Genipin-Crosslinked Gelatin Hydrogel Injection Recovers the Functional Integrity of Intervertebral Disc Secondary to a Needle Puncture: An in vitro Porcine Model Using Quantitative Discomanometry Examination

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Introduction: Loss of nucleus pulposus (NP) pressurization and impaired annulus fibrosus (AF) integrity are two important pathogeneses associated with Intervertebral Disc (IVD) disease. A punctured hole of AF induced by diagnostic or therapeutic procedures have previously thought to be harmless and without long-term consequences due to small needle diameter. However, some concerns have been raised recently with the finding that even subtle impairments of AF can have significant impact on its integrity and ability to withstand tensile strain. Furthermore, the subsequent compromise of NP due to the defect in AF has been linked to the development of accelerated IVD degeneration. It has been previously suggested that utilization of genipin-crosslinked gelatin hydrogel may have the potential to improve IVD integrity. However, the applicability of such biomaterial is yet to be proven. It is therefore the aims of the current study are to present a new protocol of injection genipin-crosslinked gelatin hydrogel into needle stabbed IVDs and to assess its capacity to amend and recover IVD integrity and functional capacity based on Quantitative Discomanometry (QD).

Methods: Fresh healthy porcine lumbar motion segments from 6-month-old swine were obtained immediately following sacrifice and randomized into 4 groups. The first group included 6 needle stabbed discs that received no treatment and served as the control group. The second and the third group both included 8 discs but one group was injected with uncrosslinked gelatin hydrogel and another group was injected with genipin-crosslinked gelatin hydrogel after stab injury. The last group included 4 discs without any needle injury or hydrogel injection and served as the intact group.

A 16G spinal needle was used to create disc stab injury models with single AF puncture. Uncrosslinked 10% gelatin hydrogel was prepared by dissolving 2 g gelatin powder into 20 ml deionized water at 60°C under continuous stirring until the solution became homogenous. Genipin-crosslinked gelatin hydrogel was prepared by further adding 0.2 g of genipin powder into foremost mentioned 20 ml stirred gelatin hydrogel. Hydrogel was moved into syringes and injected into the disc using 21G needles through the puncture holes created by the 16G spinal needle. For all procedures, particular emphasis was given to ensure immediate injection of the hydrogel once prepared in order to avoid hydrogel gelation. QD test was performed one hour after the completion of needle stab and hydrogel injection.

One-way analysis of variance (ANOVA) was used to identify QD parameters that demonstrated significant differences between the groups. This was followed by the post hoc comparisons of the variables found to have significance and either using Scheffe test with equal variance assumed or using Dunnett’s T3 test with equal variance not assumed.
**Results:** The QD parameters found to have statistical significant differences between different disc treatments included leakage volume (P=0.000), leakage pressure (P=0.000), saturate pressure (P=0.000) and Pressure/Volume (PV) slope (P=0.000). The saturate volume (P=0.069) and steady-state pressure (P=0.086) showed a tendency to be affected by different disc treatments but failed to reach statistical significance. In contrast, the intrinsic pressure (P=0.252) was not affected by different disc treatments. In terms of QD pressure, while higher QD pressure is indicative of better disc integrity, stab injured discs without treatment (control group) demonstrated significantly reduced leakage pressure and saturation pressure compared to other groups (all P<0.05). In contrast, genipin-crosslinked gelatin group achieved comparable leakage pressure and saturation pressure as the intact group; however, the uncrosslinked gelatin group only showed significantly reduced leakage pressure (P=0.000) and saturation pressure (P=0.001) (Fig. 1).

In terms of leakage volume, while larger leakage volume is indicative of better compliance of AF or larger space of NP, the control group also demonstrated significantly smaller leakage volume than all other groups (all P < 0.05). However, both the uncrosslinked gelatin and genipin-crosslinked gelatin group also found to have significantly reduced leakage volume when compared with the intact group (P=0.000 and P=0.001 respectively) (Fig. 2).

A disc with a higher PV slope can be interpreted as a disc with a stiffer AF ring. A similar trend as the QD pressures was observed for the PV slope. With higher PV slope indicative of a stiffer AF ring, the slope of the intact group was significantly higher than the uncrosslinked gelatin group (P =0.036 ) but not significantly different to the genipin-crosslinked gelatin group (P=0.711) (Fig. 3).

**Discussion:** In this study, we demonstrated a new viable method to treat small AF puncture injury and comparatively analyses of the differences between injection of uncrosslinked and genipin-crosslinked gelatin hydrogel in the recovery of functional integrity of discs. Our results demonstrated that gelatin hydrogel with genipin-crosslinks was able to achieve higher QD pressures and generally comparable outcome with the intact discs after repair. Collagen crosslinking had been widely used to increase mechanical strength and durability of biomaterials. Genipin was proven to increase the stability of motion segment and stiffness of AF through whole disc collagen crosslinking. Based on our presented protocol, it is speculated that gelatin hydrogel was effective as a scaffold to refill the puncture defect and its mechanical strength was further enhanced by genipin crosslinking between the filled matrix (gelatin) and native annular fiber. This protocol precludes concern about inconclusive long-term effect by whole disc collagen crosslinking. It is acknowledged that such preliminary finding will require further validation with ex-vivo and animal studies in the future.

**Significance:** Injection of genipin-crosslinked gelatin hydrogel was successful in regaining the functional integrity of injured disc in this porcine model. It is hoped that such biomaterial can be developed not only for repair of small AF defects related to needle procedures but also as adhesive after discectomy involving larger defects.
Fig. 1 Values of the leakage, saturation and steady-state pressures of four groups.
*Significantly different between control and uncrosslinked gelatin group, P = 0.000.
+Significantly different between uncrosslinked gelatin and gelatin in crosslinked gelatin group, P = 0.03.
Fig. 2 Values of the leakage volume of four groups.
*Significantly different between control and uncrosslinked gelatin group, P < 0.05.
+Significantly different between genipin-crosslinked gelatin and intact group, P < 0.05.
Fig. 3 Values of the slope of four groups.
*Significantly different between control and uncrosslinked gelatin group, \( P < 0.05 \).
+Significantly different between uncrosslinked gelatin and intact group, \( P < 0.05 \).