Enzymatic Treatment of Minced Porcine Cartilage Improves Cellular Outgrowth and GAG Production in 3D in vitro Cultures

Alex J. McNally, Chris Chapman, Kurt Sly, Steve Lin
Exactech, Inc. Gainesville, FL

Statement of Purpose: Several cartilage repair techniques are available clinically, but suffer from disadvantages such as multiple surgeries, donor site morbidity, and/or unstable fibro-cartilage tissue[1]. Surgical techniques utilizing particulated cartilage fragments alleviate the need for multiple surgeries; however, the fragments may contain a limited number of cells and the cells do not readily migrate out of the dense extracellular matrix (ECM)[2]. Partial disruption of the ECM of cartilage fragments may permit cell migration and improve integration of neo-cartilage with the host tissue. The aim of this study is to evaluate the effect of partial digestion with a collagenase and neutral protease blend on the in-vitro bioactivity of porcine chondrocytes in 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. Following harvesting and sieving, the fragments were divided into four groups: a control group and three different treatments all carried out at 37°C. The treatments consisted of low enzyme concentration at 0.1 mg/mL for 20 minutes (L20), low concentration for 40 minutes (L40), and high concentration at 0.7 mg/mL for 40 minutes (H40). After processing, all groups were rinsed with saline to remove excess enzyme. Twelve fragments from each group were placed in a 4 column by 3 row grid in separate dishes . The fragments were embedded in 2% low melting agarose. The constructs were cultured for 5 weeks in complete DMEM with 10% FBS. Media was changed twice a week. The cartilage fragments were monitored and photographed weekly for signs of chondrocyte outgrowth. The cellular activity and viability was monitored weekly with alamarBlue reagent. Histological analysis was performed with safranin-O and fast green staining. Immunohistological analysis was performed with collagen type II.

Results: The untreated cartilage fragments remained viable throughout the 5 week culture (Figure 1) but displayed extremely low outgrowth (1 out of 23 fragments) and sulfated glycosaminoglycan (sGAG) production (Table 1 & Figure 2). The treated fragments remained viable throughout culture as well but also showed an increase in cellular activity (Figure 1). After 5 weeks of culture a high percentage of fragments in the treated groups exhibited outgrowth (Table 1). The presence of neo-cartilage outgrowth was confirmed histologically. In addition, the neo-cartilage stained positive for safranin-O, indicating the presence of sGAG (Figure 2). Immunohistochemistry staining reveals that the neo-cartilage contains collagen type II (Figure2). There is no statistical difference in time to outgrowth between the treated groups. In addition, no differences were noticed histologically between the different treatment groups.

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

Groups	Outgrowth Ratio	Average Days to Outgrowth
Undigested	1/23	31
L20	21/24	19 ± 5
L40	21/24	20 ± 5
H40	22/24	19 ± 5

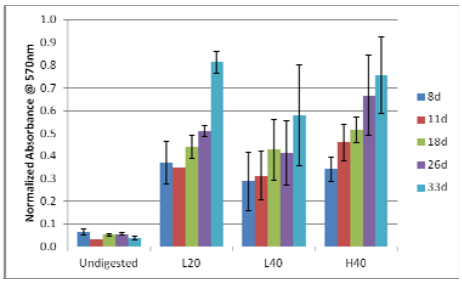

Figure 1. alamarBLUE activity of cartilage fragments

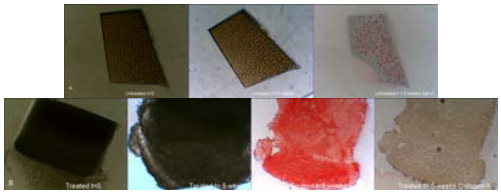

Figure 2. (A) Outgrowth and Saf-O histology of untreated cartilage fragments. (B) Outgrowth, Saf-O, and collagen type II histology of treated cartilage fragments.

Conclusions: Several studies have shown that cartilage is a source of chondrocytes which can be used to treat cartilage defects. However, adult cartilage cells cannot easily migrate out of the ECM. Treatments which disrupt the ECM of cartilage fragments may aid in cellular migration and subsequent neo-tissue formation required for integrative repair. We have investigated a treatment process which drastically increases chondrocyte outgrowth when compared to untreated controls. In addition, the cells produced sGAG and collagen type II evident by the positive staining with safranin-O and IHC for type II collagen. Future studies will characterize gene expression of chondrogenic markers in untreated and treated adult cartilage after culture from porcine and other species such as horse, goat, and human. Enzyme treatment of adult cartilage fragments results in an increase in cellular activity, outgrowth, and sGAG and collagen type II production. A single surgery technique utilizing treated partially-dissociated cartilage fragments for chondral defect repair can be envisioned.

References:

1. Smith GD, Knutsen G, Richardson JB. J Bone Joint Surg [Br] 2005;87-B:445-9.
2. Qui W, et al. Wound Rep Reg 2000;8:383-391