Effects of Micronized Cartilage Matrix on Cartilage Repair in Osteochondral Lesions of the Talus

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Category: Ankle,Arthroscopy,Sports

Keywords: osteochondral lesion, talus, OLT, biocartilage, OCD

Introduction/Purpose: A promising new technique in the treatment of osteochondral lesions of the talus (OLT) involves the use of an acellular micronized cartilage matrix (MCM), BioCartilage, to fill the lesions. The micronized cartilage matrix is thought to improve the production of hyaline-like cartilage by resident cells in a cartilage defect, but its effect on bone marrow cells remains untested. Here we hypothesized that adding bone-marrow derived stem cells to the BioCartilage would result in the chondrogenic differentiation of the stem cells. We designed an in-vitro model to mimic the clinical situation to determine if the combination of MCM and human bone marrow derived mesenchymal stem cells (MSCs) would produce a hyaline-like cartilage in-vitro to ultimately provide a reliable, one-step treatment for osteochondral lesions in the talus.

Methods: Human bone marrow-derived stem cells were obtained from consented patients and expanded in monolayer culture using standard protocols, to a maximum passage of 4. Viability was measured using Live/Dead cell viability assays (Thermofisher), and imaged on a Nikon TE2000 inverted fluorescent microscope. A custom-manufactured polysulfone device was created with four 6mm diameter 3mm deep indentations in agarose within each well of standard 6-well culture plates (Figure 1A-C). In each well, we placed chrondrogenic media with cells+micronized matrix to a depth of 2mm and covered with a 1mm layer of TISSEEL fibrin glue as is done clinically. Control groups had either no cells, or no MCM. At the end of 3 weeks, cartilage constructs were extracted and divided to perform viability, histology, and gene expression analysis (Figure 1D). Experiments were performed with 4 technical replicates, and repeated at least 3 times. Statistical analysis was performed using ANOVA with Dunnett's test.

Results: We found that stem cells were almost immediately killed when added directly to the dry micronized cartilage powder. Rehydrating the micronized cartilage prior to addition of cells was required to maintain the viability of the added stem cells, with no statistically significant difference between rehydration with serum or saline. After 3 weeks of culture in chondrogenic media, we observed that the combination of stem cells and micronized cartilage produced a cohesive structures that were easily handled, suggesting chondrogenic differentiation of the stem cells. Without the micronized matrix, the stem cells did not form viable constructs. In constructs that contained both cells and micronized cartilage, the 3-week cell viability was over 98%, with no dead cells visible in many constructs.

Conclusion: Our study demonstrates that the micronized cartilage matrix is a suitable scaffold for the chondrogenic differentiation of bone marrow-derived stem cells, given that the matrix is first rehydrated before adding cells. Technical observations include that the MCM itself generated a "dead cell" signal initially, therefore the normalized total number of live cells in each condition was used for statistical comparisons. After 3 weeks of culturing under chondrogenic media conditions, we observed robust cell survival with nearly 100% viability. Preliminary results suggest cartilage matrix deposition occurred surrounding the cells after 3 weeks of chondrogenic culture.

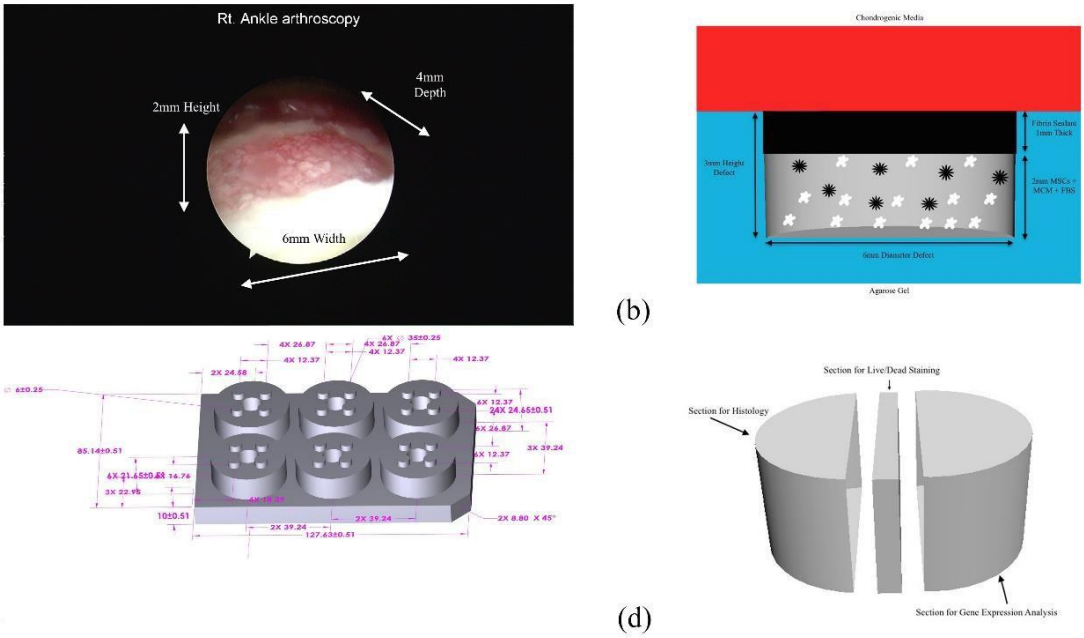

Figure 1. (a) Osteochondral lesion of the talus in vivo with implanted Biocartilage. (b) Schematic of defect replication in agarose including Mesenchymal stem cells, micronized cartilage matrix, Fibrin sealant and chondrogenic media. (c) Graphic design of custom-made polysulfone defect mold. (d) Illustration of defect harvesting technique yielding three sections.

Foot & Ankle Orthopaedics, 2(3)
DOI: 10.1177/2473011417S000055