Injection of GAG analogue hydrogel in degraded intervertebral disc to restore tissue function

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ABSTRACT

Lower back pain leads to both direct health care cost and indirect costs such as decreased productivity at work. It should therefore not only be considered as a health problem, but also as a social and economic problem. Lower back pain can be caused by different mechanisms, but it is strongly associated with degeneration of intervertebral discs (IVDs). During disc degeneration there is a loss of structure, proteoglycans and water content, which initiates a progressive degeneration cascade. This leads to a loss of mechanical function, eventually causing low back pain. The earliest biochemical changes due to disc degeneration are observed in the nucleus pulposus (NP), indicating that early stage treatments should be focused on NP tissue.

The aim of this research was to inject a GAG analogue hydrogel into artificially degraded NP explants in order to restore its equilibrium stress to a healthy range. Therefore, bovine NP explants were cultured in custom-built bioreactors in which NP degeneration was simulated. Artificial degradation of NP explants led to a decrease in both equilibrium stress and tissue GAG content. The swelling capacity and equilibrium stresses of the GAG analogue hydrogel were validated. GAG analogue hydrogel equilibrium stress within the bioreactor were more than twice as high as those measured for healthy NP tissue samples, indicating that the GAG analogue hydrogel could be used to increase equilibrium stress upon injection into tissue. However, injection of 20 µL hydrogel in artificially degraded NP explants did not lead to an increase, but a further decrease in equilibrium stress. This was probably the combined result of ongoing tissue degradation, and injection of a too low gel volume. GAG analogue hydrogel injections need to be repeated with larger gel volumes in the future to determine if they hydrogel is able to restore equilibrium stress to a healthy range.

Moreover, it is unknown if the GAG analogue hydrogel is capable of maintaining its function over longer periods of time. Incorporation of NP cells (NPCs) within the hydrogels might lead to preservation of gel function due to ECM produced by these incorporated cells. It is explored whether the GAG analogue hydrogel can also be used to deliver NPCs to the IVD and whether it supports the production of GAGs by these cells. As a first step, a method was developed to maintain NPC viability during hydrogel polymerization. Next, the GAG analogue hydrogel was dissolved by sonication and an attempt was made to determine GAG content of cultured cells by the use of a DMMB assay. However, the GAG analogue hydrogel itself interfered with the GAG content determination due to its unpredictable background noise. The GAG analogue hydrogel also interfered with histology since the hydrogel itself was also stained with Alcian blue staining. In addition, coupes of the hydrogel were difficult to stain due to immediate swelling of the hydrogel upon contact with any liquid. Moreover, sonication steps were taken to dissolve GAG analogue hydrogel in order to isolate RNA of cultured cells. qPCR indicated that the sonication process led to denaturation of the RNA, thereby preventing assessment of gene expression. Taken all together, cell-seeded hydrogel show viable cells over time, but it was not possible to evaluate GAG production of incorporated cells by histology or DMMB assay and gene expression could not be determined with qPCR.