Application of Hydrostatic Pressure Versus Vacuum in Accelerating Chemical-Based Decellularization of Cartilage

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INTRODUCTION: Decellularized allogenic or xenogenic cartilage tissue as a biological scaffold can provide an effective solution in cartilage tissue repair. Most treatments for decellularization are based on chemical agents like detergents [1]. Detergent treatment is easy to use but can have a long treatment time, residual toxicity, and a damaging effect on tissue biomechanical properties. This study aims to show the efficiency of hydrostatic pressure and vacuum for accelerating a chemical-based decellularization process of cartilage tissue in order to shorten its exposure time to the chemical.

METHODS: A chemical-based decellularization process of cartilage tissue was performed, with and without applying vacuum or hydrostatic pressure. The chemical used in this study was the detergent Sodium dodecyl sulfate (SDS, Sigma Aldrich) of 1% concentration. The application of vacuum (VAC) and hydrostatic pressure (HP) were performed by placing cartilage tissue samples in the chamber of a hydrostatic pressure generator device [2] filled with the SDS detergent. Twenty cylindrical specimens of about 8 mm diameter and 2mm thickness were cut out of the calf knee joint articular cartilage using a diamond core drill. The specimens were divided into five groups of 4 samples per each group; Group 1 (HP+SDS6) were subjected to a hydrostatic pressure of 10 MPa in SDS detergent for 6 hours; Group 2 (VAC+SDS6) were subjected to a Vacuum of 23 in. Hg in SDS detergent for 6 hours; Group 3 (SDS6) were treated in SDS detergent for 6 hours without applying any pressure or vacuum; Group 4 (SDS24) were treated with SDS detergent for 24 hours without applying any pressure or vacuum; and Group 5 (Native) were the native tissue without any treatment. The 6 hours and 24 hours treatments represent short-term and long-term treatments respectively. The extent of cell removal in the decellularization process was inspected by standard histological test, was used to determine the presence of cell nucleus in the cartilage samples [4]. The results of DNA data were expressed as mean ± standard deviation. Analysis of variance (ANOVA) with a significance level of p < 0.05 followed by Turkeys' multiple comparison test were performed to determine significant differences between any two groups.

RESULTS: Figure 1 shows the average of residual DNA content of the cartilage samples for different groups. The figure shows that compared with the native tissue (Native) there was a significant reduction of DNA content of about 73% and 56% (P < 0.05) for the short term pressure-detergent (HP+SDS6) and vacuum-detergent (VAC+SDS6) groups respectively, whereas the long term treatment with detergent alone (SDS24) was not nearly as effective, causing a reduction of about 28% (P < 0.05). Figure 2 shows Elastic Modulus values of different groups, indicating a significant reduction in the mechanical properties of the long term treatment with the detergent (SDS24) compared with those of all other treatment groups (Native, SDS6, HP+SDS6, and VAC+SDS6). Comparing the H&E staining images of cartilage sections of different groups, there were very few cell presence in the short term pressure-detergent (HP+SDS6) and VAC-detergent (VAC+SDS6) groups compared with those of Native, SDS6, and SDS24 groups. Figure 3 shows representative H&E staining images of (HP+SDS6) and (SDS6) groups.

DISCUSSION: In the present study, the application of vacuum or hydrostatic pressure enhanced the function of the chemical decellularization process, therefore, reducing the time of tissue exposure to the deteriorating effect of the SDS detergent. The short term treatment of 6 hours with detergent (SDS6) and without any vacuum or pressure did not cause any significant changes in either DNA content or mechanical properties (Figs. 1 and 2). The long term treatment of 24 hours with detergent and without any pressure or vacuum (SDS24) caused a reduction of only 28% in the DNA content (Fig. 1), with the cost of a significant reduction in mechanical properties (Fig. 2). However, with the application of hydrostatic pressure (HP) or vacuum (VAC), the short term treatment with detergent not only did not cause any damage to tissue biomechanical properties (Fig 2), but it caused a very significant reduction in the DNA content in a considerably shorter time (Fig. 1). Considering the levels of the hydrostatic pressure (10 MPa) and Vacuum (23 in. Hg) used in this study, the application of hydrostatic pressure was significantly more effective in reducing the cell content than that of the vacuum treatment (Fig. 1).

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents new data comparing the methods of using vacuum and hydrostatic pressure for enhancing a chemical-based decellularization process of cartilage tissue for producing ideal allogenic or xenogenic scaffolds of cartilage tissue.

REFERENCES:

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**Fig. 1.** Residual DNA contents of different groups. *Ω: Groups with any of these symbols in common are significantly different (P < 0.05).**

**Fig. 2.** Elastic Modulus (MPa) of different groups. *: Significantly lower than other treatment groups (P < 0.05).**

**Fig. 3.** H&E staining images selected from SDS6 and HP+SDS6 groups. Dark spots indicate presence of cell nucleus.