Dynamic Properties of Degenerated Intervertebral Disc Can Be Better Recovered by Elevated Protein Crosslinking

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INTRODUCTION:
The feasibility of using crosslinking reagent, e.g., the genipin or methylglyoxal, to enhance the biomechanical functions of the injured or degenerated intervertebral disc has been extensively investigated. Our previous study found that genipin could recover the disc dynamic properties of mild denatured disc; however, the recovery efficiency was degraded by fatigue loading. Genipin is able to crosslink the intrahelical, interhelical and intermicrofibrillar free amino peptides in collagen-based biomaterials. The degraded recovery efficiency post fatigue loading may suggest the insufficient crosslinking within the disc, especially for the intermicrofibrillar structures. The degree of crosslinking can theoretically be elevated by increasing the genipin concentration and crosslinking reaction time. The purpose of this study is to evaluate the effect of enhanced crosslinking on the recovery of dynamic properties of denatured disc post fatigue loading.

METHODS:
Specimen treatment. Lumbar spines were dissected from 6-month old pigs. After removal of muscle tissue and posterior elements, a total of 36 lumbar discs (L2-3, L4-5) were randomly assigned to one of the four treatment groups: (a) intact disc, (b) denatured disc, (c) denatured disc with low-level crosslinking, (d) denatured disc with high-level crosslinking. The intact discs were injected with 1 ml saline. For the rest three groups, the discs were injected with 1 ml 0.5% trypsin solution, which breaking the protein molecules within the disc matrix. The specimens were then immersed in saline solution for 24 hr to ensure complete protein denaturation. After the saline bath, the low-level crosslinked discs were injected with 1 ml 0.33% genipin solution, while the high-level crosslinked discs were injected with 1 ml 3.3% genipin solution. The crosslinking reaction time was one day for low-level crosslinking discs and three days for the high-level crosslinking discs.

Loading condition. All the discs were applied with a 30 min fatigue loading after the treatments. The loading magnitude was, peak-to-peak, from 190 N to 590 N, and the loading frequency was 5 Hz. An impulse loading (1.2 J impact energy at 40 ms contact time) was applied to each disc before and after the fatigue loading. Data analysis. The disc was modeled using one-dimension spring and damper model ($M \ddot{u} + C \dot{u} + K u = 0$). The disc dynamic properties, i.e., the stiffness modulus (K, N/mm) and damping coefficient (C, Ns/mm) were calculated using the displacement data during free vibration mode post impulse. Two-way ANOVA was used to test the effect of “treatment” (the independent factor) and “fatigue loading” (the repeated factor) on the stiffness modulus and damping coefficient. Tamhane’s T2 test was adapted for the post hoc analysis. All tests were considered significant at $p<0.05$.

RESULTS:
For all group of discs, the stiffness modulus increased with fatigue loading ($p<0.01$). The stiffness modulus of the intact disc was 720 (75) N/mm before fatigue and was 938 (16) N/mm after fatigue. The denaturation decreased the pre-fatigue stiffness to 612 (30) N/mm, and the post-fatigue stiffness to 885 (34) N/mm. Both low-level and high-level crosslinking was even higher than the one of the intact disc. However, after fatigue loading, the stiffness of low-level crosslinking denatured disc was lower than the one of denatured disc with no treatment, and the stiffness of high-level crosslinking denatured disc was similar to the one of denatured disc with no treatment (Figure 1A).

The damping coefficient of all discs decreased with fatigue loading ($p<0.01$). The damping coefficient of the intact disc decreased from 0.63 (0.03) Ns/mm to 0.53 (0.03) Ns/mm. Denaturation reduced the pre-fatigue damping coefficient to 0.44 (0.02) Ns/mm, and the post-fatigue damping coefficient to 0.33 (0.02) Ns/mm. Both low-level and high-level crosslinking increased the damping coefficient of the denatured disc pre- and post-fatigue. Compared to the intact disc, the damping coefficient of the low-level crosslinking denatured disc was the same before the fatigue loading, but became lower after the fatigue loading; meanwhile, the damping coefficient of high-level crosslinking denatured disc was higher than the intact disc before the fatigue loading, but decreased to the same level as the intact disc after the fatigue loading (Figure 1B).

DISCUSSION:
This study proved that the dynamic properties of the denatured discs are better recovered by the elevated crosslinking. The magnitude of disc stiffness is reversed to the degree of hydration, and fiber integrity. The fatigue loading expels the fluid of the disc hence increases the stiffness. The stiffness of denatured disc was weakened due to higher hydration of chemical denaturation and fiber weakening. The crosslinking reagent increases the stiffness by enhancing the fiber strength and lowering the nucleus viscosity. The damping coefficient quantifies the fluid flow capability of a system. The higher magnitude of damping coefficient represents either higher viscosity of the fluid or smaller pores within the matrix. The denaturation decreased the fluid viscosity of nucleus, and the fatigue loading further increased the fissure size within the fibril matrix. The crosslinking reagent increases the viscosity of nucleus pulposus by crosslinking the intrahelical and interhelical bonds, and decreases the fissure size by crosslinking the intermicrofibrillar; hence increases the damping coefficient of disc.


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