Structural and Compositional Changes of Porcine Articular Cartilage After Partial Enzymatic Digestion

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Statement of Purpose: Several studies have reported that enzymatic treatment could increase chondrocyte bioactivity and improve integrative cartilage repair. Collagens, glycosaminoglycan (GAG) and lipids of the extracellular matrix (ECM) could be hydrolyzed by enzyme, and the ECM structure altered as well. In this study, the composition and structural changes of porcine articular cartilage after enzymatic 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 enzymatic digestion was also investigated.

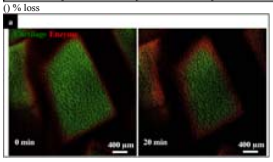
Methods: Articular cartilage was harvested from porcine knees and minced into fragments (<1.0 mm), and then ~100 mg of fragments was incubated with collagenase and neutral protease for 20 min at different treatment conditions. After the digestion, the fragments were rinsed with saline to remove excess enzyme. Their dry weights, GAG and hydroxyproline (HYP) contents were evaluated. Confocal laser scanning microscopy (CLSM) was used to observe the penetration depth of enzyme while the ECM structural changes were investigated under SEM. The cartilage integration experiment after enzymatic treatment was performed in vitro following the method described by B. Obradovic et. al. [1]. Cartilage fragments without enzymatic treatment were used as a control group (undigested fragments).

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. CLSM (Fig. 1a) confirmed the enzyme penetration and shrinkage of the fragments. The penetration depth, which was calculated from fluorescent images, was around 100 μm. SEM (Fig. 1b), revealed that partial enzymatic digestion altered 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. 2a). In contrast, integration was observed in the enzymatic treatment group, and outgrowth of tissue in the interface was also noted. In addition, a higher cell density was found in the integrative interface.

Table 1. Enzymatic digestion at different condition

	Undigested	25°C	37°C	42°C	Low Enzyme Conc. 0.25 mg/ml	Medium Enzyme Conc. 0.5 mg/ml	High Enzyme Conc. 1.0 mg/ml
		Enzyme Concentration, 0.5 mg/ml			Treatment Temperature, 37°C		
Dry Wt, mg	19.8±0.4	16.5 ± 0.9 (17%)	9.6±0.5 (51%)	9.3±1.8 (53%)	11.1±0.7 (44%)	9.2±1.0 (53%)	9.4±1.0 (52%)
HYP, μg	1966.60±259.24	1675.20±120.12 (15%)	1109.70±12.66 (44%)	1094.09±192.48 (43%)	1177.96±62.10 (40%)	1109.70±15.66 (44%)	1156.53±29.51 (43%)
GAGs, μg	3267.43±120.30	1123.87±189.55 (66%)	923.43±57.51 (72%)	725.16±154.14 (78%)	1156.57±109.07 (65%)	923.43±57.51 (72%)	845.71±74.94 (74%)

() % loss



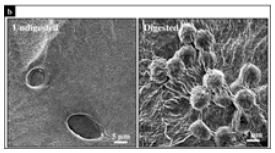
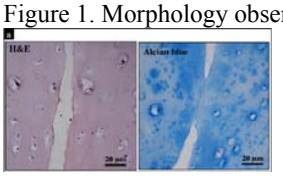


Figure 1. Morphology observation (a)CLSM, (b)SEM.



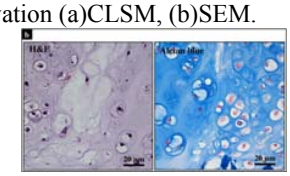


Figure 2. Cartilage integration (a)without enzymatic treatment, (B)with enzymatic treatment.

Conclusions: Several studies have shown that enzymatic digestion could increase integration of cartilage [2-4]. However, the effect of varying digestion conditions on the structure and composition of the ECM had not been systematically explored. The ECM components in cartilage tissue play a critical role in supporting chondrogenesis. Our goal in this study was to determine which structural parameter was best able to represent the degree of cartilage digestion. We observed that the change in dry weight and HYP content were highly relevant. This is a logical result, as the HYP is a major component of the protein collagen and most of the dry weight is contributed by collagen. At all the different digestion conditions, the GAG content of the fragments dropped dramatically. Increasing the temperature or enzyme concentration results in additional modest decreases in GAG levels. The results revealed that the HYP content or dry weight could be utilized as an indicator of the extent of matrix disruption. As we know, chondrocytes are embedded in a dense ECM, which limits their mobility and proliferation. In this study, cartilage fragment shrinkage could be observed by CLSM and enzyme penetration into the cartilage was calculated. SEM images further showed that the ECM of the cartilage tissue fragments was partially digested by the enzyme and the chondrocytes were exposed on the surface. This exposure, and the increase in cell density observed at the cartilage surface may facilitate the integration of cartilage fragments as seen in our experiments. The effect of different enzymatic treatment conditions on cartilage integration will be the topic of future research.

References:

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