The Effect of Storage Medium Tonicity on Osteochondral Autograft Plug Diameter

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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 solution (sterile water), isotonic saline solution (0.9% sodium chloride), and hypertonic saline solution (3.0% sodium chloride). Four osteochondral plugs were acquired (4.5-mm harvesting system) from each of 10 fresh calf femurs and randomized to 1 of 4 storage media (N = 40). Micro–computed tomography was used to evaluate the precise diameter of each plug. After a time 0 scan, each plug was placed in a designated storage medium and rescanned at 3 time points over approximately 1 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 0 plug diameter (mean ± 95% confidence interval) 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 0 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 ($P < .05$), indicating that 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 placement 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.

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 one 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 by use of a tamp, with an attempt to restore the 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

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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 4 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 whereas 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.

METHODS

Specimen Acquisition

Femurs from 10 fresh calf legs were dissected. Three osteochondral plugs were obtained from each femoral condyle by use of a 4.5-mm disposable commercial coring system (MosaicPlasty DP-Disposable Harvesting System; Smith & Nephew, Andover, MA) to a depth of 10 mm (Fig 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 5 or more plugs were sufficient, 4 were randomly chosen. The 4 specimens from each calf knee were block randomized to each of 4 storage media: air (no liquid solution), hypotonic (sterile water), isotonic (0.9% sodium chloride), or hypertonic (3.0% sodium chloride). 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 ($N = 40$).

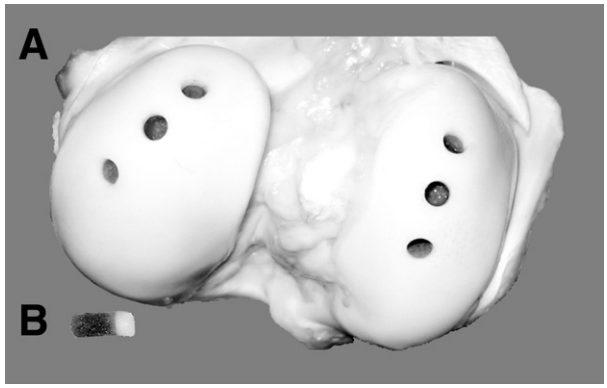

FIGURE 1. Six 4.5-mm osteochondral plugs were obtained from each specimen. (A) Osteochondral plug harvest locations. (B) Single osteochondral plug.

Imaging Technique

Each block of 4 samples from a single specimen was imaged in a single group. Micro–computed tomography (micro-CT) images (45-μm voxel resolution) of cartilage plugs were obtained by collecting 360, 512 $\times$ 512, 12-bit projection radiographs at 1° intervals around the entire specimen (80 kilo-volt potential, 450 mA). These images were acquired with the eXplore Locus in vivo micro-CT gantry-based scanner (GE Healthcare, Piscataway, NJ) with rotating X-ray source and detector (fixed anode with tungsten target source, operating from 40 to 80 kilo-volt potential at 0.5-mA maximum current). After acquisition, reconstruction of projection data was performed on a 4PC Unix Cluster (HP, Palo Alto, CA) (8 GB of RAM, approximately 40 minutes per volume) by use of a multithreaded, modified Feldkamp reconstruction algorithm.[12]

A baseline scan was performed on all specimens. This scan was taken 29.6 $\pm$ 2.3 minutes (mean $\pm$ 95% confidence interval [this notation is used throughout]) after the time of harvest. After the baseline scan, the assigned storage media were added to each microfuge tube (except the dry medium). Each specimen was then scanned 3 additional times at room temperature. Scans were performed immediately after the previous scan time for each group. These scans were performed at 14.1 $\pm$ 1.4, 40.1 $\pm$ 1.8, and 66 $\pm$ 2.8 minutes after placement in the assigned storage medium.

Diameter Calculation

All scans for each specimen were registered to one another by use of custom-designed software that uses a mutual information/joint histogram algorithm with