou, 2005b). This substitution is typically motivated by the inhomogeneity of synovial fluid, as synovial fluid is physically a colloid consisting of multiple lubricating factors and large molecules dispersed throughout. However, PBS viscosity is only 0.001 Pa s (Momen-Heravi et al., 2012), whereas synovial fluid viscosity ranges from 0.08 to 1.9 Pa s in healthy individuals (Jebens and Monk-Jones, 1959). PBS is also a Newtonian fluid, while synovial fluid is a complex non-Newtonian fluid that demonstrates shear-thinning characteristics. Since PBS and synovial fluid are mechanically different fluids, the mechanics of meniscal tissue may differ when hydrated with these two fluids. The goal of this short communication is to assess the effect of these hydration fluids on meniscus mechanical properties.

2. Materials and methods

2.1. Experimental design

Porcine medial menisci ($n=28$) and bovine synovial fluid were purchased from Animal Technologies, Inc. (Tyler, Texas) and shipped frozen on dry ice. To test the effects of hydration fluid, a paired experiment was designed, where half the meniscus was tested in synovial fluid and the remaining half was tested in PBS (Fig. 1). The assigned fluid was alternated to account for known regional variability within the meniscus (Proctor et al., 1989; Sweigart and Athanasiou, 2005a).

2.2. Tension

The halves of 12 different menisci were frozen to a cryostat stage and sliced to obtain a centrally-located, 450 μm thick section parallel to the tibial surface. Using a custom dumbbell-shaped punch, a sample aligned with the circumferential

* Correspondence to: J Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, 1275 Center Drive, Biomedical Sciences Building, Gainesville, FL 32610, United States. Tel.: +1 352 273 9337.
E-mail address: kyle.allen@bme.ufl.edu (K.D. Allen).

http://dx.doi.org/10.1016/j.jbiomech.2015.10.046
0021-9290/

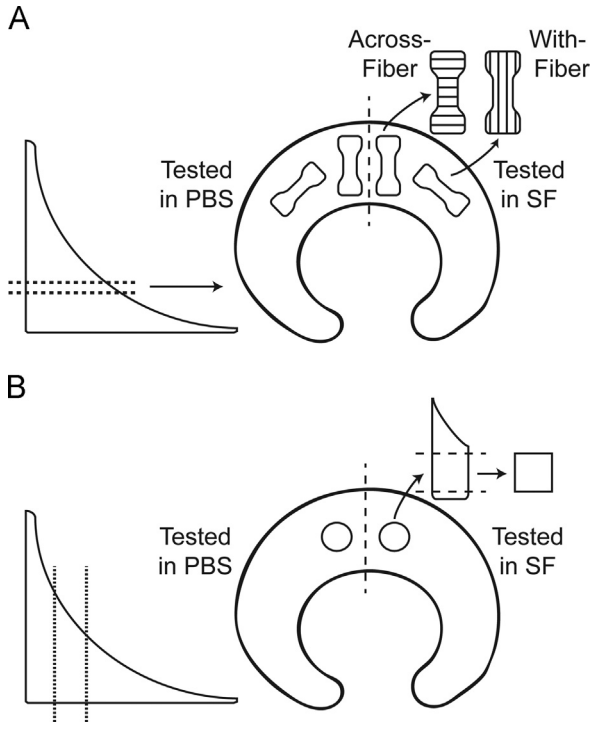

Fig. 1. Each meniscus was dissected in half to allow for one half to have samples tested in PBS and the other half to have samples tested in synovial fluid (SF). Fluid assignments were alternated between anterior/posterior regions among menisci. (A) Tensile samples were taken as both across-fiber and with-fiber in relation to the circumferential collagen fibers. Dumbbell tensile samples had a central width of 2 mm and central length of 3.7 mm. (B) For compression testing, two cylindrical samples were taken next to each other in the central portion of the meniscus and were then cut with parallel blades.

collagen fibers (with-fiber) and a sample aligned radially (across-fiber) were obtained from each half (Fig. 1A). Samples were stored in a 0.15 M NaCl solution with protease inhibitors (2 mM EDTA, 5 mM benzamidine HCl, 10 mM N-ethylmalemide, and 1 mM PMSF) (Skaggs et al., 1994; Sweigart and Athanasiou, 2005b). Prior to testing, samples were transferred to either synovial fluid or PBS for 48 h (at 4 °C).

For tensile testing, samples were secured in hemostat grips on a 5542 model Instron ($\pm$50 N load cell) and immersed in PBS or synovial fluid at room temperature. Two lots of synovial fluid were used, with half of the samples tested in each lot. For with-fiber samples, a 0.05 N tare load was applied; then, samples were preconditioned with 10 cycles of 0.65% strain followed by 10 testing cycles of 1.3% strain at 0.26%/s. For across-fiber samples, a 0.05 N tare load was applied; then, samples were preconditioned with 10 cycles of 2.5% strain followed by 10 testing cycles of 5% stain at 1%/s. Strains and strain rates were selected from work reporting circumferential strains of 1.3% and radial strains of 5% in the knee meniscus after five seconds of a physiologic load (Spilker et al., 1992). Immediately after cycling, samples underwent pull to failure at the same strain rate. From cyclic loading, area of hysteresis and peak stress were calculated, and from pull to failure tests, Young's modulus, yield stress, yield strain, ultimate tensile strength (UTS), and strain at UTS were calculated. Young's modulus was defined as the linear portion of the stress–strain curve after the toe region.

2.3. Compression

Using a 5 mm biopsy punch, samples perpendicular to the tibial surface in the central portion of the meniscus were collected from the halves of 16 menisci (Fig. 1B). Samples were cut with parallel blades to a height of 3.5 mm, with both surfaces removed. As for tension testing, samples were placed in saline with protease inhibitors, then transferred to either synovial fluid or PBS for 48 h prior to testing (at 4 °C).

For unconfined compression, samples were secured to a petri dish via cyanoacrylate, then surrounded by room temperature PBS or synovial fluid (5542 model Instron, $\pm$500 N load cell). Three different lots of synovial fluid were used, with samples 1–3 tested in the first lot, sample 4 was tested in the second lot, and samples 5–16 tested in the third lot. Samples were cycled 30 times at 10% strain at a strain rate of 2.5%/s. The first 15 cycles were considered preconditioning and the last 15 cycles were considered the repeatable response for cyclic loading. Immediately after cycling, each sample underwent stress relaxation at 20% strain until a steady-state stress was reached ($\approx$30 min). Cycling at 10% strain was based on an estimation of physiologic loading (Martin Seitz et al., 2013). The strain rate was chosen from previous testing of meniscal attachments (Maes and Haut Donahue, 2006) and preliminary testing showing higher strain rates associated with walking (Chia and Hull, 2008) exceeded our machine's capabilities. From cyclic loading, area of hysteresis and peak stress were calculated. Young's modulus was calculated from the ramping phase between cycling and stress relaxation, while the instantaneous stress and relaxation stress were calculated from stress relaxation data.

2.4. Stress-relaxation curve fitting

To further characterize stress relaxation responses, first-order decay and standard linear solid (SLS) models were fit to stress relaxation data. First-order decay provided an estimate of the time constant (63% decay from instantaneous stress). The SLS model is governed by the following equation:

$$\sigma(t) = \varepsilon_0 * \left(E_1 + E_2 e^{-t/\tau}\right) \quad (1)$$

where E_1+E_2 is the instantaneous modulus, E_1 is the relaxation modulus, and τ is the time constant for relaxation (Pruitt and Chakravartula, 2011).

2.5. Statistics

Since PBS and synovial fluid samples had a matched sample from the same meniscus, a paired t-test was used to compare samples tested in PBS versus samples tested in synovial fluid (α=0.05). To compare the variance between groups, an F-test was performed (α=0.05). Due to an experimental error during test set-up, one across-fiber and one with-fiber tensile sample was excluded from analysis (dropping from n=12 to n=11). Additionally, in compression, one meniscus sample was accidently stored and tested in the incorrect fluid (dropping from n=16 to n=15 for compression). Lastly, failure and yield occurred at a similar stress–strain, thus while the yield stress and yield strain were calculated, only UTS and strain at UTS are presented.

3. Results

3.1. Tension

For across-fiber samples, no differences were found between PBS and synovial fluid in hysteresis area (p=0.854) and peak stress during hysteresis cycling (p=0.902) (Fig. 2). Additionally, hydration fluid did not affect the across-fiber Young's modulus (p=0.362), UTS (p=0.565), or strain at UTS (p=0.995) (Fig. 3). For with-fiber samples, no differences were found in hysteresis area (p=0.507) and peak stress during hysteresis cycling (p=0.760) (Fig. 2). Additionally, hydration fluid did not affect the with-fiber Young's modulus (p=0.362), UTS (p=0.766), or strain at UTS (p=0.862) (Fig. 3). Finally, variability in tension parameters was not significantly affected by hydration fluid ($p \geq 0.077$).

3.2. Compression

Hydration fluid did not affect the mean value of hysteresis area (p=0.679) or peak stress during cyclic testing (p=0.575) (Fig. 4). Similarly, no differences were found for the Young's modulus (p=0.887), instantaneous stress (p=0.778), and relaxation stress (p=0.244) (Fig. 5). However, the variability of all measured compressive properties was reduced in synovial fluid ($p \leq 0.022$). Graphical representation of average stress relaxation curves with standard deviation bounds is shown in Fig. 6.

Quantitatively, time constants from the first-order decay model were similar in different hydration fluids (p=0.245), but lower variability was found in samples tested in synovial fluid ($p < 0.001$, Table 1). Similarly, for SLS curves, no differences were found for time constant (p=0.507), instantaneous modulus (p=0.932), or relaxation modulus (p=0.326), but the variability of these measures was generally reduced in synovial fluid, with a significant reduction in the relaxation modulus ($p < 0.001$, Table 1).