Evaluation of Geometric Fidelity of Anatomically Shaped Tissue Engineered Meniscus

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Introduction
A great advantage of tissue engineering technologies lies in the ability to generate living implants in specific shapes[1-3]. This is of potential advantage for a tissue such as the meniscus, which has a complicated geometry that requires assessment of suitability for allograft transplantation[4,5]. Previous studies have established that 10% variance from normal tissue in some critical dimensions is acceptable for meniscus transplantation[6]. This level of tolerance is likely achievable using established technologies for tissue printing[7] and injection molding[8] as well as MRI and µCT data for implant design[9]. However, determining whether these methods are sufficient to achieve the desired dimensional tolerances is hindered by the fact that no established method exists for comparing the geometry of tissues with complex shapes. The goal of this study was to use optical scanning methods to compare the geometric fidelity of meniscal implants designed from MRI and µCT data and generated using tissue injection molding and printing technologies.

Methods

Imaging: Ovine menisci (n = 4) underwent both MRI and µCT imaging to facilitate implant design. Magnetic resonance imaging of intact sheep knees was performed on a clinical 3T MR unit. Sagittal 3D spoiled gradient echo (SPGR) sequences were acquired resulting in a spatial resolution of 253.9 µm (frequency) x 253.9 µm (phase) x 0.5 mm at one excitation. The medial meniscus was dissected from each knee, soaked in Cricraque® 300mg/ml contrast agent and scanned using an Enhanced Vision Systems Model Ms-8 In Vitro Micro-CT Scanner resulting in 0.023mm/pixel resolution.

Injection Molding: Silastic molds were fabricated directly from ovine menisci as described previously[9]. ABS plastic molds were 3D printed on a Stratasys FDM 3000 from stereolithography (STL) files derived from MRI and µCT images[9]. Alginate hydrogel was prepared by mixing 2% w/v alginate with 2% CaSO4 in a 2:1 ratio[9]. Alginate hydrogel was then injected into the molds and allowed to gel for 20 minutes in 2% CaCl2 solution, and the constructs were manually removed from molds[9].

Solid Freeform Fabrication: The SFF was conducted on a custom open-architecture 3D printing platform. STL files of the meniscal geometry were imported into internal software that generated tool paths to guide deposition of alginate hydrogel[7]. The alginate hydrogel was prepared by mixing 2% w/v alginate with 0.75% CaSO4 mixed in a 2:1 ratio[7]. The gel was printed on top of a 3D printed substrate that had contours to support overhanging parts of the gel constructs. The printed constructs were post-crosslinked for 20 minutes in 2% CaCl2 solution.

Geometry Analysis: Alginate hydrogel constructs were placed onto the Z-platform of the 3D printer and scanned via a laser triangulation distance sensor. The distance sensor collected data at 40 KHz and captured distances at 100 points per square centimeter. A point cloud of the scan data was imported into Geomagic for quantitative analysis. Manual measurements of key dimensions were taken using calipers, and % errors calculated based on specific points of interest established previously[4,5]. Dimensions included total volume, span between the horns, depth across the entire meniscus, anterior and posterior horn widths and the height and width along the meniscus at 7 locations spaced 30° apart along the hoop direction.

Statistics: A total of 6 replicates were made for each meniscus for each fabrication method based on MRI, µCT, or silastic impression mold, yielding a total of 120 samples for the study. ANOVA was performed to determine significant differences in sample dimensions with post hoc Tukey test for pair wise comparisons.

Results
The seven heights and widths were pooled since only a significant difference existed among groups across all points of interest. All three molded groups were significantly more accurate in achieving the desired height than the two printed groups (P < 0.05). The µCT mold group was significantly less accurate in replicating the desired width than the MRI mold and both printed groups (P < 0.01). Silastic molds were within ±10% error for all 7 key measurements, µCT molds for 6 of 7, µCT prints for 4 of 7, MRI molds for 5 of 7, and MRI prints for 4 of 7.

Discussion
This work shows the ability to generate anatomically shaped meniscal constructs of high geometric fidelity. We have also established an effective way to compare these complex structures quantitatively, MRI and µCT injection molds are capable of achieving shape fidelity within 10% of the target, a critical threshold established previously[9]. Silastic molds and µCT molds appear to be the more accurate forms of generating meniscal constructs. The MRI image groups suffered in precision due to wide variation from knee to knee when the image scan was obtained. The 3D printed molds also suffered in surface quality due to the print resolution of the Stratasys FDM machine which could be addressed with finish polishing of the molds after printing or using a higher resolution printer. Future efforts must focus on development of higher resolution 3D printing of tissues implementing control feedback to prevent void formation due to inadequate extrusion of scaffold material. Geometric fidelity of printed or molded samples would also likely be aided by development of new processing methods to enhance the mechanical properties of hydrogels used for implant fabrication.

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Figure 1
Computationally rendered images of the surface to surface deviation between native tissue and engineered constructs showed increases in error from silastic mold to µCT mold, µCT print, MRI mold, and MRI print (Fig. 1). Gross inspection showed silastic molds had superior surface quality over ABS plastic molded and 3D printed samples (Fig.1).

Figure 2: Histograms of deviation points from color maps pooled from 6 replicates of 1 sheep knee.
Frequency histograms showed that the majority of points fell within ±10% of the native tissue as denoted by the orange dotted lines (Fig. 2). No significant difference was found among groups on the percentage of points that were within ±10% (P = 0.981) for silastic, 48% µCT mold, 47% MRI mold, 46% µCT print, and 44% MRI print (Fig 2).

References

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