Age-associated changes in the cell number of the human lumbar intervertebral disc

INTRODUCTION:
During degeneration, the intervertebral disc undergoes significant changes in the composition of the extracellular matrix. Since disc cells are responsible for matrix formation and maintenance, their proper quantity, quality and distribution in the regional compartments of the disc may be crucial in preventing degenerative changes. Although current investigations on cell therapy require knowledge about the cell number in the disc at various age groups, hardly any studies have been performed so far. As there is minimal information about cell number and density in the disc, and especially about age-related changes, the number of implanted cells into annulus fibrosus and nucleus pulposus in animal studies ranges between 40 cells/mm and 10,000 cells/mm.

As the ideal number of cells for injection or implantation for an effective treatment has yet to be determined, the aim of this study was to determine at least changes in cell density of endplate, nucleus pulposus and annulus fibrosus during ageing (birth until 86 years), and to compare changes in cell density with the histological degeneration score (HDS).

METHODS:
A total of 49 complete lumbar motion segments were harvested from 22 autopsies with a mean age of 34 years (range from birth to 86 years). The medical records did neither provide evidence of a history of spinal disease and back pain nor of major illness (e.g. chronic infection or tumour). The grade of degeneration of the samples was assessed according to the histological degeneration score.

A morphometric analysis was performed to determine the cell number per region of interest. As the nuclei of intervertebral disc cells are usually larger than 5 µm, they may appear and be counted in more than one section. Thus, cell number was adjusted as described by Abercrombie et al. In order to exemplarily calculate cell numbers for biological disc regeneration, we made the assumptions that nucleus pulposus and annulus fibrosus regions are relevant for potential cell transfer to the IVD. The total cell number of annulus and nucleus per region of interest was compared with the total area per region of interest. Using the percentage of annulus and nucleus cells at a specific age, the number of annulus and nucleus cells could be calculated. Statistical analyses: Interrater reliability of the histological assessment of the cell density and the HDS was assessed by the intraclass correlation coefficient (ICC). All correlation analyses were explored using the Spearman rank correlation test. Univariate analysis of variance (ANOVA) was performed to explore differences between the spinal levels. Differences between the anterior and posterior regions in the annulus and endplate as well as between the superior and inferior endplate within single discs were explored by paired samples t test. Significance level p < 0.05.

RESULTS:
Gender and disc level did not influence cell density. We found a significant correlation of cell density and histological degeneration score between grade 0 and 1, but not for grades ≥1 (Figure 1).

Cell density in nucleus pulposus decreased significantly from 0 to 16 years with main changes from 0 to 2 years. In contrast to cell density, the total cell number per region of interest decreased significantly only up to two years. Because of volume effects, cell density decreased thereafter without actual changes in cell number (Figure 2).

The percentage of nucleus pulposus cells in the intervertebral disc is about 15% between 30 and 60 years. Notochordal cells were found until the 3rd year.

To get a lead how many disc cells exist in a human disc, we calculated that the total cell number in the entire disc is approx. 36,000 cells and in the nucleus pulposus alone only approx. 6,000 cells of a subject in the 4th decade of life with a calculated disc volume of 7.49 cm³.

DISCUSSION:
The current study presents a systematic evaluation of disc cell density in distinct anatomical regions and total cell number per intervertebral disc area throughout all ages. It offers a basis for future cell-based therapies for degenerative disc disease by providing an estimation of the cell density in the disc and by showing that rather changes of cellular function than of cell density occur during ageing. The number of cells should be considered in cell transplantation, besides material and mechanical properties of carrier matrices, especially because of the difficult disc nutrient and metabolic environment in vivo. Our data may add to a better understanding of disc pathophysiology and may have an impact on the design of future therapeutic approaches to treat degenerated intervertebral disc tissue.

REFERENCES:
1) Boos et al. Spine, 2002
2) Abercrombie et al., Anat. Rec, 1946