

ENGINEERING OSTEOGENIC PROGENITORS FROM EMBRYONIC STEM CELLS

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Introduction: Although immune-compatible autologous adult stem cells have been proven to repair bone defects, the practical use of these cells is limited by the scarcity of available progenitors or the quality of their genetic composition. New approaches to skeletal repair involving pluripotent stem cells have thus emerged.

Results: Our lab has previously explored the *in vitro* osteogenic differentiation of murine, non-human primate and human embryonic stem cells (ESCs) as a model of skeletal development from pluripotent stem cells. One of the major bottlenecks on the path to successful clinical implementation of ESC technology in the area of degenerative bone diseases, however, is the poorly defined strategies that are currently available to induce directed differentiation of ESCs into osteoblasts.

In the past, we have identified the Wnt/beta-catenin (CtnB) signaling pathway as a regulator of osteogenesis, which may be manipulated to enhance mineralization and maturation as well as early mesendodermal specification. Our newer studies suggest that osteoblasts may be generated from ESCs from progenitors of the neural crest, paraxial mesoderm and lateral plate mesoderm. Specifically, expression of Wnt5a seems to mark neural crest derived osteoprogenitors.

Conclusion: With the desire to identify inexpensive differentiation strategies, we have also studied the metabolism of differentiating osteoblasts and will present results, which imply that hyperglycemia is detrimental to osteoblast development by interfering with Wnt signaling. Furthermore, we have begun to investigate the role of small regulatory microRNAs in osteogenesis, which may also be linked to the Wnt/CtnB signaling pathway. Results of scale-up studies will also be presented.

Keywords: Embryonic stem cells, osteoblasts, Wnt signaling

ORAL PRESENTATIONS

PREFIX (B2A-COATED CERAMIC GRANULES) FOR LUMBAR SPINAL FUSION: PRECLINICAL AND PRELIMINARY CLINICAL RESULTS

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Introduction: Synthetic osteoconductive ceramics are by themselves insufficient as grafts to facilitate spinal fusion, a treatment for chronic back pain. PreFix is composed of ceramic granules coated with the bioactive peptide B2ATM and is in development to increase spinal fusion rates without the need for autologous iliac crest bone (ICB), the gold standard.

Methods: Preclinical studies. PreFix/B2A mixed with ICB was evaluated in a rabbit posterolateral fusion (PLF) model. Fusion was assessed at 6 weeks by radiography and palpation, and confirmed by histology and CT. In a sheep model of instrumented interbody spinal fusion, PreFix/B2A plus ICB was evaluated after 4 months by CT and histomorphometry. Clinical studies. International multi-center,

prospective, randomized pilot studies were initiated to evaluate safety and preliminary effectiveness of PreFix/B2A plus local bone as compared to ICB in subjects with degenerative disc disease.

Results: Preclinical studies. In the rabbit PLF model, PreFix/B2A resulted in significantly higher fusion incidence as compared to controls. In the sheep interbody fusion model, PreFix/B2A had higher fusion rates as compared with the control. Clinical studies. After 6 months, radiographs and CT for the first 13 patients demonstrated comparable spinal fusion rates for each group. VAS pain and Oswestry Disability Index scores from 13 patients at 6 months demonstrated substantial improvements for both groups compared to baseline. No serious device-related adverse events have been observed.

Conclusion: PreFix/B2A may provide a safe, less expensive and more effective alternative to current bone grafting materials and be a reliable alternative compared to ICB.

Keywords: Growth factor, spine fusion, peptide, bone repair

BIOMECHANICAL IN-VITRO EVALUATION OF A NEW POLYMERIZING GEL

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Introduction: A new carrier material for the autologous disc-derived chondrocyte transplantation was developed. The material is based on an albumin hyaluronic acid gel which can be injected in a degenerated intervertebral disc and subsequently polymerizes *in situ*.

In order to evaluate the biomechanical behavior after injection and to evaluate the extrusion risk a biomechanical *in-vitro* study was performed.

Methods: 12 bovine lumbar functional spinal units were subjected to a complex multi-axial loading condition in order to simulate worst-case conditions. In between the segmental height was measured repeatedly. Also a possible gel extrusion was observed permanently.

Results: After injection and polymerization of the gel the median increase of height was 0.3 mm for all 6 specimens of the gel injection group. During cyclic loading a gradual decrease of height could be detected. However, this was considered to be the result of visco-elastic creep and liquid extrusion. No gel extrusion could be observed for all specimens within the whole test procedure. Finally, macroscopic sections exhibited an accumulation of the polymerized gel in the center of the nucleus.

Conclusion: The results demonstrated that injection of the new polymerizing gel has the potential to anchor injected autologous disc-derived chondrocytes as a source for regenerative cell populations in the damaged nucleus pulposus.

Keywords: Disc degeneration, ADCT, carrier material, biomechanical testing

THE DOG AS A SPONTANEOUS ANIMAL MODEL FOR HUMAN INTERVERTEBRAL DISC DEGENERATION

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Introduction: Many different animal models are used in intervertebral disc degeneration (IVDD) research and most are mechanically or chemically induced.

Aim: To investigate if spontaneous IVDD in dogs is a good model for human IVDD research.

Methods: 183 IVDs from 19 randomly selected dogs were used. The degree of IVDD was evaluated using gross pathology, MRI, histopathology, glycosaminoglycan (GAG) content and activity of matrix metalloproteinase 2 (MMP-2). All parameters were compared to previous studies of human IVDD using the same evaluation methods.

Results: On gross pathological evaluation and MRI, many similarities between the appearances of IVDD in both species were found. The notable difference was the relatively common occurrence of ventral spondylosis in dogs. Histopathology revealed thicker cartilaginous endplates in humans. This is likely due to the lack of growth-plates in developing human vertebrae, which are present in dogs. The presence of notochordal cells was observed much later in life in most (non-chondrodystrophic) dog breeds compared to humans. Notably, the notochordal cells disappear at the age of 1 year in chondrodystrophic dogs at which age IVDD starts. The relative GAG content and MMP-2 activity in canine IVDD followed a similar pattern as that of humans: MMP-2 activity increased and GAG content decreased with increasing grades of IVDD.

Conclusion: There are many similarities between human and canine IVDD, thus the dog may serve as a good model of spontaneous IVDD for human research. However, when employing the dog as a model for human IVDD it is important to recognize inter-species differences.

Keywords: Intervertebral disc degeneration, animal model, dog

LUMBAR SPINE INSTABILITY AFTER LAMINECTOMY: A HUMAN CADAVER STUDY

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Introduction: Laminectomy is a customary surgical intervention for patients with lumbar stenosis. Using this technique the affected nerves are successfully decompressed, but a reduction in residual strength and stiffness may increase the risk of postoperative spondylolisthesis and return of neurological symptoms. In this study we quantify the effects of laminectomy on the stiffness of the lumbar spines of human cadavers with the aim to determine whether stabilization after laminectomy is indicated.

Methods: Ten lumbar spines that were freshly frozen were included. From each lumbar spine two motion segments were dissected (L2/L3 and L4/L5). Laminectomy was performed in one of the two segments of each spine (equally divided between L2/L3 and L4/L5). MRI and X-ray's were used to grade the degeneration of the segments. Stiffness and ultimate shear strength of all segments (20 total) were determined using Instron testing equipment. The segments were loaded in compression with 1600 N and subsequently loaded in ventral shear with a shear rate of 2 mm/min until failure. Strength and stiffness of segments with and without laminectomy were compared using a paired student t-test.

Results: Ventral shear strength was reduced to 59% (SD = 23%) compared to the native segment after laminectomy ($p = 0.0017$). The effect of laminectomy on the strength and stiffness was dependent on the amount of degeneration; more degenerated segments showed less reduction in strength and stiffness.

Conclusion: Ventral shear strength can be reduced considerably by laminectomy. This effect is greater in less degenerated segments. In these cases stabilization of the segment using instrumentation is indicated.

Keywords: Human, Lumbar spine, Degeneration, Laminectomy, Stiffness

COMPARING AUTOGRAFT, ALLOGRAFT AND TRICALCIUM PHOSPHATE IN INSTRUMENTED POSTERIOR LATERAL FUSIONS

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Introduction: The use of a synthetic bone graft, such as TCP would circumvent the disadvantages of autograft and allograft, due to prevention of donor site morbidity, its unlimited supply, and ease of sterilization. The purpose of this study was to compare the effectiveness of TCP with and without local autograft, with iliac crest autograft and fresh-frozen allograft in spinal fusion in goats.

Methods: Nine goats underwent a two-level instrumented posterolateral fusion. Each unilateral segment was randomized to receive 10 cc of iliac crest autograft, fresh-frozen allograft, TCP with local autograft or TCP alone. After 16 weeks, CT-scans were used to evaluate the fusion qualitatively and quantitatively. In addition, histological evaluation was performed.

Results: The fusion rates were 38% and 50% for autograft and allograft, 50% in the TCP group and 63% in the TCP/local autograft group. There were no statistical differences. The volume of the fusion mass in the autograft group was significantly higher than in the TCP and TCP/local autograft group. This also accounted for the allograft vs TCP alone. Histological evaluation showed that the TCP was well integrated and almost completely resorbed.

Conclusion: TCP was capable of achieving fusion at a rate comparable to that of autograft, while almost completely resorbing. It is clear from these results that TCP is a promising alternative for the autologous bone graft, but cannot yet reach comparable bone volumes. Increase of implanted graft volume may overcome this or the bone inductive properties may be enhanced by optimizing surface microstructural properties or by addition of bioactive stimuli.

Keywords: Spinal fusion, TCP, autograft, allograft

APPLICABILITY OF LAMINECTOMY BONE SHAVINGS AS VIABLE AUTOGRAFT FOR SPINAL FUSION

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Introduction: In spinal decompression and fusion surgery, high speed burrs are often used to remove bony material. Bone shavings generated that way can serve as an autograft, but so far no data regarding successful cell delivery from shaving material exist. Aim of the presented study is an in vitro comparison of osteoblast delivery and characteristics from laminectomy bone grafts harvested either by Kerrison rongeur or by high speed burr.

Methods: Bone chips and shavings from 14 patients (13 female, 1 male; mean age 68 +/- 10 years) undergoing spinal decompression surgery were analyzed after institutional review board approval. 5 patients had concomitant diseases (osteoporosis : n = 2; diabetes mellitus: n = 2; permanent cortisone medication: n = 1) potentially affecting bone healing. Samples were aseptically placed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 µg/ml Penicillin and 100 U/ml Streptomycin for transport to the laboratory. Bone chips and shavings were then washed in phosphate buffered saline (PBS) to remove contaminating erythrocytes and sample wet weight was assessed. Samples were then cultured in DMEM supplemented with 10% fetal calf serum (FCS), 0.05 mg/ml Ascorbic Acid, 100 µg/ml Penicillin and 100 U/ml Streptomycin for three weeks. Osteoblast emigration and proliferation, viability and mineralization were analyzed.