EXOGENOUS CROSSLINK IMPROVES THE FUNCTIONAL INTEGRITY OF INTERVERTEBRAL DISC SECONDARY TO STAB INJURY

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INTRODUCTION:
Collagen crosslinks play a major role in providing the mechanical strength for load-supporting tissues. Human intervertebral disc collagen has a higher concentration of mature crosslinks than other tissues. A natural, cell-mediated crosslinking mechanism exists in response to the elevated tensile environment in biological tissues. Crosslinks have recently been described as providing “sacrificial bonds” that protect tissue and dissipate energy. Together these studies suggest that an increase in crosslinks may be advantageous for maintaining tissue integrity. Stabbing injury with large needle causes the loss of integrity of the intervertebral disc. In this study we hypothesized that exogenous crosslinking can increase the integrity of the disc tissue post the stab injury.

METHODS:
Fresh porcine thoracic spine specimens (T2/T9) were obtained immediately following death. Specimen preparation involved cutting the pedicles and removing the posterior processes. The test unit consisted of hemi-vertebrae and the intervening disc.
Quantitative Discomaneometry (QD) technique was used to measure both the injected fluid volume and the developed hydrostatic pressure within the disc to quantify the disc functional integrity. The specimen was divided into two groups, i.e. the stab injury using 16G and 18 G needles. Each group was further divided into three sub-groups. The first sub-group was the discs without any treatment. The second one was the discs soaked with saline for two days. The third one was the discs soaked with Genipin solution for two days. The three sub-groups were abbreviated as Control, PBS and Genipin hereafter. The QD test was conducted right after the stab injury was created for the Control sub-group, and at the end of second day for the Saline and Genipin sub-groups. Seven parameters were obtained from each pressure-volume (PV) curve. The seven were the intrinsic pressure, leakage pressure and volume, saturate pressure and volume, steady-state pressure, and slope between intrinsic and leakage pressure.
One-way analysis of variance (ANOVA) was used to find the significant QD parameters affected by the disc treatments in both 16G and 18G group. Post hoc multiple pairwise comparison tests (Fisher’s Least Significant Difference) were performed to determine the level differences among discs at the significance level of α = 0.05.

RESULTS:
The QD Pressures. The intrinsic pressure was not significantly changed by treatment. In both the 16G and 18G groups, all the leakage, saturate and steady-state pressures of Saline were comparable to Control. All the leakage, saturate and steady-state pressures of the Genipin sub-group were higher than both the Control and Saline (only leakage pressure is showed in Figure 1).
The QD Volumes. In both the 16G and 18G groups, both the leakage and saturate volume were not significantly changed by the treatment (only leakage volume is showed in Figure 2).
The QD Slope. In both the 16G groups, the slope was not significantly changed by the treatment. In the 18G group, however, the slope of Control was significantly higher than both the Saline and Genipin (Figure 3).

DISCUSSION:
The injured anulus fibrosus (AF) cannot hold the intradiscal pressure (IDP). The decreased IDP drops due to the fluid leaking would decrease the strength of annular fiber hence decrease the stability of motion segment. The QD test showed that the pressure decreased post stab injury, while the crosslink restored the disc integrity. The exact mechanism of genipin crosslinked the collagen of the intervertebral tissue is not well understood. It is speculated that linking is due to the amino acid groups in intramolecular, intermolecular and intermicrofibrilar bonds. It was found that genipin reacts with lysine, hydroxylysine, and arginine residue in biological tissue. The crosslink realignment of the collagen fibril may contribute to this phenomenon.

CONCLUSION:
The Genipin increased the functional integrity of disc secondary to stab injury in both the 16G and 18G needle injury models.

Figure 1: The leakage pressure for the two types of injury (16G vs. 18G needle stab injury) and three types of treatment (Control: no treatment, Saline: soaked with 2-days of saline, Genipin: soaked with 2-days of Genipin solution).

Figure 2 The leakage volume for the two types of injury (16G vs. 18G needle stab injury) and three types of treatment.

Figure 3 The steady state pressure between intrinsic and leakage pressure for the two types of injury (16G vs. 18G needle stab injury) and three types of treatment.

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