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Fabrication of Custom-Shaped Grafts for Cartilage Regeneration

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Abstract

Transplantation of engineered cartilage grafts is a promising method to treat diseased articular cartilage. The interfacial areas between the graft and the native tissues play an important role in the successful integration of the graft to adjacent native tissues. The purposes of the study were to create a custom shaped graft through 3D tissue shape reconstruction and rapid-prototype molding methods using MRI data, and to test the accuracy of the custom shaped graft against the original anatomical defect.

An iatrogenic defect on the distal femur was identified with a 1.5 Tesla MRI and its shape was reconstructed into a three-dimensional (3D) computer model by processing the 3D MRI data. First, the accuracy of the MRI-derived 3D model was tested against a laser-scan based 3D model of the defect. A custom-shaped polyurethane graft was fabricated from the laser-scan based 3D model by creating custom molds through computer aided design and rapid-prototyping methods. The polyurethane tissue was laser-scanned again to calculate the accuracy of this process compared to the original defect.

The volumes of the defect models from MRI and laser-scan were 537 mm³ and 405 mm³, respectively, implying that the MRI model was 33% larger than the laser-scan model. The average ($\pm$ SD) distance deviation of the exterior surface of the MRI model from the laser-scan model was 0.4 $\pm$ 0.4 mm. The custom-shaped tissue created from the molds was qualitatively very similar to the original shape of the defect. The volume of the custom-shaped cartilage tissue was 463 mm³ which was 15% larger than the laser-scan model. The average ($\pm$ SD) distance deviation between the two models was 0.04 $\pm$ 0.19 mm.

Custom-shaped engineered grafts can be fabricated from standard sequence 3-D MRI data with the use of CAD and rapid-prototyping technology, which may help solve the interfacial problem between native cartilage and graft, if the grafts are custom made for the specific defect. The major source of error in fabricating a 3D custom shaped cartilage graft appears to be the accuracy of a MRI data itself; however, the precision of the model is expected to increase by the utilization of advanced MR sequences with higher magnet strengths.

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Key Index Terms

Cartilage; Tissue Engineering; Custom-shaped; MRI; rapid prototyping; CAD; computer aided design; Fused Deposition Modeling

Introduction

Cartilage defects seldom heal as the chondrocytes have low metabolism and regenerative capacity (1), which may result in degenerative joint disease. Transplantation of engineered cartilage grafts is a promising method to treat articular cartilage defects (2). The implanted graft is expected to integrate with adjacent tissues, remodel itself to form the structural and biochemical tissue, and withstand mechanical stresses within the joint (3). Numerous tissue engineering methods have been described to create cartilage grafts *in vivo* and *in vitro*. The success of these procedures relies on the integration of the graft to adjacent native tissues (4). Therefore, recent studies have focused on preparing custom-shaped grafts to minimize graft-defect mismatch.

Tsuchiya et al. incorporated poly DL-lactic-co-glycolic acid sheets and collagen microsponges in their openings to repair bone defects (5). They fabricated this material into desired shapes by changing the shape of the poly sheets. Our laboratory previously reported a cartilage tissue engineering method *in vivo* using adipose-derived stem cells, fibrin glue and growth factors (6). These tissue engineering methods have begun to fabricate cartilage grafts into arbitrary shapes to match the complex shape of the defect. This allows the cartilage graft to be implanted without excising and widening the damaged cartilage area. The graft, therefore, would have minimum interfacial mechanical stresses with adjacent bones and cartilage, which will promote the viability of the chondrocytes and the formation of collagen networks (7,8).

Magnetic resonance imaging (MRI) provides a non-invasive high-resolution method to detect and create three-dimensional models (9). Detectable sizes of drill holes in articular cartilage showed that 2 mm and 3 mm holes on the distal femur were detectable in MRI with the aid of intra-articular saline and Gd-DTPA injections, respectively (10). Graichen et al. also investigated the accuracies in the determination of the 3D shapes of cylindrical holes on cartilage (11). The accuracy of measuring cartilage thicknesses (12) by MRI have been investigated but the accuracy of measuring arbitrary defect shapes using MRI has not been evaluated. In our previous study, we utilized a novel three-dimensional laser scanning technique to evaluate the accuracy of articular cartilage thickness measured in MRI (12).

The purpose of this study was to create a custom shaped cartilage graft through 3D tissue shape reconstruction and rapid-prototyping methods, and to test the accuracy of the custom shaped cartilage graft against the anatomic defect on a human knee.

Methods

Specimen preparation and MR imaging

A fresh-frozen cadaveric human knee was thawed at a room temperature and an artificial defect similar to an osteochondritis dissecans lesion, which includes injury to both cartilage and bone, was surgically created on the distal femoral surface as shown in Figure 1a. The soft tissues were surgically repaired with 2-O Vicryl, and remainder of the knee was kept intact.

MR images of the knee were obtained with a fat-suppressed Spoiled Gradient Recalled Echo sequence (13) with the parameters of TR=19.40 ms, TE=4.60 ms, Flip Angle=20 degrees and Field of View=16×16×8.6 cm in a 1.5T GE Signa scanner using an 8-channel knee coil. The slice thickness was 0.7 mm and the in-plane resolution was 0.625×0.625 mm (Figure 1b).

Creating a 3D model of the defect

The MR images were loaded into our custom software (12). The boundaries of the distal femur including articular cartilage were identified in the MR images and semi-automatically segmented using Spline tools in the software followed by manual correction for all slices of MR images. The segmented boundaries were combined and made into a three-dimensional model using the custom software. The accuracy of 3D model created using custom software was validated in a previous study (12). The defect on the distal femur was incorporated into a separate 3D model by creating a negative shape of the distal femur using 3D polygon manipulation software (Rapidform, Inus Technology, Seoul, South Korea) (Figure 4a). A smooth surface was created on top of the defect using the curvatures of the intact cartilage around the defect. The smooth surface was stitched with the polygons of the defect to create a 3D defect model.

Creating molds with computer aided design and rapid-prototyping

The 3D defect model was placed in the middle of two mold blocks consisting of top and bottom blocks (Figure 2). The size of the bounding box of the defect was $17 \times 16 \times 6 \text{ mm}^3$ and the size of each top and bottom block was $60 \times 60 \times 20 \text{ mm}^3$. The mold was designed to keep the smooth surface shape of the articular surface intact when the fragile molded cartilage tissue would be peeled off the mold, thus the boundary of the top surface of the defect model was used as a parting line (Figure 2b). The parting line along the top surface was connected to the boundaries of the top and bottom molds to create a parting plane (Figure 2c and 2d). The defect model was separated into two parts along the boundary of the surface. The top surface and the remainder of the defect were connected to the parting plane in the top and bottom molds, respectively, to create complete shapes of the top and bottom molds. A 3mm injection channel was made at the bottom mold to keep the smooth top surface uninterrupted. The matrix gel was then injected into the mold through the bottom of the defect (Figure 2e).

The computer models of the top and bottom molds were exported as .STL files for rapid-prototyping fabrication. The molds were created using the Fused Deposition Modeling (FDM) technique (Redeye RPM, Eden Prairie, MN). Briefly, plastic filament (Polycarbonate-ISO) was unwound from a coil and supplied to an extrusion nozzle which was heated to melt the material. The material was then extruded onto a platform, which was moved in both horizontal and vertical directions, and directly controlled by computer-aided manufacturing (CAM) software. The mold was produced by extruding small beads of PC-ISO material to form layers, as the material hardened immediately after extrusion from the nozzle (Figure 3a). The sterilizable thermoplastic Polycarbonate-ISO (PC-ISO) was used as it is a medical grade plastic which meets both ISO 10993-1 and USP Class VI standards.

Testing the accuracy of the custom shaped tissue created from the molds

The ability of the molds to create a custom-shaped graft was tested by the use of liquid polyurethane (VytaFlex 10, Reynolds Advanced Materials, Hollywood, CA). This material was injected into the molds through the 3 mm injection channel (Figure 3b). The molds were taken apart along the parting plane 16 hours (matrix specific) after injection and the defect shaped polyurethane was easily peeled off from the mold.

The accuracy of the 3D MRI fabrication model used to create the molds was tested against the anatomic defect shape from the distal femur. The cadaveric knee was then dissected of all soft tissues to obtain the distal femur. The surface of the distal femur with the target defect was laser-scanned using a desktop laser-scanner (Model 15, Cyberware, Monterey, California, USA) (Figure 4b). The accuracy of the scanner was 50 μm in depth and the pitch between scan lines was 300 μm . The laser-scan was performed in multiple views and combined using Cydir (Cyberware, Monterey, California, USA) to create a complete shape of the distal femur with the defect. Using the same method as the 3D MRI fabrication model, a negative shape of the distal femur was created from the laser-scan assuming a smooth cartilage surface.

The two 3D defect models from MRI and laser-scan were aligned and compared in 3D polygon manipulation software. The average distance deviation on the exterior surface and the volume difference were quantified.

The accuracy of the custom-shaped graft made of polyurethane (see above) from the laser scan prototype was tested against the 3D MRI fabrication model using Rapidform Software (Inus Technology, Seoul, South Korea). The average distance deviation and the volume difference were quantified.

Results

The volumes of the defect models obtained from the MRI and laser-scans were 537 mm^3 and 405 mm^3 , respectively, implying that the MRI model was 33% larger than the laser-scan model. The surface areas were 479 mm^2 and 431 mm^2 for the MRI model and the laser-scan model, respectively. The average ($\pm$ Standard Deviation) distance deviation of the exterior surface of the MRI model from the laser-scan model was 0.4 ± 0.4 mm. The distance deviation map is shown in Figure 5.

The custom-shaped grafts created from the molds were directly compared to the laser-scanned original defect used to create the molds. The two models were qualitatively very similar (Figure 6). The volume and the surface area of the custom-shaped cartilage tissue were 463 mm^3 and 444 mm^2 , respectively, which were 15% and 3% larger than the laser-scan model (405 mm^3 and 431 mm^2 , respectively). The average ($\pm$ Standard Deviation) distance deviation between the two models was 0.04 ± 0.19 mm.

Discussion

This study demonstrated that the creation of a custom-shaped engineered graft from an anatomic defect on a distal femur of an otherwise intact knee can be fabricated using 3D MRI data and rapid-prototype manufacturing techniques. This process was based on the assumption that the engineered grafts can be constructed using injectable matrix materials combined with autologous stem cells and growth factors as reported in our previous study (6). In many cases, the major reason for failure of cartilage grafts is increased interfacial stresses between the native and the transplanted tissues due to graft-defect mismatch, which affects cell viability and collagen formation (7,8). This type of failure may be minimized by inserting a bone-cartilage tissue graft which matches the shape of the defect.

With these assumptions in mind, the 3D defect model was created from non-invasive MRI data through manual segmentation and a 3D geometry reconstruction process. The MRI-based model was very similar to the original defect shape qualitatively, but showed 33% overestimation in the volume. Actually, the absolute volume overestimation (132 mm^3) was small, but the large percentage of error was due to the small volume of the defect. The overestimation in the volume may actually prove useful in the process of surgical

implantation, by providing the ability to press-fit the graft into the defect without fixation or to create a safe buffer in case the surrounding cartilage margin is unhealthy and needs minor debridement.

A previous study has reported the accuracy in detecting in-vivo 3D cylindrical defects in tibial cartilage with 1.5T MRI. The average error in detecting the diameters of cylindrical defects with 3, 5 and 8 mm diameters were 1.29, 1.03 and 0.10 mm, respectively (11). Our results, with an average error of 0.4 mm for the overall surface, were very similar to the 0.65 and 0.52 mm deviations in radii for the 3 and 5 mm defects in the previous study. We can also infer using results from Graichen et al. (11) that the error in detecting the shape of the defect would increase for a smaller defect. Therefore, the size of the defect should be considered when using this technique.

The resolution of MR imaging data using a 1.5 Tesla (T) magnet was $0.625 \times 0.625 \times 0.7 \text{ mm}^3$ for each voxel. The shape of the cartilage tissue from the molds was very close to the original defect used in creating the molds. The average distance deviation of the graft produced via laser scan was $0.04 \pm 0.19 \text{ mm}$ with a 95% confidence interval of -0.33 and 0.41 mm , which is very similar compared with the average distance deviation of the MRI-based model (0.4 mm). Currently, the major source of error in manufacturing a 3D custom shaped cartilage graft appears to be the accuracy of a MRI data, but the accuracy of the model is expected to increase by utilizing advanced musculoskeletal MR sequences with higher magnet strengths (3T) (14,15,16). Furthermore, since the location of the defect is often known, the MRI could target this location by using a small surface coil to provide the signal noise reduction (SNR) necessary to obtain higher resolution images. A laser-scan-based defect model was used as a standard in testing the accuracy of the MRI custom-shaped grafts. The accuracy and the methods of creating 3D models of tissue shapes from laser-scans have been comprehensively presented in a previous study (12).

A new method of creating parting line was introduced in this study for designing molds requiring a smooth surface to recreate a seamless articular surface. The position of the parting line would also allow the ability to easily peel-off of the tissue from the molds without disruption of the smooth articular surface.

The molds were created using the using fused deposition modeling (FDM) rapid-prototyping technique. Medical grade, sterilizable thermoplastic material was used to allow use in tissue engineering applications. This technique also allows the molds to be manufactured with an accuracy of $\pm 0.127 \text{ mm}$. Future improvement in thermoplastics should allow the use of 3D printing techniques, which are rapid and much less expensive than FDM.

Conclusion

This investigation proves the concept that custom-shaped engineered grafts can be fabricated from standard sequence 3-D MRI data with the use of CAD and rapid-prototyping technology. Qualitatively, the articular defect details were captured on the mold surfaces using the MRI-based 3D data, and the overall accuracy, or average distance deviation, was 0.4 mm. The custom-shaped graft was very similar to the original anatomic defect both qualitatively and quantitatively. The overall accuracy of the custom-shaped grafts should increase with more accurate 3D MRI data, which could be achieved through further advancements in the resolution, contrast and magnet strength.

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Figure 1.

(a) Artificial defect created surgically on the distal femur and (b) 2-D MRI of the defect.

Figure 2.

(a) Defect model from MRI (b) parting plane rendered as wireframes and polygons (c) top mold (d) bottom mold (e) Top and bottom molds with translucent rendering; injection port is visible in the bottom mold.

Figure 3.

(a) FDM top and bottom molds (b) Injection of polyurethane cartilage through the injection channel in the bottom mold, (c) actual polyurethane graft produced from mold.

Figure 4.

(a) The process of creating the 3D defect model from MRI and (b) the process of creating the 3D defect model from the actual distal femur with articular cartilage using laser-scanning (bottom).

Figure 5.

Distance deviation map showing the differences between the laser-scan based defect model and the MRI based defect model. Negative values illustrate that the laser-scan-based model is deviated in the surface normal direction (outward) of the MRI-based model.

Figure 6.

(a) Laser-scanned defect model and the defect model from the molds created from the 3D MRI fabrication model (b) distance deviation between the two models. The positive values represent the fact that the mold-based model deviated inwards in comparison with the MRI-based model.