EFFECTS OF GENIPIN ON DECELLULARIZED PORCINE CARTILAGE

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ABSTRACT

This research aims to develop an alternative method to osteochondral articular transfer using decellularized, crosslinked porcine osteochondral xenografts (OCXGs). Crosslinking collagenous tissue results in greater mechanical strength, more resistance to enzymatic degradation, and reduced immunogenicity. This study used genipin, a chemical substance from the Gardenia jasminoides Ellis fruit. Genipin is inexpensive and simple to use. In this study, porcine articular cartilage disks were decellularized via a previously published method for porcine nasal septal cartilage decellularization. The process extracts glycosaminoglycan to allow for greater infiltration of nutrients and host cells. The disks were then crosslinked with 0.01% and 0.1% aqueous genipin for 3 days at room temperature with agitation. Prior to decellularization, the cartilage disks' biphasic properties were determined by confined compression testing. The test was repeated after decellularization and after crosslinking. The aggregate modulus was notably lessened after decellularization but returned to, and in most cases, exceeded that of the fresh disks after crosslinking. Genipin fixation also tended to lower the hydraulic permeability. In a separate experiment, genipin fixation of fresh cartilage was shown to slow the rate of tissue destruction by collagenase. This study demonstrates that genipin seems to be a viable alternative for crosslinking OCXGs.

Keywords: xenografts, crosslinking, genipin, cartilage, mechanical properties

INTRODUCTION

Orthopaedic surgeons come into contact with numerous patients experiencing articular cartilage lesions. According to a study that anyalyzed 25,124 knee arthroscopies from 1989-2004, 60% of the patients suffered at least one chondral lesion. Of that, 7% accounted for candidates suitable for restorative procedures, being 40 years of age or younger with one to three moderate to severe cartilage lesions [1]. Currently there is only one procedure that immediately restores hyaline cartilage to the joint surface and is capable of bearing normal loads, osteochondral articular transplantation. An osteochondral autograft transplantation removes cartilage from the edges of the patient's own joint to repair the defect. Disadvantages of this method include donor site morbidity and limited supply. On the other hand, an osteochondral allograft transplantation uses cartilage pieces from other donors. Disadvantages include graft rejection and disease transmission [2,3,4,5,6]. The purpose of this research is to develop an alternative to conventional treatments which will utilize decellularized porcine osteochondral xenografts (OCXG). Osteochondral dowels could be harvested from a porcine stifle joint, cleaned, decullarized, and crosslinked. The plugs could then be used to repair articular cartilage defects during exploratory surgery using standard OAT surgical procedures. The pig is an appropriate source of human replacement tissue due to its similar size and physiology as well as its rapid growth rate [7]. In addition to the benefits mentioned, decellularized xenografts are low cost, available in abundance, low risk for disease transmission, able to support weight and provide a solid anchorage within the joint.

The long-term goal of this research is to advance the development of deceullarization and crosslinking processes to make OCXGs more biocompatible and durable. The immediate aim is to further develop a non-cytotoxic means of chemically crosslinking collagen. A previously published study on glutaraldehyde fixation of collagenous tissue showed that crosslinking simultaneously

increases mechanical strength and decreases degradation by enzymatic processes. Crosslinking also masks antigen, resulting in a less immunogenic product [8]. Glutaraldehyde has often been used to chemically crosslink bioprosthetic heart valves but is quite cytotoxic. Leaching of any glutaraldehyde residues into nearby tissue results in necrosis [9]. Therefore, this research studies the use of non-cytotoxic genipin as a means of chemically crosslinking cartilage collagen. Genipin is derived from geniposide which is harvested from the fruit of Gardenia jasminoides Ellis. A study comparing the effects of glutaraldehyde and genipin for fixation of decullarized porcine xenografts in sheep found glutaraldehyde to be extremely cytotoxic while genepin succeeded in depressing the host's inflammatory response [10]. In addition, genipin-crosslinked matrices with at least a 50% crosslinking degree were biocompatible and the cells within, viable [11]. We hypothesize that, in general, crosslinking via genipin has positive effects on mechanical strength and resistance to enzymatic degradation.

METHODS

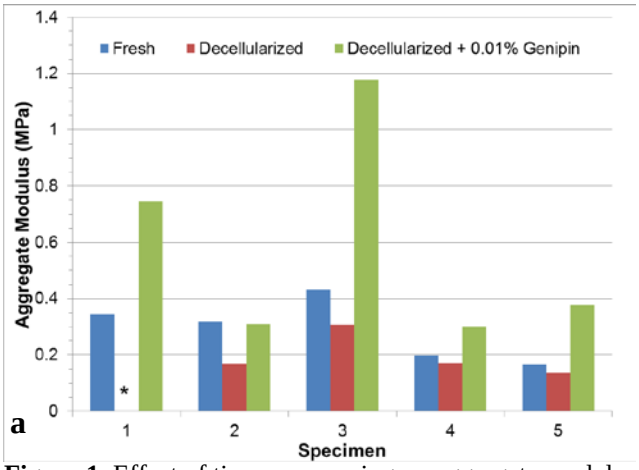
Ten samples of 5 mm articular cartilage discs were harvested from porcine stifle joints and biphasic properties (aggregrate modulus and hydraulic permeability) measured via stress-relaxation tests performed in confined compression. The cartilage samples were then decellularized according to previously published protocol for porcine nasal septal cartilage [12]. Biphasic properties were re-tested on each of the samples and compared to the fresh cartilage values. Five of the discs were crosslinked in 0.01% aqueous genipin for 24 hours at room temperature with agitation. The remaining 5 were crosslinked in 0.1% aqueous genipin under the same conditions. The biphasic properties of all 10 samples were tested in confined compression and compared to the pre-crosslinking values.

A separate experiment was conducted to investigate resistance to collagenase digestion. Articular cartilage discs (n=30) 5 mm in diameter were harvested from pig stifle joints. Ten samples were maintained as a control group, 10 were fixed in 0.01% genipin, and 10 in 0.1% genipin. All samples were incubated at 37°C with agitation for 18 hours. The non-crosslinked control group was incubated in PBS without genipin. The genipin concentration and exposure duration were determined based on a previous study of genipin-fixed bovine pericardia [13]. Collagenase resistance testing (n=10 per group) was then performed. Samples were weighed prior to exposure to collagenase treatment and placed in individual wells of 24-well plates. Samples were covered with 1 ml of 1 mg/ml collagenase type 2 (300 U/mg) solution and incubated at 37°C with agitation. Samples 1-5 of each group (control, 0.01% genipin, and 0.1% genipin) were removed from solution after 30 min, blotted, and weighed. Samples 6-10 of each group were incubated with agitation for 60 min before being weighed.

RESULTS

Figures 1 illustrates the results of the stress-relaxation tests in confined compression to determine biphasic properties. In both the 0.01% and 0.1% genipin fixed samples, the aggregate modulus was usually higher after decellularization and genipin crosslinking compared to fresh samples. Both differences were statistically significant ($p<0.05$, paired t-test). Figure 1a also demonstrates the reduction in aggregate modulus caused by decellularization alone. Figure 2 demonstrates the effect of decellularization and genipin crosslinking on the hydraulic permeability of porcine articular cartilage. At both genipin concentrations, the hydraulic permeability tended to decrease in comparison to fresh samples. Figure 3 represents the effect of genipin crosslinking on enzyme degradation resistance of the

cartilage samples. At 30 minutes, the 0.01% and 0.1% genipin crosslinked samples had lost significantly less weight than the control group ($p<0.05$, ANOVA). This trend continued through 60 minutes but the differences were not statistically significant.



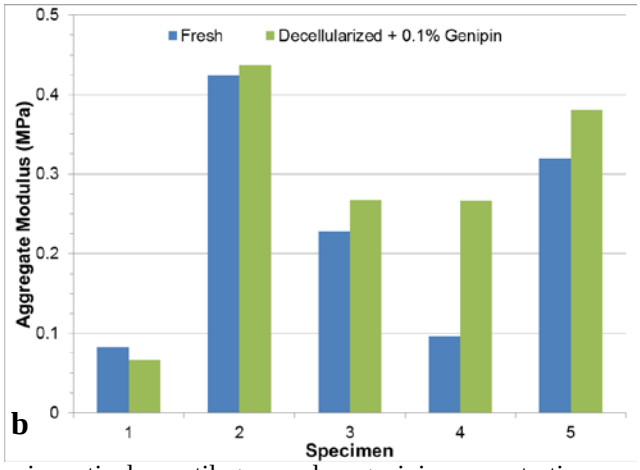


Figure 1. Effect of tissue processing on aggregate modulus of porcine articular cartilage. a – low genipin concentration; b – high genipin concentration. *Data not available due to irregular stress relaxation profile.

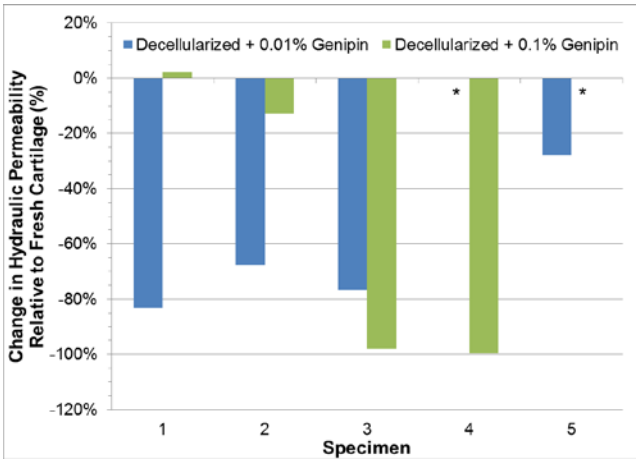


Figure 2. Effect of genipin on hydraulic permeability of porcine articular cartilage. *Data not available due to irregular stress relaxation profile.

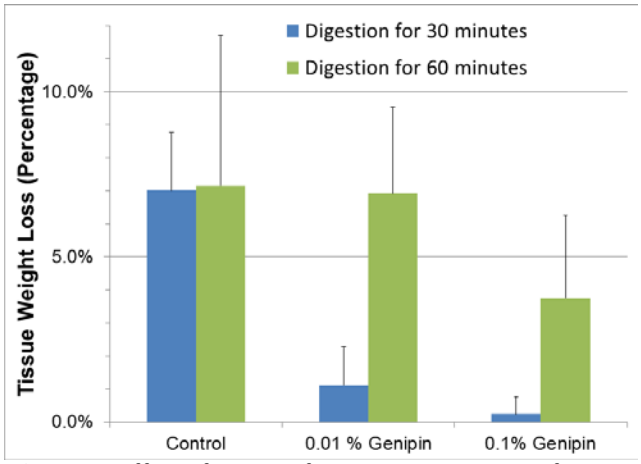


Figure 3. Effect of genipin fixation on resistance of porcine cartilage to degradation by collagenase type 2.

DISCUSSION

As hypothesized, decelluarization and genipin crosslinking increased the aggregate modulus for the 0.01% and 0.1% genipin-fixed porcine articular cartilage discs when compared to fresh samples. Simultaneously, the hydraulic permeability of crosslinked samples was decreased. A high aggregate modulus and low hydraulic permeability are what provide articular cartilage with its ability to withstand high magnitude and repetitive compressive joint loads. The samples that were chemically crosslinked with genipin performed better in the collagenase resistance test than those in the control group that were not crosslinked. The crosslinked samples were better able to resist enzyme degradation and maintain more of their original mass after being exposed to collagenase for the given time periods. Overall, these

findings are consistent with those of Okamura et al. in their *in vivo* study of deceullarized, crosslinked cartilage which suggested that crosslinked cartilage better withstood biodegradation by host enzymes than non-crosslinked cartilage [8]. Further tests will be performed with larger sample sizes to improve precision of measurements and to enhance statistical power.

CONCLUSIONS

It can be concluded that crosslinking porcine articular cartilage discs with genipin is a successful means of enhancing mechanical properties and resisting enzyme degradation. As seen in this study, the samples that had been cross-linked with 0.01% and 0.1% genipin experienced increased aggregate modulus, decreased hydraulic permeability, and greater resistance to degradation by collagenase than those that had not been crosslinked. This proves significant toward identifying genipin as a successful tool for increasing biphasic mechanical properties and reducing degradation by enzyme activity. This knowledge is consistent with the findings of literature previously cited and will be useful in developing durable osteochondral xenografts.

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