It could be shown that, compared to controls, the cell proliferation rate (+26.1%) and the proteoglycan synthesis (+20.4%) increased when the NP cells were exposed to appropriate pulsed electromagnetic fields. These results correspond to the findings of similar studies performed on chondrocytes, although the magnitude of the effect observed in the present study was substantially lower.
Despite the promising results, a large variability was observed in the response of cell cultures in individual experiments. Therefore, further trials are required to optimize the PEMF parameters. While the application of PEMF remains an attractive possibility for the noninvasive treatment of disc disorders, and indeed commercial devices already exist for patient therapy, the effective field strength and high level of field control required should be critically evaluated.

SP 45
EVALUATION OF THE BIOMECHANICAL BEHAVIOR AND THE EXTRUSION RISK OF A NEW POLYMERIZING GEL FOR INJECTION IN THE NUCLEUS PULPOSUS

HJ Wilke, H Fuchs, J Mollenhauer, K Benz, H Wurst, G Achatz, F Heuer

Institute of Orthopaedic Research and Biomechanics, University of Ulm, Ulm, Germany

Therapeutic approaches have been designed and clinically tested to utilize autologous intervertebral disc cells as a source for regenerative cell populations to be re-injected into the damaged nucleus pulposus of patients. The drawback of the current method is that cells are injected as a liquid suspension, creating the risk of leakage of cells from the injection site of the freshly injected disk when loaded. To overcome this technical problem, an in situ polymerizing gel based on chemically crosslinking albumin as a cell carrier and hyaluronic acid as a hydrodynamic additive was designed. The gel polymerizes within a few minutes after injection and is supposed to anchor the "implant" within the nucleus pulposus.
The goal of this in vitro study was to evaluate the biomechanical changes of a spinal segment after injection of the gel and to evaluate the risk of extrusion.
Twelve lumbar motion segments of 5–6 month old calves were tested. Six were treated with the in situ polymerizing gel, six served as control group. We performed a flexibility tests in the three principal motion planes. Subsequently, to provoke extrusion the specimens were exposed to 100,000 load cycles in a dynamic materials testing machine. Specimens were loaded with an eccentric load in a sinus waveform with 4–24 Nm at 5 Hz while specimens rotated with 360°/min.
The injection of the in situ polymerizing gel initially decreased slightly the flexibility in lateral bending but maintained it for flexion/extension and axial rotation compared to the intact segment. The cyclic test caused a successive increase of the flexibility in all motion planes beyond the initial ROM. The disc height showed an initial increase of about 0.25 mm after the gel injection and then a decrease of −1.4 mm due to the dynamic loading. These changes in flexibility as well as in height were the same as in an untreated control group. Most importantly, however, no extrusion of the polymerizing gel could be noticed over the 100,000 cycles. Finally, macroscopic sections exhibited a decrease of the volume of the implanted gel, probably due to loss of water.
The results suggested that injection of the new polymerizing gel might be suitable to anchor re-injected autologous intervertebral disc cells or mesenchymal stem cells as a source for regenerative cell populations into the damaged nucleus pulposus. The gel will polymerize within the center of the nucleus, thus reducing the risk of leakage of cells from the refilled defect.

SP 46
DEATH OF INTERVERTEBRAL DISC CELLS AFTER TRAUMATIC INJURY TO THE LOWER CERVICAL SPINE: THE INFLUENCE OF TIME AND DEGENERATION

I Sitte, A Kathrein, B Huber, R Schmid, F Pedross, K Pfaller, S Roberts

Traumatology and Sportsmedicine, Medical University of Innsbruck, Innsbruck, Austria

Introduction: Integrity of the intervertebral disc can be diminished by various causes including disc degeneration as well as traumatic injury. Degenerative disc disease can be accompanied by bone changes including osteophyte formation, endplate sclerosis as well as reduced intervertebral space. Degenerative changes are found histologically in the central nucleus pulposus following injury to the outer annulus in an animal model. The present study was undertaken to examine the relationship between injury and degeneration in the human intervertebral disc.
Materials and methods: Anterior discs (n = 103) from 82 patients with traumatic injuries to their cervical spines were obtained between 0 days and 9 years post-injury (divided into 2 groups: 0–7 days (n = 64) and more than 8 days (n = 39)). Injuries were classified (via Magerl's system) into those likely to have a high degree of compressive loading (cl: A-, B1-, B2- and C-injuries with cranial injury) or less compressive loading (lcl: B3- and C-fractures). Degeneration was scored radiologically (grade I–V). Cell morphology was examined histologically and ultrastructurally to identify and quantify healthy, balloon, chondroptotic, apoptotic and necrotic disc cells.
Results: In the less degenerate discs (grades I–II) the number of healthy cells increased with time post-injury, particularly in those with greatest compressive load (cl: $p < 0.001$ in the AF and $p = 0.005$ in the NP, but for the AF 0–7 days cf >8 days post-injury $p = 0.024$ in the lcl group). In contrast there were significantly more necrotic, chondroptotic and apoptotic cells 0–7 days post-fracture than >8 days. However, no significant difference in frequency of cell death or healthy cells was seen with time in the more degenerate discs (grades IV and V) (see Figs. 1, 2).

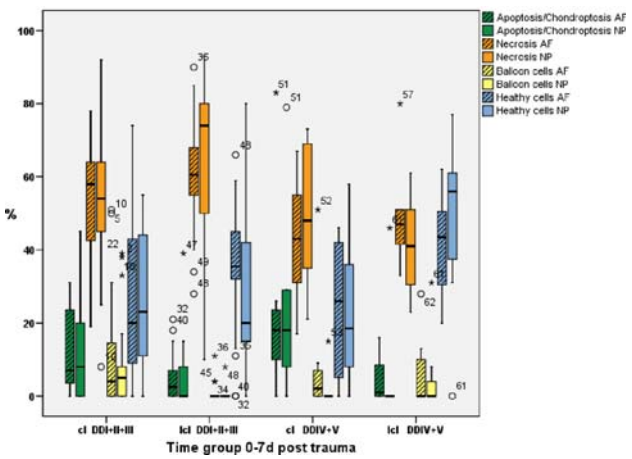

Fig. 1

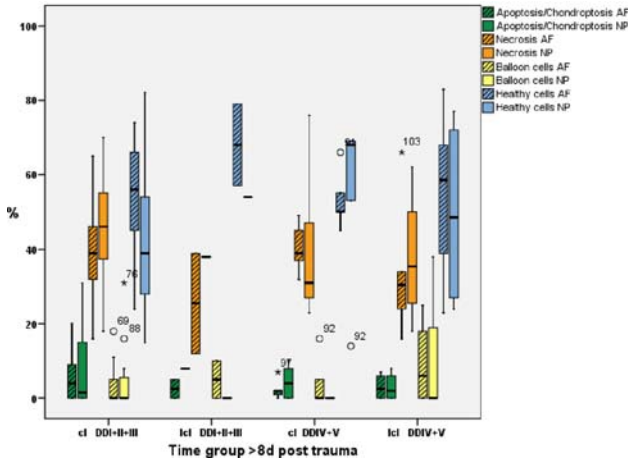

Fig. 2

Discussion and conclusion: In the first week after trauma to the cervical spine disc cell death via apoptosis, chondroptosis and necrosis is common in both the annulus and nucleus regions. In discs with a low degree of degeneration, the incidence of cell death decreases after a week and the number of healthy cells increases, particularly in discs which have had a high degree of compressive loading. It thus appears that disc cells are able to mount a proliferative response to trauma, but that this response is influenced by the type of injury and the underlying degree of degeneration present in the disc prior to injury.

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SP 47
THE INFLAMMATORY RESPONSE TO TRAUMA IN INTERVERTEBRAL DISCS AFTER FRACTURE OF THE THORACOLUMBAR SPINE

CE Heyde, I Anagnostopoulos, W Ertel, SK Tschoeke

Orthopaedic Surgery, University of Leipzig, Leipzig, Germany

Background: The optimal treatment algorithm regarding thoracolumbar fractures remains debatable, and long-term outcome of both operative and nonoperative strategies continue to show contrary results. Furthermore, the inflammatory response to trauma is known play a critical role in promoting disc degeneration, which may consecutively alter function and stability of the injured spinal segment.

Purpose: To evaluate whether a single traumatic impact causing vertebral fracture may induce migration of inflammatory cells into the adjacent intervertebral disc, similar to that observed in degenerative discs.

Methods: 18 human IVD specimens from 17 patients undergoing open reduction and anterior stabilization surgery of a thoracolumbar fracture were sampled after intervertebral cage-implantation (mean age 40.6 ± 13.3 year; 21–59 year; m:f—5:12). Samples were compared to three healthy IVD specimens from three patients, where disc resection was inevitable to perform stabilization of a vertebral tumour (mean age 46.0 ± 1.7 year; 44–47 year; m:f—2:1). Degeneration and tumour infiltration was excluded by radiographic and MR-imaging. All samples were immediately fixed in neutral buffered formalin upon resection and subsequently embedded in paraffin. Immunhistochemical analyses were performed on paraffin sections using the APAAP method and fast-red chormogen. Sections were stained with specific antibodies for neutrophil granulocytes (chloracetatesterase), CD68+ (PGM-1) and CD163+ (Ber-Mac3) macrophages, CD3+ and perforin positive cytotoxic T cells, and subsequently analysed by light microscopy.

Results: All sections from trauma IVD demonstrated enhanced positive staining for neutrophil granulocytes, CD68+ and CD163+ macrophage, as well as CD3+ and perforin positive lymphocytes. In contrast, healthy control specimens proved to be negative for these markers, thus confirming the complete absence of respective infiltrating inflammatory cells.

Conclusion: These data provide evidence that in the early phase after trauma inflammatory cells migrate into adjacent IVD tissue of fractured vertebrae. Furthermore, the consecutive inflammatory response may promote disc degeneration and critically affect the posttraumatic outcome in cases where segment integrity in fracture stabilization is considered.

SP 48
TISSUE-INTRINSIC SUPPORT OR HAMPER OF VASCULO-ANGIOGENIC PROCESSES IN THE TRAUMATIZED RAT SPINAL CORD

ON Hausmann, MF Ritz, B Gutierrez, U Graumann

Spinal Injury Research, Swiss Paraplegic Research, Nottwil, Switzerland

Acute spinal cord injury (SCI) initiates a series of cellular and molecular events in the injured tissue leading to further damage in the surrounding area. Physical and molecular disruption of the sensitive capillary networks leads to haemorrhages, inflammation, and disturbance of the blood spinal cord barrier. Although the regenerative capacity of the nervous tissue vasculature has been illustrated in experimental animal studies of spinal cord trauma it became obvious that restoration of the microvascular function is incomplete. Recent observations of participa-tion of CD133+ bone marrow-derived endothelial progenitor cells (EPC) in neo-vessel formation in peripheral organs raise the question about a possible local recruitment and the fate of these cells in ischemic SCI-tissues. Further, a question of considerable interest is that of the stability of the newly growing vessels appearing in the early phase after SCI.

In order to highlight a possible contribution of CD133+ EPCs in vessel formation after SCI, we analyzed the temporal expression of precursor cell markers (e.g., CD133, CD34, CXCR4) and of several angiogenic factors (e.g., SDF-1, HGF, VEGF) in the rat thoracic spinal cord following a compression injury using RT-qPCR and immunohistology. Blood vessel structures were visualized by staining with RECA-1 and smooth muscle actin (SMA) antibodies. The analyses of the dissected tissues were performed at various time points, from 2 h to 4 weeks after SCI. In addition, the temporal expression of key-modulators for vessel stability (e.g., TGFb1 and PDGF-BB) was investigated by RT-qPCR.

Whereas the gene expression of CD133 decreased from 6 h to 3 days post-SCI, the expression of CD34, CXCR4, and HGF increased in the injury site after 2 or 3 days, and after 3 weeks for SDF-1. CD133 expressing cells colocalized with cells also positive for SMA, while CD34 and CXCR4 were expressed mainly by endothelial cells. SDF-1 was secreted by reactive astrocytes around the lesion. VEGF and PDGF-BB expression was decreased with the lowest level at day 3 postinjury, whereas TGFb1 exhibited the highest level.

These results indicate that the expression of factors involved in neovascularization is modulated in the injured spinal cord after SCI in a time-dependent manner. The finding implicate in-sights about the