Double Mincing Process to Improve Integrative Repair of Articular Cartilage

Powei Lee1, Chun-Nan Chen1, McNally Alex2, Kurt Sly2, Chapman Chris2, Steve Lin2.
1Exactech Taiwan, Chutung, Hsinchu, Taiwan, 2Exactech Inc., Gainesville, FL, USA.

Disclosures:

Introduction: Cartilage lesion shows very little tendency for self-repair, these injuries are maintained for years and can eventually lead to progress to catastrophic arthritis. [1,2] Current treatment techniques for cartilage lesion include debridement, bone marrow stimulation, mosaicplasty and autologous chondrocyte implantation (ACI) [3]. However, currently none can predictably restore articular surface, the repair tissue eventually degenerates to fibrocartilage and the symptoms return [4]. Cartilage is an avascular tissue and chondrocytes are surrounded in a dense extracellular matrix (ECM), in which the nutritive materials are transported of by continuous diffusion instead of through the vasculature [5,6]. Hence the mobility and proliferation of chondrocytes are limited. In this study, a double mincing method, included mechanical mincing and chemical mincing, was proposed improve integrative cartilage repair. The composition, structural changes and cell viability of articular cartilage after treatment were investigated. Treatment parameters were varied and the effects on the ECM composition and the ultrastructure of cartilage fragments were observed. The cartilage integration after double mincing was also investigated.

Methods: Articular cartilage was harvested from porcine knees and minced into fragments (<1.0 mm) by a tissue pulverizer (mechanical treatment). Then, 100 mg of pulverized fragments were partially digested with a collagenase and thermolysin blend for 20 minutes at different treatment conditions (chemical treatment). Following digestion, the fragments were rinsed with saline to remove excess enzyme. The dry weight of each treated sample was measured after freeze-drying under vacuum for 3 days, GAG content in the freeze-dried samples was quantified by Blyscan assay, and hydroxyproline content was evaluated using the Hydroxyproline (HYP) assay kit (Biovision). Cell viability was evaluated by Live/Dead staining, and the diffusion of FITC-dextran in cartilage was measured by fluorescence recovery after photobleach (FRAP) to analyze the permeability of cartilage. ECM structural changes were investigated under scanning electron microscope (SEM). Pulverized but without enzymatically digested fragments were used as a control group. The cartilage integration experiment after enzymatic treatment was performed in vitro following the method described by B. Obradovic et. al. [4].

Results: Enzymatic treatment at 25°C caused the cartilage fragments to lose 17% of dry weight and 15% of HYP content respectively. The loss of the dry weight increased three fold to 52% when the treatment temperature was raised to 42°C, while the loss of the HYP content increased to 44% (Table 1). The impact of treatment temperature on GAG content was more significant. A drop of 66% was observed at 25°C treatment temperature and increased to 78% as the temperature was raised to 42°C. When treated at the low enzyme concentration, the cartilage fragments showed a loss of more than 40% in dry weight and HYP content and more than 60% in GAG content. An increase in enzyme concentration caused a further loss in dry weight, HYP and GAG. Fluorescence microscopy analysis revealed that the dead cells tend to be located around the edges. However, the majority of the cells appear to be viable (Fig. 1a). The quantitative viability assay results were 98.0% ± 0.79, 97.7% ± 1.75, 98.0% ± 1.48 for unpulverized, pulverized, and pulverized-digested cells respectively. SEM (Fig. 1b) images revealed that partial enzymatic digestion altered the ECM structure of cartilage, and exposed the embedded chondrocytes. FRAP experiments (Fig. 2) showed the partial digestion process enhanced the permeability of cartilage, and the diffusion coefficient increase from 11.28 μm2/s to 75.01 μm2/s after partial digestion. SEM images (Figure 2) showed that partial enzymatic digestion changed the ECM structure of cartilage, and exposed the embedded chondrocytes. After 3 weeks of in vitro culture, no sign of integration was observed in the group without enzymatic treatment and a gap between the fragment edges remained visible (Fig. 3a). In contrast, integration was observed in the enzymatic treatment group (Fig. 3b), and outgrowth of tissue in the interface was also noted. In addition, a higher cell density was found in the integrative interface.

Discussion: Chondrocytes are surrounded in a dense extracellular matrix (ECM), in which the nutritive materials are transported by continuous diffusion instead of through the vasculature. Hence, the mobility and proliferation of chondrocytes are limited and the cartilage lesion shows very little tendency for self-repair. In this study, a double mincing method, included mechanical mincing (pulverization) and chemical mincing (enzymatic digestion), was proposed improve integrative cartilage repair. The mechanical treatment pulverized cartilage into small fragments, which increased mass transfer area for nutritive materials. The chemical treatment partially digested the tissue and exposed the embedded chondrocytes on the surface, which may facilitate the integration of cartilage fragments. The permeability in cartilage was also enhanced after chemical treatment. The viability of the chondrocytes was not affected by either mechanical or biochemical treatment. These effects might provide with respect to improvement of functional integration for cartilage repair. Therefore, a double mincing process may provide a valuable tool for the development and optimization of methods for integrative cartilage repair. The effect of different enzymatic treatment conditions on cartilage integration will be the topic of future research.

Significance: The capacity of integrative cartilage repair was improved by double mincing technique. The mechanical treatment
increased the surface area of cartilage fragments and the chemical treatment partially digested dense ECM to exposed embedded chondrocytes and enhanced the permeability cartilaginous ECM of cartilage fragments was by collagenase and neutral protease, exposing the embedded chondrocytes on the surface.

Acknowledgments:


<table>
<thead>
<tr>
<th>Table 1. Enzymatic digestion at different condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Dry Wt, mg</td>
</tr>
<tr>
<td>HY, µg</td>
</tr>
<tr>
<td>GAGs, µg</td>
</tr>
</tbody>
</table>

Figure 1. (a) fluorescent images of cartilage fragments labeled with calcein and EthD-1; (b) SEM images of unpulverized, pulverized, and pulverized-digested cartilage fragments.
Figure 2. Comparison of undigested and digested cartilage FRAP time course.
Figure 3. Cartilage integration, (a) without enzymatic treatment, (B) with enzymatic treatment.