The Investigation of the Effect of Exogenous Crosslink to the Hydraulic Permeability of Annulus Fibrosus of Intervertebral Disc

1 Chuang, S-Y; 2 Lin, S-R; 1Lin, L-C; 1Wang, S-J; 2 Chen, W-P
+1 Department of Orthopaedic Surgery, Tri-Service General Hospital, Taipei, Taiwan; 2 Department of Biomedical Engineering, Chung Yuan Christian University
Senior author: Chen, W-P: wpchen@cyu.edu.tw

Introduction:
Although there is some controversy in the literature, several studies have demonstrated that the nutrient supply of intervertebral disc, especially small solutes like oxygen and glucose, depend on passive diffusion across the vertebral endplates and the annulus fibrosus (AF). [1-3] Further, the periphery of the outer AF is nourished by its own blood supply with no deep penetration, while the remaining inner AF and nucleus pulposus (NP) rely on fluid flow across the vertebral endplates and AF for nutrient delivery. The hydration and load-actuated (convective) fluid flow of the IVD play a role not only in nutrient transport, but also in governing the viscoelastic behavior of IVD. An approach to facilitate fluid flow and nutrient delivery to the IVD would be through affecting the permeability of the collagenous AF and endplates.

Genipin, a traditional Chinese herbal medicine extracted from gardenia fruit, has been found to be an effective collagen crosslinker. The mechanical advantages of crosslinks in intervertebral disc have been previously demonstrated in terms of improved tissue stability and mechanical properties of the motion segment[5, 6].

The objective of this study was to investigate the possible influence of exogenous collagen crosslink augmentation on the nutritional environment by measuring the effect on hydraulic permeability of annulus fibrosus. We checked the viscoelastic properties and the porosity, which are two important parameters, related to permeability of the annulus fibrosus in the study.

Materials and Methods
Using fresh porcine lumbar intervertebral discs, the testing specimens were prepared from the selected anterior, lateral, and posterior annulus fibrosus by 3 mm diameter trocar. Two groups of specimens were tested. The control group (anterior AF N=8, lateral AF N=8 and posterior AF N=8) specimens were treated with phosphate buffered solution soaking for two day in the room temperature and the crosslink experimental group (anterior AF N=8, lateral AF N=8, and posterior AF N=8) was treated with the same condition with 0.33% genipin in phosphate buffered solution.

Custom-made specimen sitting apparatus was made with metal filter on the floor of the device. Confined compression relaxation and creep test was performed with loading to 10 N, 25 N and 50 N respectively after preconditioning and lasting for 10 minutes. The load generated by an MTS 858 materials testing system (Eden Prairie, MN) was transduced using a 25 N load cell. The relaxation was analyzed by relaxation ratio (RR) which was defined by the following equation:

\[ RR = \frac{F_0 - F(t)}{F_0} \]

where \( F_0 \) is the initial compression force and \( F(t) \) is the force data gathered by the load cell over time. For creep analysis, the immediate deformation stiffness and the time-dependent viscoelastic creep (additional deformation following the initial elastic response) were quantified separately.

Another study was made to test the porosity of the specimens in the two groups with the same specimen number 16 in anterior, lateral and posterior AF. The porosity was analyzed with the formula: \( \beta = V^d / V^t \)

where \( \beta \) is the porosity, \( V^t \) is the fluid volume in the specimen, and \( V^d \) was the specimen volume.

Result
The stiffness was higher in posterior annulus fibrosus as compared with the anterior and the lateral ones in the control group. After exogenous crosslink reaction, the AF exhibit viscoelastic properties as the control group. At lower load to 10 N, the stiffness was about half lower in genipin crosslinked group as compared with the control group in all the anterior (p=0.024), lateral (p=0.013) and posterior (p=0.006) AF (Figure). While loading to 25N and 50N, the trend for the stiffness was the contrary to the 10 N groups with higher in the genipin group. The viscoelasticity properties including creep and relaxation did not show significant change.

The porosity of crosslink group slightly decreased as compared to the control group in anterior (3.2%), lateral (4.6 %), and posterior (4.0%) but without statistical significant.

![Figure The stiffness of annulus fibrosus before and after crosslink.](image)

Discussion
Resisting compressive loading of annulus fibrosis depends not only on collagen fiber strength, but the fluid matrix interaction of the extracellular matrix. After exogenous crosslink reaction, the biomechanical viscoelasticity behavior had no significant different as compared with control ones. After crosslink, the annulus fibrosus increase stiffness in all anatomical area of annulus fibrosus. According to porosity test, under unloaded situation the mean porosity diminished slightly after crosslink with no statistical significant.

Conclusions
Utilizing exogenous crosslink reagent can alter the mechanical properties of the AF without change viscoelasticity and porosity much.

Reference