Vertebral Transplantation of Mesenchymal Stromal Cells for Intervertebral Disc Repair Outperforms Intradiscal Injection in a Rat Tail Disc Degeneration Model: A preliminary study

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INTRODUCTION: Cell therapy has shown promising results for intervertebral disc (**IVD**) repair in human clinical trials.[1] However, contemporary methods rely on intradiscal injection, involving puncture (thus additional damage) to the IVD.[1] Multiple animal models and ex vivo studies have highlighted the potential of mesenchymal stromal cells (**MSC**) to migrate into degenerating IVD.[2-4] As such, this pilot study aims to examine whether this process could be exploited by injecting therapeutic MSC into the vertebrae to limit deterioration of their adjacent IVD in a disc degeneration rat model. Vertebral transplantation of MSC was compared to degenerating discs treated through intradiscally injected MSC on their ability to retain disc height index (**DHI**) and histological features.

METHODS: All animal experiments were approved prior to initiation by the Tokai University School of Medicine committee for safe animal experimentation (#214033) conforming to national laws and regulations. Transgenic ubiquitously GFP expressing mice were sacrificed and MSC were obtained from the compact bone of both femur and tibia. The cells were expanded in MesenCult (mouse; STEMCELL Technologies) at 5% O_2. Next, 20 female SD rats were assigned to 0 (n=2), 1 (n=6), 5 (n=6), or 14 (n=6) days cohorts. Each rat was subjected to induced disc degeneration in 2 coccygeal discs (assigned within Co2/3 – Co7/8 range) by nucleus pulposus (**NP**) aspiration using a 22G needle. MSC were transplanted via a 27G needle in 10µL containing 250.000 MSC for vertebral and 100.000 MSC for intradiscal injection. Each rat had 4 conditions assigned to their tail (Fig 1A); i.e. (**HT**) one healthy IVD and (**DT**) one IVD induced to degenerate, were treated with MSC in their caudal vertebrae. Additionally, a (**CON**) degeneration-induced IVD was treated through intradiscal MSC injection. A healthy and untreated (**REF**) was used as a reference. Following transplantation, the rats were kept up to 2 weeks according to their allocated cohort. Radiographic images were taken and DHI was assessed in a blinded manner.[5] Finally, rats were sacrificed and 17 rats were used for preparing cryo-sections and histological assessment for tissue quality. Finally, GFP-positive cells were detected using immunohistochemistry applying rabbit-anti-GFP (ab290) and goat-anti-rabbit Alexa 633 (A21071). Images were obtained through confocal microscopy. Statistical analysis was performed using Two-way ANOVA, post-hoc Tukey's multiple comparison test using Prism 9 (GraphPad Software LLC.).

RESULTS SECTION: Transplantation could successfully be performed without signs of complications. DHI confirmed that DHI of REF and HT conditions remained stable, while the CON conditions showed a continuous decline up to 71% (±11%) at 2 weeks. (Fig 1B) Interestingly, DHI for the DT cohort showed capable of limiting DHI loss, resulting in significant improvement at week 2 (96±13%) compared to CON (p=0.009). Macroscopic scores[6,7] and histological scores[8] showed a trend of enhanced outcomes of the DT cohort compared to the CON. (Fig 1C) GFP positive cells could be detected in different conditions, including within the NP. (Fig 1D) Overall, larger fractions of GFP-positive cells were detected in the DT and CON conditions compared to HT and REF conditions.

DISCUSSION: The process of transplanting cells into the vertebrae next to an IVD induced to degenerate proved beneficial for maintaining the integrity (histological and DHI) of the IVD. Moreover, vertebral transplantation led to a significantly enhanced outcome compared to the IVD treated through intradiscal transplantation. Notably, however, the CON condition involved a second puncture to allow for transplantation of the MSC that could have worsened the degeneration cascade. Although the enhanced regenerative outcome for the DT condition could be explained by the MSC homing mechanism, the GFP tracing outcomes did not demonstrate an evident enhancement in GFP positive cells in the DT samples. Whether this might be due to short survivability or due to limitations of the immunohistochemistry approach remains to be determined. Alternatively, the observed outcomes might also be a result of paracrine signaling of the MSCs from the vertebra or alternatively a result of the repair mechanism of the vertebrae in response to the transplantation defect in the bone. These questions remain to be explored in future experimentation as well as assessment of long-term regenerative outcomes needs to be determined.

SIGNIFICANCE: Vertebral injection of MSC as a method to resolve induced disc degeneration proved effective in a short (2 weeks) time frame, and outperformed intradiscal injection. This might offer an alternative strategy to transplant therapeutic cells for IVD repair.

REFERENCES: (**1**) Schol & Sakai (2019, PMID 30498909), (**2**) Illien-Jünger et al (2012, PMID 22433498), (**3**) Sakai et al (2015, PMID 25459743), (**4**) Croft et al (2021, PMID 33805356), (**5**) Hiraishi et al (2018, PMID 31463441), (**6**) Thompson et al (1990, PMID 2363069), (**7**) Nukaga et al (2019, PMID 31463464), (**8**) Han et al (2008, PMID 18708924)

Figure 1 (**A**) Overview of the different transplantation conditions, involving MSC transplantation into the IVD or vertebrae. (**B**) Measurement outcomes of the relative disc height index. (**C**) Histological scores assigned to each of the sagittal cut specimen. Bars represent mean and error bars represent standard deviation. (**D**) An example of GFP expressing cells detected in a DT sample, one day following transplantation. (scale = 50µm) DHI: Disc height index, IVD: intervertebral disc, MSC: mesenchymal stromal cells. * p<0.05, ** p<0.01, *** p<0.005, and **** p<0.0001.