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THE EFFECT OF STORAGE MEDIUM TONICITY ON OSTEOCHONDRAL AUTOGRAFT PLUG DIAMETER

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Abstract

Purpose—The purpose of this study was to investigate the effect of differing storage medium on osteochondral plug diameter.

Methods—Four storage conditions were evaluated: air, hypotonic (sterile water), isotonic saline (0.9% NaCl), and hypertonic saline (3.0% NaCl). Four osteochondral plugs were acquired (4.5 mm harvesting system) from each of 10 fresh calf femurs and randomized to one of four storage media (n=40). Micro-CT was used to evaluate the precise diameter of each plug. Following a time zero scan, each plug was placed in a designated storage medium and rescanned at three time points over approximately one hour. A region of interest was identified from approximately 1 to 6 mm proximal to the tidemark. Custom software automatically calculated the diameter of each plug.

Results—The time zero plug diameter (mean ± CI) for all specimens was 4.66 ± 0.01 mm. There were no significant differences between any of the groups at the baseline scan. There were also no significant differences between the time zero and subsequent scans of the unsubmerged specimens. However, all of the liquid solutions (hypertonic, isotonic, and hypotonic) resulted in a significant increase in diameter from their baseline scans (p < 0.05), indicating a cause may be increased extracellular matrix fluid pressure.

Conclusions—Placing an osteochondral plug in a liquid solution increased the diameter of the subchondral bone. Size increase from the storage medium appeared to level off within 14 minutes after being placed in solution.

Clinical Relevance—Increases in diameter of the plug may alter the ease of insertion of the graft, possibly increasing contact pressure on cartilage during plug implantation.

Keywords

Osteochondral; Plug; Bone; Cartilage; OATS; Mosaicplasty

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INTRODUCTION

Many surgical techniques exist for the treatment of focal cartilage lesions. These include marrow stimulation techniques, cell-based repair techniques, and osteochondral transplantation techniques.¹⁻⁴ Osteochondral transplantation is the only of these techniques to immediately replace defects with mature, hyaline articular cartilage.⁵ Transplanted plugs can be harvested from a non-weight bearing region (autograft) or from a cadaveric donor (allograft). Prolonged storage necessary for allograft transplantation procedures has been shown to decrease chondrocyte viability, metabolic activity, and viable cell density.⁶ Autograft transplantation procedures eliminate the need for prolonged storage and avoid risks of disease transmission. These procedures typically require harvesting multiple small plugs from the periphery of both femoral condyles with a cylindrical cutting device.⁷⁻⁹ Recipient site tunnels are drilled and the osteochondral plugs are inserted using a tamp, attempting to restore native curvature of the joint.¹⁰ Grafts are temporarily stored between the time of harvest and implantation.

Impact loads during the time of insertion of osteochondral allograft plugs can produce damaging loads that have been shown to cause chondrocyte death.¹¹ Impaction of these plugs can increase cell death in the superficial zone by 47% within 48 hours.¹¹ The magnitude of cell death is linked to the impaction force required to implant these plugs. Oversized plugs require more impaction force which likely results in increased cell death and potentially compromises the successful outcome of the osteochondral reconstruction. Therefore, maintaining or reducing the diameter of the plugs during temporary storage would likely reduce the amount of impaction force needed to implant the plug and would therefore minimize chondrocyte death. It has been observed that saline soaked plugs are more difficult to implant than plugs stored dry.⁷

The purpose of this investigation was to quantify changes to plug diameter over time for four storage media (hypertonic, isotonic, hypotonic, and dry) *in vitro*. Our hypothesis was that plug diameter would be affected by storage medium. We expected that a plug placed in liquid solution would increase in diameter, while a plug left unsubmerged (“dry”) would not significantly change in diameter. Furthermore, we hypothesized that hypotonic storage solution would result in larger increases to plug diameter if these increases are mediated by intracellular swelling pressures.

MATERIALS AND METHODS

Specimen Acquisition

Femurs from 10 fresh calf legs were dissected. Three osteochondral plugs were obtained from each femoral condyle using a 4.5 mm disposable commercial coring system (MosaicPlasty DP-Disposable Harvesting System, Smith & Nephew, Andover, MA) to a depth of 10 mm (Figure 1). The 4.5 mm coring diameter was verified with multiple digital caliper measurements. Four specimens were selected from six harvested specimens to eliminate plugs that were noticeably oblique or lacked sufficient bone. In cases where five or more plugs were sufficient, four were randomly chosen. The four specimens from each calf knee were block randomized to each of four storage media: air (no liquid solution), hypotonic (sterile water), isotonic (0.9% NaCl), or hypertonic (3.0% NaCl). Plugs were placed into microfuge tubes at room temperature with no solution (solution was added later after the baseline measurement). Harvest position was recorded for each specimen. This resulted in a total sample size of 40 osteochondral plugs (n = 40).

Imaging Technique

Each block of four samples from a single specimen were imaged in a single group. Microcomputed tomography (micro-CT) images (45 μm voxel resolution) of cartilage plugs were obtained by collecting 360, 512 x 512 12-bit projection radiographs at 1° intervals around the entire specimen (80 kVp; 450 mA). These images were acquired using the eXplore Locus in-vivo micro-CT (GE Healthcare, Piscataway, NJ) gantry based scanner with rotating X-ray source and detector (fixed anode with tungsten target source, operating from 40–80 kVp at 0.5 mA max current). Following acquisition, reconstruction of projection data was performed on a 4PC Unix Cluster (8GB RAM, ~40 minutes per volume) using a multi-threaded, modified Feldkamp reconstruction algorithm.¹²

A baseline scan was performed on all specimens. This scan was taken 29.6 ± 2.3 (mean $\pm$ 95% confidence interval, this notation is used throughout) minutes after the time of harvest. Following the baseline scan, the assigned storage media were added to each microfuge tube (except the dry medium). Each specimen was then scanned three additional times at room temperature. Scans were performed immediately following the previous scan time for each group. These scans were performed at 14.1 ± 1.4 , 40.1 ± 1.8 , and 66 ± 2.8 minutes after being placed in the assigned storage medium.

Diameter Calculation

All scans for each specimen were registered to one another using custom designed software that utilizes a mutual information/joint histogram algorithm with simplex optimization. Prior to algorithm implementation, both volumes were rendered in 3D with the volume to be registered pseudocolored in blue. The pseudocolored volume was then translated and rotated using mouse inputs for rough alignment to the original (fixed) volume. The algorithm was initiated with volume positioning updated for each iteration. Following convergence, the volume to be registered was transformed, re-sampled, and resized to the resolution of the fixed volume. Registration accuracy was verified within the program by simultaneously scrolling thru 2D slices (sagittal, coronal, and transverse) of the registered volumes. This ensured that all measurements of diameter were taken in the same locations.

Following registration, a region of interest was manually defined from approximately 1 mm to 6 mm proximal to the tidemark (Figure 2a). This region of interest was thresholded to segment bone from storage medium, creating a binary mask (MicroView version 2.1.2, GE Healthcare, Niskayuna, NY). A custom software program aligned a best-fit circle to the convex hull of this mask using simplex optimization and a mean-squared-error metric. This best-fit circle algorithm was applied to each slice in the region of interest, resulting in approximately 100 measurements for each specimen (Figure 2b). These measurements were averaged to form a single diameter measurement for each specimen. The root mean square (RMS) error was calculated for each diameter measurement.

Statistical Analysis

Minitab (State College, PA) was used for all statistical analysis. An ANOVA was used to statistically analyze the effect of time on each storage medium. A second set of ANOVAs were used to statistically analyze the effect of solution type within each time point. Tukey post hoc analyses were used to evaluate differences between groups for all ANOVAs.

RESULTS

All plugs were harvested using a 4.5 mm inner diameter disposable harvesting system. The 4.5 mm inner diameter was verified by multiple measurements with a digital caliper. The baseline measurement for all specimens was 4.66 ± 0.01 mm (mean $\pm$ 95% CI). There was

not a significant difference between any of the storage medium groupings at this baseline measure.

An ANOVA was performed for each of the storage media (dry, hypotonic, hypertonic, isotonic), and Tukey post hoc analyses were used to compare differences between the four scans for each specimen (Figure 3). For the dry group, there were no statistically significant differences between time points, i.e. the diameter of the plugs in the dry medium stayed approximately constant throughout all scans. For all liquid media (hypertonic, hypotonic, isotonic), there was a significant change from baseline for each of the time points. P-values and plug diameters are reported in Table 1.

A second set of ANOVAs were performed within time points to analyze statistically significant differences between storage media at each time point. For the first two time points following baseline, there was a statistically significant difference between only the dry and hypotonic storage media ($p = 0.012$ for time point 1 and $p = 0.024$ for time point 2). For the final time point, there were statistically significant differences between the dry and hypertonic media ($p = 0.003$), and between the dry and hypotonic media ($p = 0.009$). At all time points there were no statistically significant differences between any of the liquid media.

DISCUSSION

Clinical experience has suggested that osteochondral plugs swell when exposed to saline.⁷ We tested this hypothesis and further investigated whether the tonicity of the solution affects bone swelling. Hypertonic solutions have higher solute concentrations than those inside cells resulting in a net movement of water out of cells. Hypotonic solutions have a lower solute concentration than that inside cells resulting in a net movement of water into cells. We therefore hypothesized that our plugs stored in a hypotonic solution would swell more than those placed in the hypertonic solution if mediated by intracellular swelling pressures. Our hypothesis regarding swelling in solution was confirmed in that we did see a significant increase from baseline plug diameter for all plugs placed in liquid solution. Furthermore, we found no significant difference between the baseline plug diameter and later scans for the dry storage medium group. However, we did not find a relationship between solute levels and changes to plug diameter. We also did not find a significant difference between any of the liquid media at any of the time points.

Traumatic impact on joints has been shown to lead to chondrocyte death in both human clinical studies^{13–15} and experimental animal models¹⁶. In experimental animal models, loading rate¹⁷ and contact stress¹⁸ are independently associated with chondrocyte death. Boranzjani et al¹¹ investigated the effect of impact load on chondrocyte viability specifically in osteochondral allograft plugs. They found that rates and stresses generated during impaction of the plugs for implantation were sufficient to cause chondrocyte death. Plugs that increase in diameter between the time of harvest and time of implantation require more force to implant. It seems likely that these plugs, which require more impact force, would exhibit increased chondrocyte death. This study showed that plugs placed in any of the liquid solutions studied (hypotonic, hypertonic, and isotonic saline) increase in diameter and may therefore be predisposed to decreased chondrocyte viability due to increased impaction loads.

It was noted that the removal of osteochondral plugs from the 4.5 mm inner diameter harvesting system led to a rapid expansion in plug diameter (an increase of 0.18 mm) that did not change with further “dry” storage. One explanation for this finding is that the osteochondral tissue at the femoral condyle maintains a state of pre-compaction *in vivo*. This

state of compaction remains after the tissue is removed so long as it remains inside the harvesting device. However, when the osteochondral tissue plugs are removed from the harvesting device, this natural state of compaction is released and the tissue expands to a new equilibrium state even when stored without submersion in a liquid solution.

Consistent with the clinical experiences of Morelli et al⁷, the results from our study indicate that submerging osteochondral plugs in the liquid solutions studied further increased the diameter of the plugs. Submersion of these plugs in a liquid solution would seem to permit bulk water to flow into the tissue space in these plugs causing further swelling pressures that lead to an increase in tissue volume. Such an increase in tissue volume via hydration could occur as a result of intracellular fluid pressures and/or extracellular matrix fluid pressures.¹⁹

One means to distinguish between these two sources of fluid pressure is to slightly alter the amount of dissolved solutes in solution, which will modulate preferentially intracellular fluid movements, and less so for extracellular matrix fluid movements. Our results indicated that osteochondral plug diameters increased independent of the amount of dissolved solutes in the bathing fluid solution, and suggest that water movement into/out of cells is not likely to be the mechanism for the increase in diameter of these plugs. Instead, the data suggest that increases in extracellular matrix fluid pressure within the soft tissue regions within the cancellous bone volume, such as those mediated by proteoglycans¹⁹, are likely to be one contribution for the cause of the increase in plug diameter when they are stored submerged in a fluid solution. Additionally, it is conceivable that cancellous bone in vivo may exist in a pre-compressed state that could expand in a limited fashion when explanted out of its natural location.

Our first post baseline micro-CT scan of the plugs was taken approximately 14 minutes after being placed in solution. The specimens were then rescanned two additional times at approximately 40 and 66 minutes after being placed in storage medium. The plug increase in plug diameter appeared to level off by the first measurement. This rapid increase in plug diameter is consistent with our proposed mechanism of increased hydration of the tissue's extracellular matrix leading to elevated fluid pressure. This indicates that even short exposures to liquid media may increase the diameter of the plugs as much as those soaked an hour or more.

There were limitations to our study. First, we acknowledge that this study was performed on bovine explants. While it is possible that human samples will respond to storage media differently, we find this unlikely given the similarities between bovine and human osteochondral explants. Furthermore, the methods used could not be employed on human subjects, and cadaveric explants cannot be attained immediately following death. Second, although we obtained sequential MicroCT measurements as rapidly as possible, it appears the change in plug diameter occurred between the baseline and first time points (approximately 14 minutes). Future studies should focus on how size changes during this interval. Finally, our study investigated changes to bone plug diameter and did not directly measure chondrocyte viability, so we are unsure of the effect of the storage media themselves on chondrocyte viability. Previous research has documented that changes to irrigation solution may inhibit the normal synthesis of proteoglycan by chondrocytes.²⁰ Furthermore, while dry storage may protect against plug expansion, the resulting desiccation of the cartilage may also lead to cell death.

CONCLUSIONS

Placing an osteochondral plug in a liquid solution increased the diameter of the subchondral bone. Size increase from the storage medium appeared to level off within 14 minutes after being placed in solution.

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Figure 1.

Six 4.5 mm osteochondral plugs were obtained from each specimen. (A) Osteochondral plug harvest locations, (B) single osteochondral plug.

Figure 2.
(A) Region of interest selection was manually selected from approximately 1 mm to 6 mm proximal to the tidemark, (B) illustration of circle-fit. Approximately 100 diameter measurements were made on each specimen.

Figure 3.

Effect of time on plug diameter for the four storage media (mean $\pm$ 95% confidence interval). Time points after being placed in the assigned solution medium were #1 (14.1 ± 1.4 minutes), #2 (40.1 ± 1.8 minutes), and #3 (66 ± 2.8 minutes). Statistical significance notes a significant change from the baseline measurement of each storage medium (* $p < 0.05$) and significant differences between storage media at each time point († $p < 0.05$). Plugs were acquired with a 4.5 mm disposable harvesting system.

Table 1

Plug diameters by time point.

	Baseline	2nd Scan	3rd Scan	4th Scan
Dry storage medium diameter (mm)	4.68 ($\pm$ 0.03)	4.68 ($\pm$ 0.04) (p = 1.000)	4.68 ($\pm$ 0.03) (p = 0.983)	4.68 ($\pm$ 0.03) (p = 0.998)
Hypertonic storage medium diameter (mm)	4.66 ($\pm$ 0.03)	4.70 ($\pm$ 0.05) (p = 0.024)	4.72 ($\pm$ 0.05) (p = 0.002)	4.73 ($\pm$ 0.04) (p < .001)
Hypotonic storage medium diameter (mm)	4.65 ($\pm$ 0.05)	4.73 ($\pm$ 0.05) (p = 0.001)	4.73 ($\pm$ 0.06) (p = 0.001)	4.72 ($\pm$ 0.05) (p = 0.003)
Isotonic storage medium diameter (mm)	4.66 ($\pm$ 0.05)	4.71 ($\pm$ 0.05) (p = 0.018)	4.72 ($\pm$ 0.05) (p = 0.005)	4.71 ($\pm$ 0.05) (p = 0.022)

NOTE: Data are presented as mean ($\pm$ 95% confidence interval of the mean). P-values indicate the Tukey post hoc comparison to the baseline value specific to each storage medium.