The effect of severity of disc degeneration on mesenchymal stem cells' ability to regenerate the intervertebral disc: a rabbit model

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INTRODUCTION: Degenerative disc disease (DDD) is a consequence of alterations in the extracellular matrix composition of the intervertebral disc (IVD). Studies suggest that mesenchymal stem cells (MSCs) have the ability to arrest degeneration but not regenerate it. We hypothesize that this is related to the severity of degeneration. This study investigates the effect of different severity of degeneration on the ability of MSCs to halt or regenerate the IVD.

METHODS: Disc degeneration was induced in 9 New Zealand white rabbits at 4 consecutive lumbar levels by annular puncture[1]. The degeneration was allowed to progress for 1 month (early group, n=5) or 7 months (late group, n=4). Autologous MSCs were then isolated, expanded *ex vivo* and labeled with bromo-deoxyuridine (BrdU). $1x10^5$ MSCs were injected into 2 of 4 degenerated discs per rabbit. The other 2 levels were used as control levels that were either sham (induced but no further treatment) or injected with culture medium only. Serial radiographs of the spine were taken every 2 weeks. All rabbits were sacrificed at 16 weeks post-injection, the discs were retrieved for histological and immunohistochemical exam-ination. Disc heights on radiographs were measured[2]. A histological grading[3] was used to assess the severity of degeneration (Grade 0 = normal to Grade 5 = severe degeneration).

RESULTS: MSCs could be detected in all IVDs 16 weeks post-injection. For the early group, there were no significant differences in disc height between the sham discs, the medium-injected discs and the cell-injected discs. For the late group, all discs showed loss of height. The loss was significantly ($p<0.05$) greater for the cell-injected discs and the medium-injected discs than the sham discs (? because of double puncture). Histological grading of annular degeneration revealed no significant differences in the early group, whereas for the late group, the cell-injected discs were significantly ($p<0.05$) less degenerated than the medium-injected discs and sham discs (Table 1). This finding was further supported by histological confirmation of a near normal proteoglycan level within the posterior annulus fibrosus of the cell-injected discs (Figure 1).

DISCUSSION & CONCLUSIONS: Contrary to intuitive belief, MSCs injection has a more significant effect when introduced into more severely degenerated discs. Although evidence of halting progression of degeneration can be demonstrated, they do not have the ability to regenerate the disc fully or restore disc space height. It is not possible to conclude from the current study whether this failure to regenerate the disc is a problem of the model itself, an insufficient number of cells, a lack of a scaffold material, or that MSCs lack this ability. Further studies will be needed to ascertain the stage of disc degeneration that would benefit most from stem cell-based therapy.

Disc treatments	Early group	Late group
Sham	2.8	4.5
Medium-injected	2.5	3.8
Cell-injected	2.4	2.1

Table 1. Mean degenerative grades for IVDs subjected to different treatments.

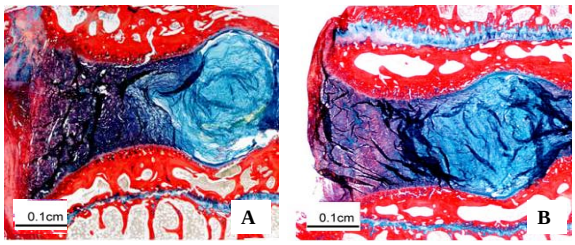

*Fig 1. Cells-injected discs (**A**) restored the proteoglycan level (stained blue)[4] when compared to sham discs (**B**) in the late group.*

REFERENCES: (1) KS. Kim, ST. Yoon, J Li, et al. (2005) Spine 30(1):33-7. (2) J. Mochida, K. Nishimura, T Nomura et al (1996) Spine 21(13): 1556-623. (3)K. Nishimura, J. Mochida (1998) Spine 23(14): 1531-1538. (4) HE. Gruber, J. Ingram, EN. Hanley et al (2002) Biotech Histochem 77: 81-3.

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