Enzymatic Treatment and Cryopreservation of Minced Porcine Cartilage

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Statement of Purpose: Several methods currently available to improve cartilage repair include debridement, bone marrow stimulation, mosaicplasty, and autologous chondrocytes implantation. However, these current cartilage repair techniques still suffer from disadvantages such as unstable fibro-cartilage, donor site morbidity, and/or multiple surgeries. The ideal cartilage repair method would have chondrogenic potential via cells, chondro-inductive potential, and be chondro-conductive such as using a scaffold capable of supporting chondrocyte adhesion and the production and retention of cartilage-specific ECM. Current treatments do not possess all of these attributes. Allograft cartilage potentially satisfies the requirements of being conductive, inductive, and chondrogenic while eliminating donor site morbidity. However, adult cartilage fragments contain a limited number of cells and the cells do not readily migrate out of the dense ECM. In addition, viable allograft cartilage tissue has a short shelf-life. Partial disruption of the ECM of healthy cartilage fragments will allow cells to migrate out of the ECM and begin to divide and produce new ECM and improve integration of neo-cartilage with the host tissue. Cryopreservation of cartilage fragments will allow for a longer shelf-life of viable allograft cartilage. The aim of this study is to evaluate the effect of partial tissue digestion with a collagenase and neutral protease blend followed with cryopreservation, on the in-vitro bioactivity of porcine tissue fragments as compared to untreated tissue.

Methods: Cartilage was harvested from adult porcine condyles. The cartilage was minced and sieved between 0.1 and 1.2mm. Then, the fragments were divided into two groups: a control group and a treated group. The treatment consisted of a collagenase and neutral protease enzyme blend concentration at 0.7 mg/ml for 40 minutes at 37°C. After processing, the treated groups were rinsed with saline to remove excess enzyme. The fragments were suspended in DMEM with 10% DMSO and frozen overnight to -80°C at a rate of -1°C per minute. Then, the fragments were transferred and stored in the vapor phase above the liquid nitrogen. Both control and treated fragments were cryopreserved for 9 weeks. Nine fragments from each group in duplicate were placed in a 3 column by 3 row grid in separate dishes. The fragments were embedded in 2% low melting agarose. The constructs were cultured for 6 weeks in complete DMEM with 10%FBS. Media was changed twice a week. The cartilage fragments were monitored and photographed for signs of chondrocyte outgrowth.

Results: The untreated cartilage fragments displayed low outgrowths of 12 out of 18 fragments (Table 1). The cellular density of the fragments displaying outgrowth in the control group remained low (Figure 1). The first sign of outgrowth for the control group occurred at 14 days, with an outgrowth ratio of 2 out of 18 fragments. The average time to outgrowth in the control group was 29 days. The treated fragments exhibited a higher ratio of outgrowth of 17 out of 18 fragments. In addition, the average time-to-outgrowth in the treated groups was only 12 days, with the first sign of outgrowth occurring at 6 days and an outgrowth ratio of 6 out of 18 fragments. Consequently, the cellular density around the treated fragments with outgrowth was visibly higher compared to treated group (Figure 2).

Table 1. Visible cellular outgrowth ratios and average days to outgrowth throughout fragment culture.

Groups	Final Outgrowth Ratio	Final Average Days to Outgrowth
Control	12/18	29 ± 10
Treated	17/18	12 ± 11

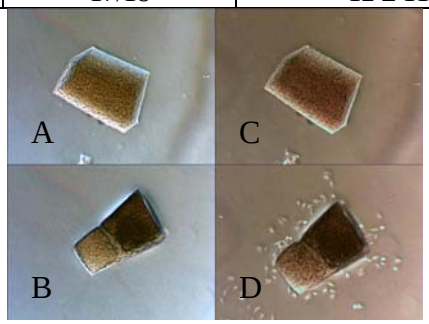

Figure 1. Representative image of control fragments (A-B) at t=0 and (C-D) at t=6wks of culture.

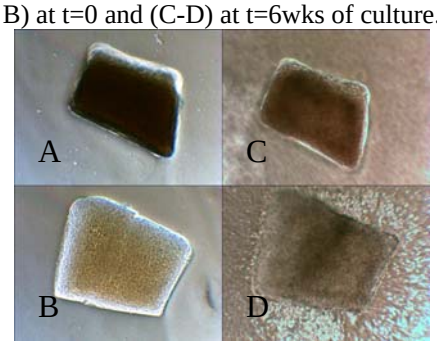

Figure 2. Representative image of treated fragments (A-B) at t=0 and (C-D) at t=6wks of culture.

Conclusions: The ideal cartilage repair strategy would encompass a treatment that is chondrogenic, chondro-inductive, and chondro-conductive. The use of allograft cartilage is a potential treatment which satisfies all three requirements. Potential pitfalls are the short shelf-life of viable cartilage allograft and the inherent bio-inactivity of adult chondrocytes encapsulated in the dense ECM. However, our study has shown a partial digestion treatment drastically increases chondrocyte outgrowth when compared to untreated controls. In addition, partially digested cartilage fragments remained bioactive after cryopreservation. Future studies will evaluate the effect of fragment size on bioactivity after cryopreservation. In addition, biochemical analysis and histological analysis need to be performed to determine the type of ECM that is being produced by the enzymatically treated and cryopreserved cartilage fragments.