O.01
MSCs SECRETOME DECREASES MATRIX DEGRADING ACTIVITY OF ANULUS FIBROSUS CELLS UNDER MECHANICAL LOADING AND AN INFLAMMATORY ENVIRONMENT
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Introduction: Cell-based therapies for low back pain and intervertebral disc (IVD) degeneration have been emerging specially using MSCs, despite the poor knowledge on their full mechanism of action. As failure of the AF is often associated with IVD herniation and inflammation, the objective of the present study was to investigate if the secretome of MSCs influences AF cells exposed to mechanical loading in a pro-inflammatory environment.

Materials/Methods: Human IVD biopsies were isolated from patients with scoliosis or IVD degeneration, after Hospital’s ethical approval and patients’ informed consent. AF cells were expanded and exposed to physiological cyclic tensile strain (CTS) during 72h with or without the presence of IL-1β (10 ng/mL). AF cells stimulated with CTS and IL-1β were treated with MSCs secretome during 48h. Metabolic activity, gene expression and MMPs activity of AF cells were evaluated.

Results: Similar gene expression profile, metabolic activity and apoptosis of AF cells from patients with scoliosis and IVD degeneration were observed. CTS stimulation up-regulated COL1, while IL-1β significantly stimulated the expression of IL-6, IL-8, MMP1, MMP3 and PGE₂ production, and down-regulated COL1 gene expression. The combination of CTS+IL-1β had a similar outcome as IL-1β alone. MSCs secretome reduced MMP1/3 gene expression and MMP2/9 activity of CTS+IL-1β-stimulated AF cells.

Conclusions: The results obtained demonstrate the impact of the inflammatory milieu on the human AF, evidencing a novel aspect of MSCs mechanism of action in degenerated IVD that consists in the modulation of MMPs activity by AF cells, which could have an impact in AF tissue weakening and consequently in IVD herniation.
NOSE TO BACK: COMPATIBILITY OF NASAL CHONDROCYTES WITH ENVIRONMENTAL CONDITIONS MIMICKING A DEGENERATED INTERVERTEBRAL DISC
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Introduction: One therapy dedicated to treating intervertebral disc (IVD) degeneration is cell therapy. In recent years, nasal chondrocytes (NCs), have gained a reputation for cartilage tissue regeneration. To assess whether NCs can be considered as an autologous cell source for cell therapy of disc degeneration, NCs were compared to mesenchymal stromal cells (MSCs) and articular chondrocytes (ACs), two cells sources presently used in phase two clinical trials.

Methods: To mimick the milieu of the degenerated IVD in vitro, cells were cultured in low glucose media (1gr/L) in hypoxia (2% O₂) for 28 days. The media was adjusted to pH 6.8 and/or supplemented with proinflammatory cytokines TNFα, IL1β, and IL6. Furthermore, cells underwent chondrogenic instructional priming by addition of TGFβ1 for the first seven days of culture.

Results: Analysis of histology, immunohistochemistry, biochemistry and quantitative rtPCR demonstrated that, harsh environmental conditions and donor variability play a crucial role in the successful survival and chondrogenesis of MSCs. ACs could accumulate glycosaminoglycans (GAG), however, lack the ability to synthesize Collagen type II (Col2). Commendably, primed NCs produce ECM rich in both GAG and Col2 in nonacidic conditions. Even though an acidic environment reduces GAG production of NCs the same as ACs, the supplementation of the inflammatory cytokines affect the NCs to a lower extent compared to ACs.

Conclusion: Our data indicate that NCs are more resident to harsher environments than ACs or MSCs upon chondrogenic priming. These findings encourage the assessment that employing NCs in a cell therapy treatment of degenerative disc disease.
Introduction: Disease modifying anti-rheumatic drugs (DMARDs) have shown pain relieving effects in the treatment of degenerative disc disease. However, their underlying anti-inflammatory and regenerative activity is poorly explored. The present study aimed to investigate the effects of the TNF-α inhibitor Etanercept and the selective JAK3-inhibitor Tofacitinib in a degenerative, inflammatory intervertebral disc (IVD) organ culture model.

Methods: Whole bovine caudal IVDs were cultured within a bioreactor. The control group (PHY) was cultured under physiological loading and high glucose medium. In the degenerative group (DEG+TNF-α), detrimental loading and low glucose medium was applied together with TNF-α intradiscal injection (100 ng/IVD). Etanercept (3.5 mg/70 µL/IVD) or Tofacitinib (2.5 µg/mL) was applied under DEG+TNF-α conditions by intradiscal injection or medium supplement respectively. After 4 days, the effect on cellular gene expression and molecule release from IVDs was analysed.

Results: DEG+TNF-α treatment upregulated the expression of catabolic enzymes MMP-1, MMP-3, and proinflammatory cytokines IL-1β, IL-6, IL-8; whereas Etanercept and Tofacitinib partially reduced these effects. DEG+TNF-α conditions induced ECM degradation, as indicated by markedly elevated GAG release; and was partially attenuated by both drugs. NO and IL-8 release from IVD were increased under DEG+TNF-α conditions. Etanercept partially attenuated the release of NO and IL-8 protein, whereas Tofacitinib had no effect on it.

Conclusion: The combination of detrimental dynamic loading, nutrient deficiency, and intradiscal TNF-α injection synergistically triggered a proinflammatory and degenerative situation within the IVD. Etanercept and Tofacitinib showed the ability to slow down the degenerative response and reduce inflammation in the organ culture model.
O.04
INTERVERTEBRAL DISC DEGENERATION FROM A BIOMECHANICAL POINT OF VIEW: WHAT DO WE NEED TO FIX?
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Introduction: Low back pain is viewed as multi-factorial, and the predominant interventions are physiotherapy and remaining active. These biomechanical stimuli possibly stimulate cells to maintain the extracellular matrix. To provide biomechanical parameters which regenerative therapies need to address, this series of ex-vivo experiments investigates the determinants of ‘healthy’ disc biomechanics.

Methods: In four studies, caprine lumbar intervertebral discs were tested in a loaded bioreactor for hours upto days: 1) Intradiscal pressure (IDP) changes under physiological loading were identified; 2) The effects of proteoglycan loss were mimicked by increasing medium osmolality with polyethelene glycol (PEG); 3) We used healthy and degenerated human discs to validate our results; 4) IDP measurements and PEG medium effects were combined to isolate the effects of loading and osmolality.

Results: 1) Axial loading affects IDP twofold, initially; pressure rises. Over time, water-content reduces and IDP and disc height decline. Upon unloading, this is reversed; 2-3) Biomechanical behaviour after PEG addition closely resembled changes seen in degenerated human intervertebral discs: disc height, recovery and overall creep where reduced. However, stiffness was not affected and time-constants increased; 4) Changes in axial loading affect disc height, IDP and nucleus water-content, whereas changes in osmolality only affected disc height and annulus water-content.

Conclusion: Osmotic pressure generated by the disc’s proteoglycans is largely responsible for normal axial disc biomechanics. Permeability of the annulus and nucleus is the second most important parameter. Finally, the intervertebral disc remains highly hydrated (~68%) under prolonged loads of ~1.0 MPa. These unique properties need to be mimicked with regenerative therapies.
O.05
PERCUTANEOUS ENDOSCOPIC DECOMPRESSION FOR CALCIFIED THORACIC DISC HERNIATION

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Introduction: To investigate the efficacy of a modified percutaneous endoscopic technique for the treatment of calcified thoracic disc herniation.

Methods: Eleven patients (8 males, mean age 42.1 years) with single-segmental calcified thoracic disc herniations were treated with percutaneous endoscopic surgeries from January 2012 to January 2017. The surgical outcomes were assessed using the visual analog scale (VAS), the modified Oswestry disability index (ODI), and the modified Macnab criteria.

Results: The mean follow-up period was 24.2 months (8–52 months). The mean VAS score of leg/back pain improved from 8 to 1.75 at the final follow-up. The mean ODI score improved from 65 to 10.5 at the final follow-up. The modified Macnab criteria was excellent in 6 patients (54.5%), good in 4 (36.4%), and fair in 1 (9.1%). None of the patients required conversion to open procedures.

Conclusions: Percutaneous endoscopic decompression under local anesthesia is a safe and reproducible surgical procedure for the treatment of calcified thoracic disc herniation.
O.06
CLINICAL AND RADIOLOGIC OUTCOMES OF SINGLE-LEVEL DIRECT LATERAL LUMBAR INTERBODY FUSION IN PATIENTS WITH OSTEOPENIA
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Introduction: The direct lateral interbody fusion (DLIF) is a useful surgical approach for many spinal diseases. Subsidence of the interbody graft into the vertebral bodies is one of the major complications of DLIF, resulting in pseudoarthrosis and severe pain. Low bone mineral density (BMD) has been known to increase the risk of subsidence. This study aims to determine whether or not the osteopenia should influence the surgical results in DLIF surgery.

Methods: We reviewed collected data retrospectively on all patients who underwent sing-level DLIF at our institution. A total of 62 patients were included in this study, including 28 in the osteopenia group and 34 in the normal group. The clinical and radiologic characteristics were compared at pre-operative and at 1 and two years postoperatively.

Results: At postoperative two years, there was a statistically significant difference in disc height and intervertebral foramen height at index level between the two groups (p = 0.039, 0.044, respectively), but there was no significant difference in total lumbar lordosis (p = 0.179). At 24-month postoperative, 92.9% of osteopenia group and 97.1% of normal group were solidly fused (p = 0.585). All postoperative clinical scores (VAS back pain, VAS leg pain, and Oswestry disability index) improved similarly in both groups without significant difference. The difference in the rate of subsidence between the groups was not significant (7.1% in the osteopenia group and 2.9% in the normal group, p = 0.585).

Conclusions: Patients with osteopenia who undergo single-level DLIF exhibited comparable clinical and radiological outcomes as patients without osteopenia.
MICRODISCECTOMY ANNULAR REPAIRMENT AND AUTOLOGOUS CONDITIONED PLASMA INTRADISCAL INJECTION: A NEW SERIAL THERAPEUTIC MODEL FOR THE TREATMENT OF LUMBAR DISC HERNIATION

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Aim: Recurrent intervertebral disc herniation and degenerative disc disease have been identified as the most important factors contributing to persistent pain and disability after surgical discectomy. Annulus fibrosus closure device can immediate closure of the annulus fibrosus rupture combined with Autologous Conditioned Plasma (ACP) intradiscal injection which can promote numerous growth factors repair, restores disc height, reduces further disc degeneration and enhances intervertebral disc self-repair and regenerative capacities.

Methods: This study recruited 7 patients with lumbar disc herniation who underwent tubular microdiscectomy annulus fibrosus repairment and Autologous Conditioned Plasma intradiscal injection. The pre/post-operation neurological function and pain status were evaluated by the visual analog scale (VAS) score and the Oswestry disability index (ODI). The assessment data also including: operation time, the quantity of bleeding and intra/post-operative complications were recorded. Patients were followed up at intervals of preoperative, postoperative 1 week, 1 months, 3 months, last follow-up.

Results: The procedure was successfully performed in all cases. The mean age of patients was 36.6 years. Average operation time was 85 minutes, average blood loss was 35.3±6.2mL. The preoperative symptoms were alleviated significantly after surgery. All the standardized measures improved significantly. At the last follow-up, including VAS score (7.9±1.2 to 1.1±0.5; p < 0.001) and Oswestry Disability Index (75.3 to 9.6; p < 0.001). There was no postoperative complication and recurrence of disc herniation.

Conclusion: Early results showed the use of tubular microdiscectomy annulus fibrosus repairment and Autologous Conditioned Plasma intradiscal injection are beneficial for short term outcomes demonstrating reduction in symptomatic disc reherniation with low intra/post-operative complication rates. Long-term studies are required to further investigate the efficacy of such devices. Direct mechanical annular repair and ACP biological therapy may promote degenerative intervertebral disc regeneration and remodeling after MISS discectomy.
L5/S1 lumbar disc herniation

Tubular microdiscectomy and annular repair

Intradiscal injection of ACP
O.08
COMPARISON OF FORAMINOTOMY AND INTERVERTEBRAL DISTRACTION FOR NERVE ROOT DECOMPRESSION IN DEGENERATIVE SPINAL DISEASE

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Introduction: Degenerative spinal disease (DSD) can involve nerve root compression with consequent back pain. Surgical techniques for nerve root decompression include foraminotomy and intervertebral distraction with/without spinal fixation. The objective of this study was to compare the effects of foraminotomy and intervertebral distraction on the volume of the lumbosacral intervertebral foramen (IVF) using the dog as a model for DSD.

Methods: CT images were obtained from 7 canine lumbosacral (L7-S3) specimens in the following sequential conditions: 1) native spine, 2) after partial discectomy of L7-S1, 3) after L7-S1 foraminotomy, 4) after distraction with an L7-S1 interbody cage, 5) after distraction with cage stabilized with pedicle screw-rod fixation (PSRF), and 6) after distraction with cage stabilized with PSRF fixed in flexion. Total, cranial, and caudal compartmental IVF volumes were calculated using the CT images and statistically compared between conditions.

Results: Total IVF volume was significantly increased after foraminotomy (mean±SD: 149.0 ± 27.9%, p < 0.01) and after intervertebral distraction (120.0 ± 25.0%; p = 0.01) with no difference between the distraction conditions. Foraminotomy induced a significantly larger increase in total IVF volume compared to intervertebral distraction (p < 0.01). Foraminotomy, but not distraction, induced a significant increase in the cranial compartmental IVF volume (161.2 ± 34.6%; p < 0.01).

Conclusion: Foraminotomy and intervertebral distraction significantly expand the lumbosacral IVF. Foraminotomy is more effective in increasing the foraminal volume and especially the cranial IVF compartment, which is where the nerve root transverses the IVF. Hence, foraminotomy may be more effective for nerve root decompression in patients affected by DSD.
O.09
UPDATE ON CUSTOM MADE 3D PRINTED TITANIUM IMPLANTS FOR ANTERIOR COLUMN RECONSTRUCTION FOLLOWING EN BLOC RESECTION FOR SPINAL TUMOURS
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Introduction: Reconstruction of the segmental defect after en bloc resection for spinal tumours aims at immediate stability and secondary solid fusion. The present communication provides an update on the results of an ongoing study concerning the use of 3D printed custom made implants for anterior column reconstruction.

Methods: In 17 patients submitted to en bloc resection for spinal tumour between November 2015 and June 2018 at the same Institution, anterior column reconstruction was performed using 3D printed custom made implants. Resection was planned according to Enneking and Weinstein-Boriani-Biagini staging systems. Implants were designed according to the preoperative planning of the resection on CT-scan.

Results: At an average 18 months follow-up (range 1-28), one major mechanical complication occurred requiring the implant removal and one implant was replaced due to recurrence of the disease. Mechanical complication consisted in a massive subsidence of the prosthesis into the adjacent vertebral body and occurred with development of progressive distal junctional kyphosis. Critical analysis of the construct revealed insufficient posterior instrumentation, but custom made implants itself did not show post-operative mechanical complications (breakage or migration of the implant). However, because of the necessity of a surgical revision of the construct, it was considered a major mechanical complication. The removed implant was processed and sectioned for histological analysis that revealed the presence of new bone formation into the implant.

Conclusion: Custom made 3D printed titanium implants seems to be a viable option for restoration of the anterior column after en bloc resection for spinal tumor. Longer follow-up will be needed for fusion rates and long-term complication rates.
COMPARATIVE STUDY OF THE CROSSTALK BETWEEN MICROGLIA AND DEGENERATED VERSUS NON-DEGENERATED HUMAN INTERVERTEBRAL DISCS: IMPLICATION OF SPHINGOSINE-1-PHOSPHATE MODULATION IN NEUROINFLAMMATION

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Introduction: Low back pain (LBP), the most common cause of disability worldwide, is typically associated with intervertebral disc (IVD) degeneration (IDD). Although its aetiology is largely unknown, neuroinflammation exerts a pivotal role, due to the contribution of pro-inflammatory cytokines and the activation of neuroinflammatory-like cells. Using human IVDs, our study investigated the crosstalk between microglia and degenerated (D-IVDs) versus non-degenerated IVD tissues (nD-IVDs), and the possible implication of sphingosine-1-phosphate (S1P), as pro-inflammatory mediator.

Methods: Seven D-IVDs and adipose tissues (ATs) were obtained after surgery from patients affected by LBP. Three nD-IVDs were collected from organ donor’s spines with no history of LBP and spinal deformities. To assess the degenerative and non-degenerative microenvironment effect on neuroinflammatory-like cells, microglia were co-cultured with conditioned media (CM) by D-IVD, nD-IVD and AT. Viability, proliferation, migration, chemotaxis and inflammatory gene expression were evaluated. Further, to dissect the implication of S1P, FTY720, a modulator of S1P receptors signalling, was administered to D-IVD/microglia co-culture. Concomitantly, the exclusive effect of S1P was examined in nD-IVD/ and AT/microglia co-cultures.

Results: We observed that human D-IVDs caused microglia activation, increasing chemotactic activity and migration compared to AT or nD-IVD, releasing pro-inflammatory mediators (RANTES, TGF-β, IP-10). Interestingly, we reported that the pro-inflammatory effect of D-IVD on microglia, involves S1P signalling and that FTY720 strongly inhibits microglial activation. Similarly, exogenously administration of S1P in nD-IVD/ and AT/microglia co-cultures, mimics detrimental D-IVD effects.

Conclusion: Our data suggest that D-IVD induces microglial activation and this effect is counteracted by FTY720 through S1P signalling modulation.
A NOVEL TREATMENT FOR LUMBAR FACET JOINT PATHOLOGY THE PRP CT-GUIDED INJECTION

Philippe Adam

Introduction: Facet joint pathology can be caused by a mechanical disease, inflammatory issue or neuropathic disease. The clinical experience described, aims to assess whether CT-guided lumbar injections of PRP might be an effective alternative treatment option for facet joint pathology.

Methods: Study group: 19 males and 16 females (35 patients, mean age 50 years)
- 10 patients suffering from pure Lower Back Pain (LBP)
- 25 patients suffering from LBP associated with Leg Pain (LP)

Targeting of injection sites by MRI

Treatment: One CT-guided injection of RegenKit® BCT A-PRP (5 to 10 ml).

Outcome measures: Visual Analogic Score for LBP and LP, Roland-Morris Disability. Questionnaire and Oswestry Disability Index at d0, m1 and m3 MRI assessment of facet joint enhancement by intravenous Injection of contrast medium (21/35, d0 and m1).

Results: Mean pain score for LBP: d0 7.1/10, m1: 3.6/10, m3: 4.4
Mean pain score for LP: d0 6.4/10, m1: 2.3/10, m3: 1.2/10
RMDQ : d0 12.1/24, m1 8.1/24, m3 6.9/24
ODI : d0 18/50, m1 11.7/50, m3 12.1/50
Reduction of facet joint enhancement: 19/21 at m1
14 patients were followed-up for 9 months with therapeutic effect of PR still effective in 11

Conclusion: PRP CT-guided injection represent an effective non-surgical treatment for facet joint pathology (81% decrease of LP and 38% decrease for LBP) after failure of standard medical treatments. PRP acts as an autologous antalgic and anti-inflammatory agent specially for diabetic patients.
ABLATION OF THE BASIVERTEBRAL NERVE: PILOT STUDY RESULTS AND LONG – TERM RESULTS OF AN INNOVATIVE TREATMENT OF BACK PAIN

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Introduction: The basivertebral nerve has been described as being the origin of innervations of the disc and one of the major factors of back pain. The study shows the results of a RF – ablation of this nerve (Intracept – system).

Methods: Prospective, multicenter clinical study, long – term monocenter follow - up. 16 patients were prospectively treated between 2008 and 2009 in a multicenter trial. The follow – up nine patients in a single center (max. 5.5y). All patients were originally presenting for surgical therapy (fusion or TDR. All initial patients showed erosive osteochondritis (Modic 1 /2) of a minimum of two, maximum of three adjacent vertebrae. Treatment was performed using real-time fluoroscopy. Using a transpedicular or extrapedicular approach, bipolar RF energy was used to ablate the BVN. Treatment was limited to L3, L4, L5 and S1 vertebrae.

Results: The initial 16 patients showed a significant decrease of ODI scores (31 points) within the first year (p=0.001) with the biggest drop after surgery of 29 points (p < 0.01). The mean follow – up (3 – 5.5 years) showed a continued improvement of all parameters (VAS pre 6.3, max F/U 1.1; SF36 PCA pre 36.8, max F/U 47,4; MCA pre 47,4, max F/U 50,2; ODI pre 53,1, max F/U 18,8).

Conclusion: The basivertebral nerve is an important factor in back pain. The ablation of this has no negative effect on spine stability whilst improving back pain. This research has received the Lodwick award 2018 from Harvard University for the best research and paper in musculoskeletal medicine.
O.13

DOES THE TERMINAL COMPLEMENT COMPLEX PLAY A ROLE IN DISC DEGENERATION?

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Introduction: The present work focuses on the characterization of terminal complement complex (TCC) deposition in human intervertebral disc (IVD) tissues and its correlation with different disc pathologies.

Methods: Disc tissues were collected from healthy donors (Healthy, n = 6, age 12 ± 7), patients with scoliosis (Sc, n = 10, age 16 ± 5, 7F/3M) displaying no signs of disc degeneration, or with disc degeneration (DD, n = 36, age 60 ± 13, Pfirrmann grade 3-5). TCC deposition was investigated in AF, NP and EP. Randomly selected Sc and DD expanded cells (passage 2-5) were analyzed for gene expression of TCC-inhibitors CD46, CD55 and CD59, apoptosis, pro-inflammatory markers, extracellular matrix proteins and degrading enzymes. Cellular TCC deposition was determined by ELISA. An erythrocytes lysis test was performed to confirm TCC’s lytic activity. Statistical analysis was performed with Kruskal-Wallis test.

Results: Although TCC immunopositivity was observed with high variability, a significantly higher frequency of TCC+ cells was found in Healthy and DD compared to Sc group (p < 0.05). After cell expansion, CD46 and aggrecan were down-regulated in NP cells of DD compared to Sc (p < 0.05). Moreover, no significant differences were observed between DD and Sc expanded cells regarding TCC deposition and cell lysis.

Conclusion: The data suggests that TCC is formed in IVD cells both in very young or strongly degenerated discs, which might correlate with vascularization. TCC deposition was shown to be induced in vitro. Ongoing studies explore the functional relevance of TCC in DD as possible target for new therapeutic approaches. Acknowledgement: DFG (NE_549/6-1, BR_919/12-1), Ulm University (L.SBN.0157).
INVESTIGATION OF INFLAMMATORY AND PAIN-RELATED PATHWAYS IN CANINE DEGENERATIVE SPINAL DISEASE

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Introduction: Degenerative spinal disease (DSD) involves degeneration of the intervertebral disc (IVD) and the ligamentum flavum (LF). Besides people, dogs also commonly suffer from spontaneous DSD and hence this species is an accepted animal model for DSD. Based on the recent scientific literature, inflammation is a key process that can cause degenerating spinal structures to become painful. The aim of this study was to investigate a wide array of inflammatory and pain-related agents and to identify key inflammatory pathways in dogs suffering from DSD.

Methods: LF and IVD tissue was collected from 13 dogs affected by lumbosacral DSD and 16 healthy dogs. A qPCR gene array was used to investigate the expression of 84 inflammatory genes (n=4 per group). Specific gene targets were further investigated in 4 additional samples per group using qPCR. Protein expression validation of nerve growth factor (NGF) was performed using Western Blot analysis.

Results: Tumor Necrosis Factor (TNF) Superfamily and Nuclear factor kappa B (NF-kB) signaling were identified as major pathways activated in DSD. Significant regulation (N-fold ± SD) of 10 different inflammatory mediators involved in these pathways was found, including NGF (-8.2 ± 10.2, p < 0.01) and TNFSF ligand 10 (9.4 ± 7.6, p < 0.01) and 11 (85 ± 73.5, p < 0.01). Protein expression of NGF was significantly upregulated in degenerated LF and IVD tissue.

Conclusion: DSD involves the regulation of various inflammatory and pain-related pathways, including TNFSF/NF-kB signaling. These findings may open new doorways to counteract the inflammatory and pain-related processes involved in DSD.
THE UNFOLDED PROTEIN RESPONSE MEDIATED BY PERK IS CAUSALLY RELATED TO THE PATHOGENESIS OF INTERVERTEBRAL DISC DEGENERATION

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Introduction: Although the number of patients with intervertebral disc (IVD) degeneration is increasing in aging societies, its etiology and pathogenesis remain elusive and there is currently no effective treatment to prevent this undesirable condition. The unfolded protein response (UPR) is a cellular machinery that plays critical roles in handling endoplasmic reticulum (ER) stress, a condition caused by the accumulation of unfolded proteins in the ER lumen. This study aimed to elucidate the potential role of the UPR mediated by pancreatic endoplasmic reticulum kinase (PERK), one of the major ER stress sensors in mammalian cells, in the development of IVD degeneration.

Methods: IVD degeneration was artificially induced in Wister rats by percutaneously puncturing the coccyx IVDs and human IVDs were collected from patients who underwent spinal surgery.

Results: Expression of the UPR target genes was elevated in degenerative IVDs in both humans and rats. The induction of ER stress in annulus fibrosus cells significantly increased the transcripts for tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) in a nuclear factor (NF)-κB pathway-dependent manner. The expression of TNF-α and IL-6 was significantly reduced by treatment with a selective PERK inhibitor, GSK2606414 and by gene silencing against PERK and activating transcription factor 4 (ATF4) transcripts.

Conclusions: Our findings indicate that the UPR mediated by the PERK pathway is causally related to the development of IVD degeneration, suggesting that PERK may be a potential molecular target for suppressing the degenerative changes in IVDs.
0.17
PRELIMINARY SAFETY, TOLERABILITY AND EFFICACY OF INTRADISCAL STA363 IN PATIENTS WITH CHRONIC DISCOGENIC LOW BACK PAIN

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Introduction: Intradiscal injection of lactic acid (LA) has been shown to induce sclerosis of pig intervertebral discs (IVD), an effect accompanied by increased flexural rigidity. Since such an effect theoretically might alleviate symptoms in patients with discogenic pain, the safety and preliminary efficacy of intradiscal LA injection was evaluated in a Phase Ib study.

Methods: The study design was double blind, randomized, single ascending dose. Fifteen patients with discogenic low back pain were included. Up to 2 lumbar discs were injected with LA in Omnipaque (STA363) or placebo (Omnipaque). Injection volume was 1.5 mL and LA concentrations were 30, 60 or 120 mg/mL. Six patients were treated with placebo and 9 patients with LA. The follow-up period was 12 weeks. Primary objectives were safety and tolerability, secondary objective was MRI changes and explorative objectives were VAS and ODI.

Results: All treated IVDs were Pfirrmann grade 3. No serious adverse events occurred and there were no treatment-related AEs that persisted beyond the end of the study. Preliminary MRI data suggested loss of hydration of the nucleus pulposus (presumably indicative of sclerosis) in some STA363-treated patients. Average VAS was reduced by 50% in the highest dose group and by 20% in the placebo group. VAS in the two other groups was unchanged. ODI changes followed the same pattern.

Conclusion: The data preliminarily suggest that STA363 is safe and tolerable. Phase II studies with longer follow-up period are required to confirm safety and assess efficacy.
Intervertebral disc (IVD) degeneration is associated with an inflammatory response. Recent studies of mesenchymal stem/stromal cells (MSCs)-based therapies for low back pain indicate a key role for MSCs’ immunomodulatory potential, being their paracrine competence frequently appointed as main therapeutic factor. Thus, this work aims to evaluate whether MSCs immunomodulatory effects on degenerated IVDs can be replicated using their secretome (secMSC).

Methods: Human bone marrow-derived MSCs were pre-conditioned for 48h with IL-1β (10 ng/mL) and in hypoxia (6% O₂). Using a pre-established ex vivo model, pro-inflammatory/degenerative bovine IVD tissues (stimulated by needle-puncture, IL-1β supplementation and hypoxia) were cultured in secMSC for 48h to 14 days. Non-stimulated IVDs and IVDs co-cultured with MSCs were performed as controls.

Results: MSCs preconditioning shifted their secretome towards a pro-inflammatory profile with increased production of pro-inflammatory cytokines. After 48h, the levels of pro-inflammatory markers of degenerated IVDs treated with this secMSCs were down-regulated (IL-6, IL-8), comparatively to non-treated degenerated discs, mimicking the results obtained with the MSCs-treated IVDs. The IVDs expression of matrix degrading enzymes MMP1 and MMP3 was significantly altered only when treated with secMSCs (downregulating MMP1 and upregulating MMP3). At day 14 of culture, only secMSC was able to increase aggrecan deposition relatively to the degenerated IVDs that showed consistent loss of aggrecan.

Conclusion: The results demonstrate that MSCs immunomodulatory effect on degenerated IVD is paracrine and can be recapitulated with the cells’ secretome. The link between this effect and the remodeling of the IVD matrix should be further explored.
LIPID NANOCAPSULES FOR THE SUSTAINED RELEASE OF THERAPEUTIC MIRNA: NEW PERSPECTIVE IN REGENERATIVE MEDICINE OF INTERVERTEBRAL DISC

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Introduction: Dysregulation of miRNAs eg miR155 [1,2] has been associated with disc degenerative disease (DDD)[3]. The relevance of unprotected miRNAs for therapeutic applications suffers from their fast in vivo degradation. Thus, the development of nanocarriers is a prerequisite for miRNA therapeutic use. Lipid nanocapsules (LNCs) offer a suitable strategy thanks to their ability to encapsulate nucleic acid [4]. The purpose of this work was to formulate and fully characterize innovative miR155-LNC for a potential use in DDD treatment.

Methods: miR-155-LNCs were formulated by phase inversion process. After purification, miR155-LNCs were fully characterized (size, polydispersity index (PDI), zeta potential). Encapsulation efficiency (EE) and drug loading (DL) were assessed by Quant-IT-dye® quantification. miRNA-155 release and enzymatic protection were investigated by dialysis and gel electrophoresis respectively. miR-155-LNCs internalization and impact on cell viability in human adipose stromal cells (hASC) were assessed by confocal/FACS analysis and MTT assay, respectively.

Results: miR155-LNCs exhibit a diameter of 75.0±1.3 nm, a PDI of 0.06±0.03 and a positive zeta potential. EE and DL were estimated to 75.2±1.2% and 590±9.3µg/g of LNC respectively. miR155 sustained release from LNCs was observed (23.3±7.1% compared to 94.8±5.4% for unencapsulated miR-155, after 4h). miRNA-endonuclease protection by LNC was confirmed by electrophoresis. After 24h of incubation with fluorescent miR155-LNCs, hASC viability was about 70%. Internalization of miR-155-LNCs in hASC cells was also demonstrated.

Conclusion: LNCs could be a promising approach to protect, release and transfect therapeutic miRNA. Further experiments (in vitro and in vivo) will be needed to confirm the interest of this innovative nanoplatform to vectorize new therapeutic in order to counteract DDD.

FETAL EXTRACELLULAR MATRIX OF NUCLEUS PULPOSUS AS A NATURAL SCAFFOLD FOR INTERVERTEBRAL DISC REJUVENATION

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Introduction: The main challenge for intervertebral disc (IVD) regeneration is to develop a tissue engineering-based therapy, aiming to restore tissue function. Engineering a natural nucleus pulposus (NP) microenvironment, represents a novel strategy to target IVD degeneration. Since fetal NP extracellular matrix (ECM) has shown an increased expression of pro-regenerative proteins [1], we hypothesize that fetal NP ECM can support IVD rejuvenation.

Methods: NPs, obtained from bovine tails of different ages, were decellularized using an SDS-based protocol and processed for histology, glycosaminoglycans (GAGs), DNA quantification and western blotting for fetal ECM proteins (Collagen XII and XIV). Adult bovine NP cells were cultured on the decellularized NPs from different ages and their response was evaluated by Live/Dead, histology and gene expression after 7 days of culture.

Results: An SDS-based protocol was effective in removing cells from NPs, while preserving native ECM architecture and composition. The fetal pro-regenerative proteins were retained after decellularization. Cell behavior in the different matrices is currently being addressed.

Conclusion: Acellular scaffolds from different aged animals were developed using an optimal decellularization protocol, efficient at maintaining both biochemical and structural cues. In the future cell performance of adult NP cells will be evaluated, envisaging the ability of the fetal microenvironment to support a notochordal cell phenotype.

O.21
CONTROLLED RELEASE OF BIOLOGICAL FACTORS FOR PROGENITOR CELL-MEDIATED ENDogenous REPAIR OF INTERVERTEBRAL DISCS
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Introduction: The recent description of progenitor cells in degenerated intervertebral discs raised the possibility of harnessing their regenerative capacity for endogenous repair. We develop an intradiscal polysaccharide microbead-based delivery system for the sequential release of chemokines and nucleopulpogenic factors. This delivery system would contribute to 1) the recruitment of resident progenitors (CXCL12 or CCL5), followed by 2) the differentiation of the mobilized progenitors (TGF-β1 and GDF5), and 3) the subsequent regeneration of Nucleus pulposus (NP).

Methods: Pullulan microbeads (PMBs) (100µm) were prepared by a simultaneous crosslinking protocol coupled to a water-in-oil emulsification process. Freeze-dried PMBs were loaded with biological factors then release assays were performed at 37°C for 21 days. To determine the bioactivity of released chemokines and growth actors, on in vitro cell recruitment and synthesis of NP-like extracellular matrix (ECM), respectively, human mesenchymal stem cells (hMSCs) were cultured in Transwells during 14 days and histological analyses were performed on loaded PMBs and hMSCs aggregates.

Results: All factors were successfully adsorbed on PMBs and a burst release within the 1st day was observed. At day 14, 17% and 68% of CXCL12 and CCL5 were released, respectively and at day 21, 20% and 100% of TGF-β1 and GDF5 were released, respectively. MSCs exhibited an increased tendency to migrate and synthesize an NP-like ECM when cultured in presence of PMBs loaded with chemokines or growth factors.

Conclusion: PMBs are suitable micro-carriers for the controlled loading and release of biological factors that could boost intervertebral disc endogenous repair.
TARGETED PROTEOMIC ANALYSIS TO EXPLORE THE ANTI-INFLAMMATORY EFFECTS OF PORCINE NOTOCHORDAL CELL-DERIVED MATRIX

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Introduction: Porcine notochordal cell-derived matrix (NCM; containing biologic factors secreted by NCs) induces in vitro and in vivo regenerative effects and ameliorates local inflammation. The aim of this study was to better understand the, yet elusive, NCMs mode-of-action in the pro-inflammatory degenerative IVD environment.

Methods: Canine nucleus pulposus (NP) cells were cultured 72 hours in monolayers: Control (basal chondrogenic medium), +TNFα (10ng/mL), or +NCM (10mg/mL), or +NCM+TNFα. Prostaglandin E₂ (PGE₂) medium levels were measured (Cayman). Targeted proteomics was performed by DigiWest®, a proprietary immunoassay technology employing 90 antibodies for NP (including T, CTK18, CTK19) and inflammation signalling (including NFκB, Jak/Stat, MAPK) markers. Log fold-changes (logFC) were tested using pairwise exact Tests (edgeR-v3.20.9).

Results: PGE₂ levels increased by four-fold in +TNFα vs control, indicating that inflammation was successfully induced. After filtering, followed by group-wise normalisation of the DigiWest data, 66 proteins were further analysed. NCM induced reduction of T by 2.5-logFC vs control. NCM (±TNFα) resulted in robust induction of well-known NP-markers (CTK8: 7.8-logFC; CTK19: 10.0-logFC). NCM treatment (±TNFα) vs control induced upregulation of Dual-specificity MAPK phosphatases (DUSP5: 9.5-logFC; DUSP7: 2.8-logFC). Furthermore, Protein Kinase C Alpha (PRKCA) was significantly downregulated (-1.6-logFC).

Conclusion: NCM treatment induces DUSPs known to inactivate ERK1/2 through dephosphorylation and thereby inhibit inflammatory gene expression. These possible anti-inflammatory effects are further supported by the reduction of PRKCA which is involved in MAPK signaling. Altogether, targeted proteomics in preliminary in vitro studies provide indications for an anti-inflammatory role of NCM, next to its well demonstrated anabolic effects.
0.23

REGENERATIVE MEDICINE OF INTERVERTEBRAL DISC: COMPARISON OF THE TRANSPECULAR AND TRANSANNULAR APPROACHES OF OVINE LUMBAR INTERVERTEBRAL DISC IN A LONGITUDINAL FOLLOW UP

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Introduction: In the context of cell-based therapy to treat intervertebral disc (IVD) degeneration, modalities to inject cells into the Nucleus pulposus (NP) have to be clarified. The two current approaches to reach the NP are the transannular (TAA) and the transpedicular approach (TPA). TPA has recently been tested in ovine. However, its long-term consequences on IVD have not yet been addressed. This work aims at comparing potential lesions induced by the two approaches in a long-term longitudinal study.

Methods: TPA or TAA were performed in 12 healthy lumbar IVD in sheep (1yo). Approaches were performed with or without injection of iodine contrast agent (ICA). The injection feasibility and IVD integrity were assessed by MRI (T2wsi), micro-CT scans and histological Boos’ scoring. IVD were examined at 1, 3 and 7 months.

Results: The two approaches allowed easy access to the NP. TPA induced no pedicular fracture sequel. Endplates were crossed without secondary endplate fracture. Unexpectedly, the two approaches induced an accelerated degeneration of the IVD (decrease in T2wsi and increase in Boos’ scoring). Moreover, end-plate defects induced by TPA were not healed after 7-months. Herniation of NP tissue and presence of osseous fragments in the NP were also observed. Degeneration seemed to be amplified by the injection of ICA.

Conclusion: Despite its intrinsic AF preserving ability, the TPA induced deleterious effects on lumbar IVD that seemed to be exacerbated by ICA. The delayed healing of IVD after TPA could drastically jeopardizes the efficacy of cell-based therapy.
O.24
MRI – SPECTROSCOPY, BIOCHEMICAL EVALUATION OF DISC BIOLOGY
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Introduction: The main problem in clinical management of chronic low back pain (CLBP) patients is to accurately diagnose the painful spinal level. In a conventional MRI, this diagnosis is not possible in the absence of Modic signs. A modern development to overcome this issue is MRI spectroscopy (MRS), whereby it is possible for the first time to measure biochemical changes, such as lactate content, proteoglycan content, collagen content, etc., noninvasively and precisely on MRI.

Methods: For the first time in Europe, MRI spectroscopy (Nociscan MRS) was performed in 30 patients with suspected discogenic back pain. In total, 105 discs were examined with MRS in 17 male patients and 13 female patients. In 7 patients, the examination was performed after surgery (PRP therapy, ozone therapy, nucleoplasty and micro discectomy). After designing and performing a MRS-based Nociscan algorithm, 8 patients were operated respectively on the affected disc (5 nucleoplasties, 3 PRP) and 14 patients were treated conservatively.

Results: Pain-relevant changes in the intervertebral discs were found in 26 patients. In 4 cases a discogenic pain could be excluded. The eight invasively treated patients showed significant improvement in the symptoms after 6 weeks and three months in 7 out of 8 cases (Vas pre 7.6 - ODI pre 56.7, VAS 6 w 2.6, ODI 34.7, VAS 3 mo 1.9, ODI 16). A targeted conservative therapy taking into account the biochemical conditions in the intervertebral disc showed a significant improvement in 13 cases (VAS pre 5.6, ODI 36.5; VAS 6 w 1.8, ODI 12; VAS 3 mo 1.2, ODI 8.3).

Conclusion: Non - invasive biochemical analysis of the condition of lumbar intervertebral discs using MRI spectroscopy is a landmark investigation for the future. It is not only able to detect potential pain generators, but also to provide new information about the degenerative state of the intervertebral disc. For future innovations, such as cell therapy, as well as for the assessment of adjacent segment risk after fusion etc., this investigation will find its place in the therapy and research of discogenic spinal disorders.
O.25
MSC HOMING FACILITATES IVD CELL SURVIVAL, PROLIFERATION AND ENHANCES THE TIE2 POSITIVE IVD PROGENITOR CELL SUBPOPULATION

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Introduction: Homing of mesenchymal stem cells (MSCs) has been described as potential alternative to MSC injection, aiming to enhance the regenerative capacity of the intervertebral disc (IVD). The present study investigated the effect of MSC homing on the Tie2 (Angiopoietin-1 receptor) positive IVD progenitor cell population, the IVD cell survival and proliferative response.

Methods: Bovine caudal IVDs were cultured under free swelling conditions. PKH labeled human MSCs (1x10⁶) were placed on the endplates; IVDs without MSCs were used as controls. Human non-degenerated, traumatic and degenerative IVD tissue was obtained according the local ethical regulations. IVD tissue was separated in two equal portions and MSCs (1x10⁵) were added onto one tissue portion using the second portion as untreated control. After 5 days, IVD cells were isolated by tissue digestion and percentages of Tie2+, dead (DAPI+) and proliferating (Ki-67+) IVD cells were evaluated by flow cytometry. MSCs were excluded by a gate.

Results: MSC homing significantly
- increased the proportion of Tie2 positive progenitor IVD cells in bovine IVDs (2.4±1.3-fold; p=0.04) and 7/10 human IVDs;
- decreased the fraction of dead IVD cells in bovine IVDs (0.8±0.4-fold; p=0.02) and 7/10 human IVDs;
- induced a proliferative response in bovine IVDs (2.5±1.9-fold; p=0.013) and 5/6 human IVDs.

Conclusion: Our findings suggest a prominent role for paracrine stimulation, indicating that homed MSCs may represent “biological factories”, secreting growth- and survival factors to help resident cells to reverse or slow down a potential ongoing degenerative process.
O.26
VIABLE ALLOGRAFT AS A SUPPLEMENTAL THERAPEUTIC FOR DISC REGENERATION
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Introduction: Degenerative changes of the lumbar spine can contribute to chronic back pain. Mechanisms of intradiscal degeneration have been linked to genetic, metabolic, and mechanical imbalance. A clinical study protocol was developed to determine whether supplemental cellularized allograft disc matrix could be used to repair degenerative disc tissue. After IRB approval, a study of 224 patients was initiated (ClinicalTrials.gov NCT03709901). This study assesses the first 24 subjects for safety as a preliminary to the larger study.

Methods: Subjects were randomized to allograft, saline placebo, or to continue conservative treatment. The primary outcome of the study evaluated Oswestry Disability Index (ODI) 12 months after transplant, and the Visual Analogue Scale of Pain Intensity (VASPI) at 6, and 12 months. Structural outcomes were assessed by x-ray and Magnetic Resonance Image (MRI) at 12 months. Safety was assessed by the incidence and severity of adverse events (AEs) and clinically relevant changes in laboratory tests. Subjects consisted of 5 females and 19 males; demographics of age 35.7 (27-62), and BMI 27(17.7-35.4) were similar between the groups.

Results: There were no reports of infection or inflammation, none of the subjects withdrew from the study, and no subject required a surgical intervention during the 12-month study.

Conclusion: The target of this safety study was to assure that prescriptive intervention that is clinically efficient and effective could be delivered without risk. Although the data is preliminary, it shows that cellular allograft administration is safe and that attaining clinical improvement for painful degenerative disc disease is possible.
IMPLANTATION OF BONE-MARROW DERIVED MESENCHYMAL STEM CELLS FOR INTERVERTEBRAL DISC REGENERATIVE THERAPIES MAY REDUCE THE RISK OF NEUROVASCULAR GROWTH AND REDUCE DISCOGENIC PAIN

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Introduction: Clinical trials are underway to test the efficacy of cell based therapies for the regeneration of IVD tissue. Currently there is a lack of knowledge surrounding the relationship between naïve stem cells and the inflammatory niche of the degenerate disc. This study tests the hypothesis that the expression of neurovascular factors from ASCs and MSCs is regulated by cytokines and low oxygen, thus exacerbating pain in those patients that have the presence of sensory nerve fibres within the IVD.

Methods: Patient-matched ASCs and MSCs (n=3) were stimulated with IL-1β or TNFα in either 21% or 5% O₂. qRT-PCR was performed to assess expression of trophic factors involved in the survival of nerves (NGF, BDNF & NT3), blood vessels (VEGF, FGF-2, Ang-1 & Ang-2) and pain related peptides (Substance P & CGRP). Secreted proteins were analysed using Luminex® assay.

Results: We report for the first time that patient-matched ASCs and MSCs express constitutive levels of key neurotrophic factors, and that stimulation of ASCs with hypoxia triggers increased secretion of NGF & NT-3 and angiogenic factors Ang-1, Ang-2 FGF-2 and VEGF-A compared to MSCs. We report increased transcriptional regulation of neuropeptides in hypoxia stimulated ASCs compared to normoxia. We demonstrate upregulation of NGF, Ang-1 and FGF-2 in response to cytokines in ASCs in 21% and 5% oxygen.

Conclusion: This work highlights differences between the neurovascular secretome of patient-matched ASCs and MSCs, overall suggesting the preferential use of MSCs for therapeutic use in reducing the risk of ectopic nerve stimulation following delivery to the disc.
O.28
INTRADISCAL INJECTION OF AUTOLOGOUS ADIPOUS TISSUE DERIVED MESENCHIMAL CELLS: A COMPARISON WITH SIMULTANEOUS NUCLEOPLASTY OR ALONE
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Introduction: BACK PAIN is associated with a degeneration of the intervertebral disk. New strategies for regeneration of intervertebral disk, such as mesenchymal stem cells, have been considered. Adipose tissue is an ideal source of these naturally regenerative cells, due to its abundance and easy harvest.

Methods: 6-months outcome of a single intradiscal injection of autologous, micro-fragmented and minimally manipulated adipose tissue after nucleoplasty in patients with chronic back pain. Nucleoplasty performed with minimally invasive approach (Disc-FX®) and micro-fragmented adipose tissue was obtained using a minimal manipulation technique (Lipogems®). Clinical outcomes were determined comparing Oswestry Disability Index and Magnetic Resonance Imaging baseline and 6 months follow-up. Safety of the procedure was assessed. Results compared with a control group of patients treated with a single injection of Lipogems®.

Results: A gradual reduction in disability was observed during the 6 months period, where the median Oswestry Disability Index was 28% (IQR 16-39%), statistically significantly lower (p < 0.001) than at baseline (38% [IQR 28-50%]). No adverse events were recorded. Magnetic Resonance Imaging did not indicate any statistical change.

Conclusion: Micro-fragmented adipose tissue injection after nucleoplasty of the intervertebral disc is a safe and innovative method for treatment of chronic low back pain, in terms of pain relief and disability reduction.
O.29
WHY WE USE PEKK? MATERIAL’S CHARACTERISTICS AND CLINICAL RESULTS
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Introduction: PEKK is a semi-crystalline thermoplastics having high mechanical strength. From previous work, PEKK appears to provide good surface adhesion to support cell activity.

Methods: We present the mechanical properties of OXPEKK® compared to bone: elasticity, wettability by tissue fluids, and design of devices allow us to obtain a mechanical behavior equivalent to its bone environment. We compare the surface conditions between the machined OXPEKK® and the sandblasted OXPEKK®. SEM observations and an AFM-microscopy study allowed us to compare the surface conditions of OXPEKK® implants before and after gamma-ray sterilization. The OXPEKK® implants are often used with MBCP™ bone substitute. MBCP™ is a biphasic synthetic bone graft substitute, osteoconductive thanks to its micro and macro porous structure. Soluble and resorbable, it promotes bone regrowth.

Results: We present the results of an OXPEKK®-vs-PEEK rabbit implantation study and compare the osseointegration kinetics on both materials. We show the results of a clinical study (30 patients, 1-3 years follow-up): it demonstrates safety and effectiveness of the OXPEKK® medical device used with the MBCP™ substitute for the treatment of lumbar degenerative diseases regarding the lordosis recovering as well as the fusion rate. Finally, some long-term results of implantation of OXPEKK® cages on scoliosis cases are shown.

Conclusion: OXPEKK® has mechanical characteristics comparable to human bone, good mechanical dynamic behavior, and its physical-chemical surface properties guarantee rapid osseointegration of implants. The radioluency of OXPEKK® makes possible to visualize new bone regrowth, thanks to MBCP™ bone substitute which has a resorption rate similar to human bone.
O.30
STIFF ROD IS A SUBSTANTIAL RISK FACTOR OF PROXIMAL JUNCTIONAL KYPHOSIS AFTER ADULT SPINAL DEFORMITY SURGERY: COMPARATIVE STUDY BETWEEN COBALT CHROME- AND TITANIUM ALLOY-ROD CONSTRUCTS

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Introduction: The use of titanium alloy (Ti) rods is frequently associated with rod fracture after spinal fixation. To address this issue, cobalt chrome (CoCr) rods, which are advantageous because of their greater strength and resistance to fatigue relative to Ti rods, have been introduced. Little is known about the effect of rod stiffness as a risk factor of proximal junctional kyphosis (PJK) after adult spinal deformity (ASD) surgery. The purpose of the present study was to compare radiographic outcomes focusing proximal junctional kyphosis (PJK) after the use of Ti versus CoCr rods in a matched cohort of patients undergoing posterior spinal fusion for treatment of spinal instability.

Methods: We retrospectively reviewed data from patients who had undergone spinal fusion to the sacrum involving more than 3 levels for ASD at a single institution between 2004 and 2015. Patients were matched for age, diagnosis, 3-column osteotomy, levels fused, and T score. Fifty patients with Ti rods were identified and appropriately matched to 50 consecutive patients with CoCr rods. Radiographic outcomes were measured on the standing lateral radiographs before surgery, 1 month postoperatively, and at ultimate follow-up. The outcome measures were composed of pre- and postoperative sagittal vertical axis (SVA), pre- and postoperative lumbar lordosis (LL), pre- and postoperative thoracic kyphosis (TK)+LL+pelvic incidence (PI), pre- and postoperative PI minus LL, level of uppermost instrumented vertebra (UIV), evaluation of fusion after surgery, the presence of PJK, and the occurrence of rod fracture.

Results: The patients of the groups were similar in terms of age, gender, diagnosis, number of 3- column osteotomy, levels fused, bone mineral density, preoperative TK, pre- and postoperative TK+LL+PI, SVA difference, LL change, pre- and postoperative PI minus LL, and location of UIV (upper or lower thoracic level). However, there were significant differences in length of follow-up (CoCr, 25.0 vs. Ti, 28.5 months; P<0.001), fusion rate (CoCr, 45 [90%] vs. Ti, 33 [66%]; P=0.004), occurrence of rod breakage (CoCr, 0 vs. T, 8 [16%]; P=0.006), and PJK (CoCr, 24 [46%] vs. Ti, 9 [18%]; P= 0.003). The time of PJK was less than 12 months after surgery in the CoCr group. However, 55.5% of PJK developed over 12 months after surgery in the Ti group.

Conclusion: Our findings indicate that the use of CoCr rods is effective in ensuring stability of the posterior spinal construct and accomplishment of spinal fusion. Furthermore, our results indicate that PJK may occur more frequently in CoCr systems than in Ti systems. Increasing the rod stiffness by the use of cobalt chrome rod and can prevent rod breakage but adversely affects the occurrence and the time of PJK.
Clinical effect of the nano-hydroxyapatite/polyamide66 cage in reconstruction of cervical stability: a midterm study

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Introduction: To evaluate the mid-term clinical effect of nano-hydroxyapatite/polyamide66 (n-HA/PA66) cage in the anterior cervical spinal reconstruction, comparing with polyetheretherketone cage (PEEK cage).

Methods: 174 patients who undergone the anterior decompression, fusion internal fixation between January 2010 and January 2012 included were retrospectively studied. They were divided into two groups: 124 patients in group A were implanted n-HA/PA66 cage, and 50 with PEEK cage in group B. Sex, age, surgical blood loss, operation time, complications rate, postoperative images and clinical effect were compared between two groups. The X-ray and CT were followed in all these patients to observe the fused segmental height, fused segmental alignment, cervical spine alignment and the rate of n-HA/PA66 cage and peek cage subsidence and displacement and to evaluate bone fusion by Brantigan scores. The clinical outcomes were evaluated by visual analogue scale (VAS) scores, Japan Orthopaedic Association (JOA) scores and Neck disability index (NDI).

Results: There were no significant differences (P > 0.05) in sex, age, preoperative VAS, preoperative JOA, preoperative NDI, operation time, hospitalization time, surgical blood loss between two groups. In group B, 2 patients showed temporary sore throat disappeared 72 hours without dysphagia. No cerebrospinal fluid leakage, hematoma and wound infection were found in all patients. All the patients had been followed-up for an average of (group A: 52.10 ± 24.30 months - group B: 49.50 ± 26.50 months), without significant difference (P > 0.05). Two patients were observed the n-cage subsidence in group A and 3 in group B, the subsidence rate had significant difference (P < 0.05). Other patients didn’t show the displacement, breakage of cage, screw, and plate. The rate of fusion was satisfied (all were more than 3 points according to Brantigan score), the fusion time were 4.2 ± 1.8 months in group A, and 4.1 ± 2.0 months in group B, which wasn’t significant between two groups (P > 0.05).

Conclusion: The n-HA PA66 cage has good similar rate of osseous fusion comparing to PEEK cage and can provide effective restoring and maintaining for fused segmental height, fused segmental alignment and lower rate of the complications n-HA / PA66 cage is an ideal reconstructed bioactive material for anterior cervical column.
BIOACTIVE 3D COMPOSITE SCAFFOLDS FOR BONE TISSUE REGENERATION

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Introduction: Bone diseases still remain a significant clinical challenge worldwide, accounting for half of all chronic conditions in people over the age of 50. The tissue engineering approach offers an effective strategy to regenerate bone and overcome the limitations associated with conventional treatments. Polymeric composites scaffolds made of hydrogel and electrospun fibers, resembling the overall structure of the extracellular matrix, have a great potential as scaffolds for bone tissue engineering.

Methods: Composite scaffolds were developed, made of electrospun fibers of PLGA (75:25 mol%) blended with PEG, sandwiched between two layers of crosslinked gelatin loaded with Tantalum nanoparticles. Fiber composition, gelatin layer thickness and crosslinking were optimized in order to achieve the desired swelling degree, degradation kinetics and mechanical properties. To evaluate the material biocompatibility, cytotoxicity tests were performed by direct contacting the materials with MG63 osteoblast-like cell line. Gene expression of the most common markers of hMSC osteoblastic differentiation was evaluated to assess bioactivity.

Results: The presence of PEG blended with PLGA in the fibers enhanced dimensional stability and mechanical properties of the composite. Absence of cytotoxicity was demonstrated. Viability and bioactivity of hMSC cultured on the scaffold were evaluated up to 14 days. Results showed that hMSC adhered and grew onto the scaffold with regular morphology and full colonization of biomaterial and demonstrated good cell viability and osteoblastic differentiation. The addition of Tantalum enhanced cell performances.

Conclusion: The overall characteristics of the scaffold developed in this work make it an excellent candidate for the regeneration of bone tissue.
THE E. COLI DERIVED RHBMP-2 WITH HYDROXYAPATITE IN SPINAL FUSION

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Introduction: Since the osteoinductive properties of demineralized bone matrix with bone morphogenetic proteins (BMPs) was discovered by Urist in 1965, huge advancement on research and development of BMPs has been achieved. Especially, rhBMP-2 development using recombinant technologies enabled its commercialization in clinical fields. CGBio Inc. and Daewoong Pharmaceutical Ltd. have succeeded in manufacturing of E. coli derived rh-BMP-2 with hydroxyapatite carriers (Novosis™) in 2005 and conducted the pivotal study for Posterolateral Lumbar Fusion (PLF).

Methods: The pivotal study was designed as an open, active-controlled, randomized, multicenter trial and included 93 patients who underwent single-level lumbar or lumbosacral PLF. Patients who underwent 1-level PLF from L1 to S1 for severe spinal stenosis or grade I spondylolisthesis were randomized and received Novosis™ (Experimental Group) or autogenous iliac bone graft (Control Group). Thin-section CT (< 2 mm), VAS, ODI, and SF-36 were obtained pre- and postoperatively at 12 and 24 weeks. Safety of Novosis™ was evaluated by occurrence and severity of all adverse events.

Results: CT-based fusion rate were 100.0% (41/41) for the experimental group and 90.2% (46/51) for the control group (p = .062) at 12 weeks. At 24 weeks, 100.0% (41/41) for the experimental group and 90.2% (46/51) for the control group (p = .251). Fusion grade showed non-inferiority of the Experimental group compared with the Control group. There was no difference in changes in VAS, ODI, or SF-36 between the groups. No serious adverse event related to E. Coli derived rh-BMP-2 was found.

Conclusion: E.coli derived rh-BMP-2 showed non-inferior efficacy comparing to AIBG with no serious adverse event for PLF. With this clinical result, E. Coli derived rh-BMP-2 with hydroxyapatite for dental clinical efficacy in sinus floor elevation was also proved and recently newly developed E.coli derived rh-BMP-2 with b-TCP carrier proved its superior efficacy in socket preservation. CGBio Inc. continues to develop new types of E.coli derived rh-BMP-2 products.
ELECTROSPUN COLLAGEN COUPLED WITH BONE MARROW-DERIVED EXOSOMES

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Introduction: There are many instances demonstrating that mesenchymal stem cells (MSCs) can be modified towards an osteogenic path by uptake of exosomes from other cells. It is less clear whether nascent vesicle placement on a scaffold in the absence of cells will facilitate site-specific delivery of biopotential. A consideration of lateral transfer that might incorporate osteogenic potential while retaining thermostable properties might afford a more efficient and valuable conveyance of osteogenic potential than cell proliferation. An electrospun fleece was combined with bone marrow-derived exosomes in the absence of cells to evaluate osteoinductive potential.

Methods: Exosomes were isolated from human spines by ultrafiltration and subsequent differential ultracentrifugation and attached to collagen fleeces before lyophilization and drying. Collagen fleeces alone or including hydroxyapatite (HA) were used as a scaffold for the delivery of the exosomes. The expression of alkaline phosphatase was measured following treatment of C2C12 cells with spine-derived exosomes alone or in combination with collagen fleece.

Results: Spine-derived exosomes positively expressed flow cytometry markers tested. ALP activity on the scaffold with HA demonstrated an approximate 10-fold increase to that of the collagen scaffold alone. While a dose-dependent effect, with higher doses of exosomes resulting in a greater amount of alkaline phosphatase expression, expression did not match the 50ng BMP-4 control.

Conclusion: An admixture of bone marrow-derived exosomes combined with an electrospun fibrillar collagen demonstrated osteoinductive potential that offers an asset to achieve near immediate access to host tissue, retain a scaffold armature for modeling, and provide site-specific placement of biologic potential.
O.35
APPLICATION OF BIO-CERAMIC(BGS-7) ARTIFICIAL ZYGOMA BASED ON ADDITIVE MANUFACTURING

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Introduction: The defects of the bone tissue are manifested by inherited or acquired factors, and acquired bone tissue damage can be caused not only by degenerative diseases caused by aging but also by accidents such as traffic accidents and leports. Development of artificial tissue and replacement for bone tissue is very important because the defect of bone tissue may cause secondary damage to other organs. Especially, cheekbones defect has a lot of aesthetic effects, which can lead to deterioration of quality of life. Therefore, research and development of artificial tissue for reconstruction of cheekbones should be continuously performed.

Methods: It is the apatite-wollastonite crystallized glass developed by Kokubo in 1982 that took advantage of bioactive glass and hydroxyapatite ceramics. Biologically active crystallized glass (BGS-7) has a property of directly binding to bone and has better bioactivity than calcium phosphate-based ceramics. However, above all, it is superior in strength to hydroxyapatite ceramics, which is the most widely used bioactive ceramics, and has similar physical properties to the above-mentioned apatite-wollastonite crystallized glass. Especially, commercialization of a part to withstand loads such as spacers It is a very favorable material. We have tried ceramics for cheek bone defect patients through 3D printing.

Results: BGS-7 is effective in promoting mechanical stability at the transplant site and has a wide range of human body due to its direct attachment to the surrounding bone. Since BGS-7 binds to the bone by surface reaction after transplantation, it is preferable that the BGS-7 be grafted to the bone defect site as customized. Since the conventional ceramic manufacturing technology can not satisfy both the anatomical custom design and the mechanical characteristics, it has been suggested as an alternative to the manufacturing of the customized medical device using the ceramic. Since it is a medical device manufactured by modeling based on a medical image of a patient, it is possible to improve the satisfaction to both the medical person and the patient, such as shortening the operation time and aesthetic improvement compared to the ready-made article. Clinical application of customized medical devices to patients with defects in cheekbone through 3D printing, satisfying anatomical and clinical requirements.

Conclusion: At present, 3D printing customized medical devices using mostly metals are being applied to clinical applications. Recently, 3D printing applications of bioactive materials have been studied in order to overcome the disadvantages of inactive materials, and customized medical devices through printing of bioactive crystallization glass, BGS-7, have shown clinically very useful results. With 3D printing technology, it is possible to make bone model, fracture guide and aggregate implant in a short period of time, and it is possible to minimize the operation time and complications by pre-operation simulation. In addition, it will be possible to create new added value in accordance with application fusion technology development and mass production technology.
O.37
INSTRUMENT VERTEBRAL ARTHRODESIS IN SCOLIOSIS CORRECTION WITH LOCALLY HARVESTED BONE AUTOGRRAFT WITHOUT USE OF BONE PRECURSORS
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Introduction: Bone scaffolds advantage is enhancing arthrodesis biologic support without further autologous bone graft harvested from other skeleton sites, as from posterior iliac crests; however in our experience bone scaffold integration is often incomplete. High initial stability granted by modern pedicle screws instrumentation and bracing after surgery, as in our experience, obtains a stable correction and use of autologous bone graft from local vertebral harvesting, not enhanced by graft from iliac crest or by bone scaffolds, can obtain complete arthrodesis.

Methods: From 2013 to 2017 we operated 70 patients by posterior instrumented vertebral arthrodesis in adolescent idiopathic scoliosis (AIS) correction, mean main curve 85° Cobb, mean age 12 years and 8 months, with all pedicle screws instrumentation; bone graft has been harvested only at vertebral level, without bone scaffold or autologous graft from other patient sites or allogenic bone graft.

Results: After surgery all patients have been braced for mean 10 weeks. At mean 2 years and 6 months follow up all patients have complete and stable arthrodesis without loss of correction (mean curve 25° Cobb), instrumentation failure or non union. Bone substitutes are a further cost in arthrodesis surgery and suboptimal scaffold integration leaves foreign bodies on vertebrae. Our experience shows that all pedicle screws instrumentation and bracing after surgery obtain complete arthrodhesis with autologous bone only, harvested at local site, without bone scaffolds or further bone graft.

Conclusions: Instrumented vertebral arthrodhesis can be obtained by local harvested autologous bone graft only, without cost of bone scaffolds or allogenic bone graft.
O.39
THE OUTCOME OF MULTILEVEL ANTERIOR CERVICAL DISCECTOMY AND FUSION
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Introduction: Anterior cervical discectomy and fusion (ACDF) with plating is a widely performed procedure for cervical disc diseases. Multi-level ACDF tend to have low fusion rate than a single level. We report the outcomes on 3 or 4 level ACDF and the strategies to increase the fusion rate in multi-level ACDF.

Methods: A total of 106 patients who underwent 3 or 4 level ACDFs from 2010 to 2018 was included in this retrospective study. All patients were operated by a single surgeon. Patients were divided into three groups according to the levels and method of operation. Assessment of fusion was done by flexion/extension X-ray images and post-operative cervical computed tomography(CT) scan.

Results: Seventy-nine patients (75%) underwent 3 or 4 level anterior cervical discectomy and fusion with plating. The overall fusion rate was 32% at 4 months, 58% at 1 year, and 71% at 2 years after surgery. The fusion rate of ACDF involving C7 and below (Group B) was significantly lower (67%) than the group of segments above C7 (Group A 100%). 18 patients (17%) whom underwent additional posterior fixation at the same time as ACDF involving the C7 and below (Group C), showed high fusion rates (100%) after 2 years (p < 0.05).

Conclusion: The 3 or 4 level ACDF with plate is clinically stable, safe and effective surgery. However, the fusion rate and radiologic outcomes of ACDF including C7/T1 at the lower end were relatively low. In these cases, circumferential procedures like additional posterior fixation, can obtain better fusion rate and radiologic outcomes.
O.40
COMBINED ANTERIOR/POSTERIOR APPROACH IN DEGENERATIVE ADULT SCOLIOSIS
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Introduction: A harmonic correction of the adult deformities can be obtained by the association of minimally invasive lateral lumbar interbody fusion (LLIF) surgery techniques with the posterior open stabilization. Through this combination invasive osteotomy’s complications can be avoided. In this study we evaluate the patients treated in our hospital using Extreme Lateral Interbody Fusion technique (XLIF) and posterior open fixation and fusion, analysing the correction of the spino-pelvic parameters, clinical outcome and the rate of complications associated with the use of this surgical approach.

Methods: Our retrospective study investigated results of 31 patients affected by degenerative adult scoliosis and undergoing to corrective procedure by using XLIF followed by open posterior fixation. Radiographical and clinical follow up was made at a minimum time of 1 month and maximum time of 6 years. Clinical evaluation included the Oswestry low back pain disability questionnaire (ODI) and Visual Analogue Scale (VAS).

Results: 87 lordotic cages were implanted, pre and post operative spinopelvic parameters were compared, intra and perioperative complications were evaluated. Mean fusion levels were 8. Sagittal Vertical Axis (SVA) improved from 48.1mm (12–77) to 29.2 mm (11–51). Patiens reported improvements of ODI and VAS too.

Conclusion: Minimally invasive lateral lumbar interbody fusion, combined with posterior open stabilization, allows to obtain a good correction of the deformity on the sagittal and coronal plane, reducing complications related to more invasive procedures. We think that this surgical approach is useful for the treatment of mild or severe deformities in adult patients.
0.41
CAPACITIVE COUPLING ELECTRIC FIELDS (CCEF) IN THE TREATMENT OF VERTEBRAL COMPRESSION FRACTURES (VCFs)
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Introduction: Capacitive coupling electric fields (CCEF) is a non-invasive kind of biophysical stimulation, FDA-approved. CCEF acts by increasing the synthesis of Prostaglandin-E2 and the TGF-beta expression, thus promoting mesenchymal staminal cells proliferation and their following osteogenic differentiation. The following prospective randomized trial aims to assess the efficacy of CCEF in the treatment of vertebral compression fractures (VCFs).

Materials and Methods: From January 2016 to September 2018, 224 patients with amielic type A1 VCFs were conservatively managed at our institution. Inclusion criteria were: maximum 10 days post-trauma interval; pain at the fracture level; presence of Vertebral Bone Marrow Edema (VBME) on MRI, in no more than two vertebrae. Exclusion criteria were: neurologic lesion; posterior vertebral wall or pedicle rupture on CT; previous vertebroplasty on the affected vertebra; bone tuberculosis or other spine infections; pregnancy; BMI > 30; scoliosis > 40° and any contraindications to MRI. The patients recruited, by applying the inclusion and exclusion criteria, were divided into two groups: Group-A patients were managed with conservative treatment only, while Group-B patients were instructed to use CCEF stimulation 8 h/day for 90 days as an adjunct to conservative treatment. All the patients used a C35 hyperextension brace for three months, with bed rest for the first twenty days, and took antiresorptive therapy (Risedronate 35 mg twice a month) and vitamin D supplementation. At 0 (T0), 30 (T1), 60 (T2) and 90 (T3) days, the patients underwent MRI evaluation and clinical evaluation, using Visual Analogue Scale (VAS) for Pain and Oswestry Disability Index (ODI). VBME was evaluated on MRI using the method by Piazzolla et al. The patients’ compliance in the use of CCEF was calculated as follows: Adh = (Recorded treatment hours/Expected treatment hours) × 100. Unpaired t test after ANOVA and paired t test were performed; p values of less than 0.05 were considered significant.

Results: 41 patients (32 female; 9 male; mean age: 69.4) were recruited. Twenty patients were randomized in Group-B and twenty-one patients in Group-A. A good compliance was observed in the use of the CCEF device. No adverse reactions were reported. One lost at follow-up was recorded in Group-A. In Group-B an early VBME reduction was observed, compared with Group-A. A positive correlation was recorded between VBME and clinical scores improvement. In Group-B, 14 patients out of 20 removed the brace at T2.

Discussion and Conclusions: Our data show CCEF stimulation is clinically and MRI effective in treating acute VCFs. Patients treated with capacitive stimulation have an early improvement of clinical symptoms, depending on a faster fracture healing.
AUGMENTED REALITY SUPPORTED CERVICAL TRANSPEDICULAR FIXATION ON 3D-PRINTED VERTEBRAE MODEL; AN EXPERIMENTAL EDUCATION STUDY

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Introduction: Transpedicular fixation via screw is used widely for vertebrae stabilization because its stabilization includes three columns of the vertebrae. But applying this implantation to upper cervical vertebrae is a difficult surgical procedure, even though the practical education of this procedure can be done through animal or cadaver studies, every educative clinic cannot provide equal requirements. Augmented Reality (AR) is a combination of the ‘real time’ digital vision and 3D virtual shapes that prepared earlier. This technology can be used by widely spread mobile devices. Using of this technology in spinal surgeries might reduce the error ratio. It also might reduce the exposure of medical staff and the patient to radiation caused by fluoroscopy and CT.

Method: In the study, 10 candidates, with anatomy education and no surgical experience, applied 36 pedicle screws (EF Spine®) with C2-C3 posterior transpedicular fixation technique to 9 vertebrae models produced via 3D-Printer.

Result: In total, 36 pedicle screws were applied to 9 -3d printed- vertebrae models. 28 of all pedicle screws (%77,8) were measured Gertzbein Grade 0+1/Kaneyema et al.’s class 1 accurate screws.

Conclusion: In the study results; using AR at applying pedicle screws to experimental vertebrae model reduced the malposition ratio significantly. The resemblance between our results and the results of previous studies that researched supportive systems indicates that our 3D-printed vertebrae model might be a helpful experimental method.
ROADMAP FOR DEVELOPMENT OF BRAND NEW SPINE SURGERY ROBOT IN SOUTH KOREA
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Introduction: This topic will present current, global status of development of spine surgery robot, technical demands for spine specialized robot.

Methods: Representative spine surgery robots in the worldwide market are Mazor X by Medtronic Inc. and Excelsius by Globus medical Inc., which can be competitive models to our newly developing robot system. Disadvantage of present system should be overcome in the new system; short robot arm coverage field, invasive procedure, no self-screw insertion system, complex workflow. Newly developed system should include these features; real-time visualization, best verification, simple workflow, wireless screw inserting system, AI integrated system.

Results: Spine surgery robot is under developing by the collaboration of Yonsei University Health System and Curexo Company in South Korea. Our initial works for developing new spine surgery robot system include spine anatomical registration, special consideration for bone works, and graphic user interface. The system composed of 5 axis robot arm, Accuracy of robot arm is 0.58mm and needle tip 0.88mm. Basically this system is navigation based robot by OTS camera, utilizing intra operative CT imaging system or C-arm fluoroscope image for registration. Robot and UI were already built and current status of Robot development is completion of in vitro feasibility test, under in-vivo test, planning cadaveric test. South Korean FDA and US FDA approval are expected by 2021.

Conclusion: Spine surgery robot in South Korea will be introduced in near future and it has the potential to improve patient safety, operator comfort, reducing radiation hazards and procedure efficiency in the field of spinal surgery.
O.44
DESIGN AND EVALUATION OF ELECTROSPUN STRUCTURED POLYCAPROLACTONE BIOMATERIALS FOR ANNULUS FIBROSUS REPAIR

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Introduction: Extensive Annulus fibrosus (AF) radial tears lead to intervertebral disc (IVD) herniation. While unrepaired defects in the AF are associated with high IVD degeneration prevalence, current surgical strategies disregard the structural integrity of the AF. This study aims at i) designing polycaprolactone (PCL) electrospun implants that mimic the AF multi-lamellar fibrous structure and ii) assessing their ability to properly repair an AF defect in a sheep model.

Methods: Oriented and non-oriented PCL implants were produced by electrospinning. In vitro apposition of ovine annular explants was characterized by cell morphology (nucleus and F-actin staining) and extracellular matrix (ECM) deposition (collagen, aggrecan). In vivo study was carried out on 6 sheep in which 5 lumbar discs were exposed using a left retroperitoneal approach. Defects (2x5mm, 2mm depth) were created in the outer AF, with randomized conditions including 10-layer implants, untreated and healthy groups. X-ray and MRI examinations were performed at 1, 3 and 6 months, followed by immuno-histological analysis.

Results: PCL implants with average fiber diameters of 1µm and a tensile modulus (55±1MPa) matching the one of a native human AF lamella (~47MPa) were obtained. In vitro spontaneous colonization of PCL implants by ovine AF cells was demonstrated. In sheep, successful in vivo implantations of PCL implants were achieved. We observed cell infiltration between and within the implants, and a continuous type I collagen tissue formation between the implants and the surrounding AF tissue.

Conclusion: These results highlight that a multi-layer PCL electrospun implant is a promising biomaterial for AF repair.
0.45
A NOVEL BIOACTIVE GLASS-CERAMIC SPACER FOR SPINAL FUSION: NOVOMAX
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Introduction: Since launched in 2015, NovoMax, an interbody fusion device using bioactive glass-ceramic material (BGS-7) has been clinically used in South Korea. In this study, to improve the quality of NovoMax, CAD/CAM process is applied to realize a complex design implant that was impossible in conventional ceramic process.

Materials and Methods: The interbody fusion devices using BGS-7 were manufactured by first sintering at low temperature, then shaping by CAD/CAM process, and final sintering. The size error generated in this process was analyzed, and the fatigue and migration test of the final product were performed.

Results: The size error of the final product manufactured by the new process was within 1%. The final product with complicated design was not broken by the fatigue test of 3000N of 5,000,000 cycles and showed an ultimate expulsion load equivalent to that of a commercial PEEK cage used as stand-alone.

Conclusion: The CAD/CAM process has been successfully introduced into the BGS-7 material. Even with more complicated/anatomical design, the advanced NovoMax has passed the fatigue test of 3000N for 5,000,000 times, and migration test showed the same level of frictional force as that of the commercially available PEEK cage. As a result, the advanced NovoMax is expected to show better clinical results, furthermore, CT-based patient-specific implants can be manufactured by this process.
P01
DEVELOPMENT AND CHARACTERIZATION OF 3D PRINTED SCAFFOLDS FOR ANNULUS FIBROSUS REGENERATION
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Introduction: The goal of this work is to apply 3D printing to the fabrication of multi-lamellar, angle-ply scaffolds composed of polycaprolactone (PCL) and gelatin methacrylate (GELMA) for annulus fibrosus (AF) tissue engineering. We hypothesize that our design will advance AF repair by providing both biomechanical restoration to the degenerated IVD and support for targeted AF cell differentiation and matrix expression.

Methods: Multi-lamellar scaffolds were 3D printed on a RegenHU 3DDiscovery (RegenHU, Villaz-Saint-Pierre, Switzerland) by depositing PCL struts in an angle-ply arrangement, mimicking the architecture of the native AF tissue. In order to produce a range of mechanical properties, the angular orientation of the struts (θ) was varied between ±45° and 30°. Additionally, the strut spacing was varied between 600 and 1000 µm. The circumferential tensile modulus of the printed constructs was measured by stretching at a constant rate of 2mm/min. In parallel, precursor GELMA solutions were prepared composed of 5 % (w/v) GELMA and 0.5% (w/v) Irgacure. Human bone marrow mesenchymal stem cells (MSCs, female donor age 55) were suspended in the precursor solutions at a density of 7x10⁶ cells/mL. Cell encapsulation was achieved via exposure to UV light of wavelength 365 nm for 10 minutes. Fluorescent, histological and immunocytochemical (ICC) images were taken after 14 days of in vitro culture in medium composed of high-glucose DMEM, ITS+1, 1% pen/strep, non-essential amino acid, 50 mg/mL of ascorbate 2 phosphate, 10-7 M of dexamethasone, and 10 ng/mL transforming growth factor-β3.

Results: The median circumferential tensile modulus of the PCL constructs (600 µm spacing, θ = ±30°) was found to be 12 MPa, coinciding with the range of values reported for the native AF tissue, 11-29 MPa. The histological and ICC results of the cell study indicate that GELMA hydrogels support the expression of collagen I, collagen II, and proteoglycan, the major ECM components of the AF.

Conclusion: GELMA hydrogels are permissive to the expression of AF matrix components by encapsulated MSCs when cultured in chondrogenic medium. Further, the multi-lamellar angle-ply PCL scaffolds have potential to mimic the mechanical properties of native AF tissue. In future work, GELMA and PCL will be printed into a single, multicomponent scaffold in an alternating strut configuration. The advantage of this design is that the architecture of the PCL framework can be tailored to meet mechanical requisites for AF replacement, while variation of the GELMA composition can enable spatial control of MSC behavior to region-specific AF phenotypes and ECM expression.
P02

WHY WE USE PEKK? MATERIAL’S CHARACTERISTICS AND CLINICAL RESULTS
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Introduction: PEKK is a semi-crystalline thermoplastics having high mechanical strength. From previous work, PEKK appears to provide good surface adhesion to support cell activity.

Methods: We present the mechanical properties of OXPEKK® compared to bone: elasticity, wettability by tissue fluids, and design of devices allow us to obtain a mechanical behavior equivalent to its bone environment. We compare the surface conditions between the machined OXPEKK® and the sandblasted OXPEKK®. SEM observations and an AFM-microscopy study allowed us to compare the surface conditions of OXPEKK® implants before and after gamma-ray sterilization. The OXPEKK® implants are often used with MBCP™ bone substitute. MBCP™ is a biphasic synthetic bone graft substitute, osteoconductive thanks to its micro and macro porous structure. Soluble and resorbable, it promotes bone regrowth.

Results: We present the results of an OXPEKK®-vs-PEEK rabbit implantation study and compare the osseointegration kinetics on both materials. We show the results of a clinical study (30 patients, 1-3 years follow-up): it demonstrates safety and effectiveness of the OXPEKK® medical device used with the MBCP™ substitute for the treatment of lumbar degenerative diseases regarding the lordosis recovering as well as the fusion rate. Finally, some long-term results of implantation of OXPEKK® cages on scoliosis cases are shown.

Conclusion: OXPEKK® has mechanical characteristics comparable to human bone, good mechanical dynamic behavior, and its physical-chemical surface properties guarantee rapid osseointegration of implants. The radiolucency of OXPEKK® makes possible to visualize new bone regrowth, thanks to MBCP™ bone substitute which has a resorption rate similar to human bone.
Introduction: Combination of surgery and radiotherapy is becoming more frequent for the treatment of bone tumours of the spine [1]. Metallic hardware interfere with the postoperative radiotherapy due to the artefacts on imaging and due to the scattering effects. The risk of over-irradiation of neighbouring structures limits the dose delivered making treatment less effective. Composite materials such as carbon-fiber-reinforced (CFR) polyethil-ether-ether-ketone (PEEK) have been used since many years for interbody and body replacement cages [2,3]. This material is biologically compatible and promotes osteoblastic activity [4]. These cages are radiolucent at the standard radiograms, barely visible on TC scan and MRI, allowing easy planning CT scan [5], early detection of local recurrence and very useful to avoid any scattering effect during radiotherapy. A CFR-PEEK spine fixation system has been proposed few years ago.

Methods: We retrospective evaluate the first 60 consecutive tumor patients, treated in a single institution who underwent spinal surgery including a composite CFR-PEEK fixation system (CarboclearTM, produced by Carbofix OrthopedicsÒ Ltd., Herziliya, IL).

Results: There were 33 male and 27 female, mean age 58 years (range 18-78). 37 cases were primary tumour (24 recurrence) and 23 were metastases (13 recurrence). A separation surgery has been performed in 41 cases, a gross total excision in 11 cases and en-bloc resection in 8 cases. Only one intraoperative complication related to the implant occurred: a screw breakage during the third surgical procedure of the series. Weight-bearing was encouraged in the immediate post-operative course for all the patients without orthosis. No rod breakage, neither any screw/rod disconnection was found during the follow-up. One cases of loosening of sacral screws were found at 12 months in one patient submitted to previous surgery and revised with CFR-PEEK screws. This were a multi recurrent malignant tumour and loosening was found at the time of the local recurrence provoking instability of the construct. One case of screw mobilization with pull out of the distal screws at 6 months was recorded. After the surgery in 41 patients a postoperative radiotherapy has been performed (23 cases with particle and 18 with photons).

Conclusion: Thanks to radiolucency CFR-PEEK stabilization devices are more suitable in patients eligible for radiotherapy: the absence of image artefacts together with significantly less dose perturbation improve the treatment accuracy. Moreover, the radiolucency is useful in the follow-up of patients allowing early detection of local recurrence. The advantage of using CFR-PEEK composite implants in terms of overall results and patients’ outcome needs to be prospectively defined with larger patient series and longer follow-up. In this perspective, even the final prognosis could be positively affected by combination of less aggressive surgery and appropriate courses of radiotherapy.
MOLDABLE ALLGRAFT BONE GEL INFUSED WITH SPINE-DERIVED EXOSOMES TRIGGERS OSTEOGENIC INDUCTION

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Introduction: Exosomes are nanoscale vesicles that support cell-to-cell communication via transporting molecular cargo from a source cell to a target cell. A moldable bone gel was developed to determine whether osteoinductive properties could be obtained through exosome induction as a component of a novel bone graft material.

Methods: Exosomes were obtained from human spine bone marrow by ultrafiltration and subsequent differential ultracentrifugation of the supernatant. Exosomes were characterized by flow cytometry with known exosome markers, CD63, CD81 and CD9. Bone forming potential was assayed with a C2C12 myoblast cell line using expression of alkaline phosphatase following treatment with spine-derived exosomes alone or in combination with bone gel. Treatment with 50ng of BMP-4 was used as a positive control.

Results: The mean concentration of the spine-derived exosomes obtained was $1.22 \pm 1.09 \times 10^{10}$ exosomes/mL of supernatant. The mean number of exosomes per microgram of protein was $3.31 \pm 2.33 \times 10^8$ indicating relatively high purity. Results revealed a dose-dependent effect, with higher doses of exosomes resulting in a greater amount of alkaline phosphatase expression. All doses indicated an osteoinductive effect at doses ranging from $2 \times 10^8$ to $1 \times 10^{10}$ exosomes alone or with bone gel.

Conclusion: In vitro osteoinductive effect was obtained with spine-derived exosomes alone or infused in bone gel on C2C12 cells that was equivalent to a concentration (50ng) of BMP-4 as our positive control. Future studies will include miRNA analysis to better understand the molecular mechanism of exosome-delivered therapy in the context of bone regeneration.
COCULTURE OF NUCLEUS PULPOSUS MICRO-TISSUE WITH NASAL CHONDROCYTES AS AN IN VITRO MODEL FOR INTERVERTEBRAL DISC REGENERATION

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Introduction: Human nasal chondrocytes (hNCs) were identified as a possible alternative cell source for the treatment of intervertebral disc (IVD) degeneration due to their ability to survive and produce extracellular matrix in an IVD environment. This study aims to investigate the interaction of hNCs with human nucleus pulposus cells (hNPs) in a 3D in vitro co-culture model.

Methods: In vitro nucleus pulposus micro-tissue was fabricated by pooling eight hNPs micro-aggregates (25’000 cells per micro-aggregate, cultured for two weeks) together. GFP-labelled hNCs were then added to the hNP micro-tissue either as single cell suspension (200’000 cells per micro-tissue) or as 16 micro-aggregates (12’500 per micro-aggregate, cultured for three days). Monoculture hNPs and co-culture hNPs-hNCs were cultivated for two weeks. Preliminary analysis was conducted upon macroscopic investigations during co-culture time.

Results: Macroscopic investigations showed that: (i) control hNPs micro-aggregates in monoculture did not fuse into a stable construct, (ii) hNPs micro-aggregates in co-culture with hNCs suspension or with hNCs micro-aggregates merged to create one accumulative mass (indicating positive interactions between the two cell types), (iii) metabolic activities was high and similar in the two co-culture groups (indicating new matrix formation).

Conclusions: Preliminary results indicate that the experimental approach to investigate the interaction of hNCs with hNPs is feasible. Visual evaluation suggests that co-culture of hNCs and hNPs has an advantageous effect on matrix production. Histological and biochemical analysis will be performed to validate and quantify the impact of hNC cells on the viability, proliferation and extracellular matrix production of hNPs and vis-versa.
INVESTIGATION OF THE EFFECT OF NICOTINAMIDE RIBOSIDE ON THE CELLULAR SENESCENCE OF PRIMARY HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS IN VITRO

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Introduction: Mesenchymal stromal cells (MSC) have been identified as the most prominent cell-based therapy candidates for cartilage, bone and intervertebral disc diseases due to their multipotency. One of the major problems involved in cell therapy is the necessity of expansion to obtain sufficient cells. During prolonged expansion MSC become senescent, which impairs their therapeutic potential. Here, we investigated whether extracellular nicotinamide riboside (NR), a precursor of nicotinamide adenine dinucleotide (NAD), is beneficial for MSC expansion in terms of growth kinetics, mitochondrial activity and senescence.

Methods: MSC were isolated from human bone marrow aspirates by gradient centrifugation and subsequent expansion in α-MEM + 10% FBS + 2.5 ng/ml bFGF-2. Immunophenotyping was performed by flow cytometry. The cytotoxicity of NR was measured at day 4 for 29 concentrations in a range from 0.005 to 4’000 µM. The long-term effect of NR was tested at concentrations of 10, 100 and 1’000 µM by measuring the population doubling level (PDL), relative confluence, mitochondrial activity, and senescence.

Results: The cells displayed a typical MSC-immunophenotype by flow cytometry. Furthermore, no acute cytotoxicity was found. MSC treated with >3 mM NR had a significantly higher mitochondrial activity (N=3). At all passages, the percentage of β-galactosidase positive cells was under 5%. All experimental groups treated with NR had a higher NAD/NADH ratio, which exhibited a dose-dependent trend (N=1).

Conclusion: NR is not cytotoxic within 4 days of culture at concentrations up to 4,000 µM. Long-term culture with 10 µM NR improved the growth kinetics and mitochondrial activity markedly.
DIFFERENT ISOLATION METHODS FOR NUCLEUS PULPOSUS PROGENITOR CELLS
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Introduction: Nucleus Pulposus Progenitor Cells (NPPCs), positive for the angiopoietin-1 receptor (Tie2), were demonstrated in human, mouse, canine and bovine NP tissue. Tie2+ NPPCs possess a multi-lineage differentiation potential, and regeneration potential is attributed to them. However, the isolation of Tie2+ NPPCs can be cumbersome. Hence, three isolation methods were compared.

Methods: Bovine NP cells were isolated from 10-14-month-old animals. Cell sorting was performed with an antibody against Tie2 (bs-1300R, Bioss) using FACS, magnetic-activated cell sorting (MACS) and pluriSelect, a size-based sorting method. Outcomes were evaluated by cell yield of Tie2+ cells, the ability of sorted cells to form colonies and tri-lineage differentiation assays.

Results: FACS resulted in the highest Tie2+ cell yield (5.0 ± 4.0%) followed by MACS (1.6 ± 2.9%) and pluriSelect (1.1 ± 1.4%). Colony forming ability did not differ between Tie2+ and Tie2- cells for any isolation method. However, Tie2+ cells obtained by MACS resulted in more colonies than pluriSelect (p < 0.05). Osteogenic and adipogenic differentiation of Tie2+ and Tie2- cells did not result in a clear distinction for MACS and pluriSelect; Tie2+ FACS-sorted cells demonstrated superior osteogenic and adipogenic differentiation over Tie2- cells. Also for chondrogenesis, the Tie2+ FACS-sorted NPPCs tended to produce more proteoglycan vs Tie2- NPPCs, whereas for MACS and pluriSelect no difference was found.

Conclusion: Isolation of Tie2+ NPPC is possible with all three methods tested. However, FACS resulted in the highest cell yield and a clearer separation after differentiation making it the method of choice for Tie2+ NPPC isolation.
P08

CAN PPARδ AGONIST INCREASE CELL YIELD OF NUCLEUS PULPOSUS PROGENITOR CELLS POSITIVE FOR ANGIPOIETIN-1 RECEPTOR (= TIE2) AFTER CELL ISOLATION?

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Introduction: Nucleus pulposus progenitor cells (NPPC), Tie2+ cells (positive for angiopoietin receptor), which possess multi-lineage differential potential is a potential cell population for cell therapy. However, the number of Tie2+ cells in NP is extremely limited. Referring to the recent research of Tie2+ hematopoietic stem cells we attempted to increase the Tie2+ cell sub-population in nucleus pulposus cells (NPC) by PPARδ agonist treatment and increasing mitophagy.

Methods: Cells were isolated from fresh human IVD tissue from spinal surgery with written consent. The passage 1 human NP cells were cultured in low glucose Dulbecco’s Modified Eagle’s Medium media containing PPARδ agonist (GW501516, Sigma), i.e., 25 µM, or vehicle control (N = 2 donors). After 10 days NP, the Tie2 marker expression was then detected by flow cytometry cells and relative gene expression was determined by real-time qPCR, i.e. at ACAN, col1, col2, and PTEN-induced kinase 1 (PINK1).

Results: PPARδ-agonist-treated NP population had ~3 times more Tie2+ cells and PINK1 gene expression tended to be higher than in the vehicle control group.

Conclusion: PPARδ agonist possibly increases the Tie2+ cell population in NPC by increasing mitophagy similar to hematopoietic stem cells.
HERNIATED DISCS AT THE CERVICOThoracic JUNCTION
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Introduction: Disc herniations at the cervicothoracic junction (C7-T1 level) are unusual, and there have only been a few studies of patients with herniated C7-T1 discs. In addition, previous studies did not focus on the mechanism and causes of solitary cervicothoracic junction disc herniation. The authors investigated the characteristics, symptom duration, clinical course, and biomechanics of cervicothoracic junction disc herniation by comparing patients with C7-T1 disc herniation (C7-T1 group) with control groups.

Methods: 36 patients who underwent solitary C7-T1 single-level disc surgery between 2006 and 2015 were included. For radiographic comparison, patients in a herniated C5-C6 disc group and the healthy control group were cohort matched.

Results: In the C7-T1 group, the disc herniation mainly occurred in the foraminal space (p < 0.0001). The C7-T1 group was significantly associated with a history of trauma (p < 0.0001).
In addition, the cervical vertebral body was more readily observed on plain lateral radiographs in the C7-T1 group. Patients in the C5-C6 group tended to have the sternal notch more frequently located above the T2-T3 disc space than other groups.

Conclusion: C7-T1 disc herniation demonstrates unique characteristics. Understanding the features of disc herniation at the cervicothoracic junction would be helpful for optimal care.
P10
IS DECOMPRESSIVE C1 LAMINECTOMY WITHOUT FUSION FEASIBLE FOR THE TREATMENT OF CRANIOVERTEBRAL JUNCTION STENOSIS WITH MYELOPATHY
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Introduction: Trans-oral approach or occipitocervical/atlantoaxial fusion with/without posterior decompression has been considered to be an appropriate surgical strategy for craniovertebral junction (CVJ) stenosis with myelopathy. However, decompressive C1 laminectomy without posterior stabilization was reported recently for treating retro-odontoid pseudotumor. This study aimed to evaluate surgical outcomes of the patients treated with decompressive C1 laminectomy without posterior stabilization for CVJ stenosis with myelopathy.

Methods: Ten patients underwent decompressive C1 laminectomy without posterior stabilization for CVJ stenosis with myelopathy from August 2007 to December 2016. We investigated clinical outcomes such as pain according to the visual analog scale (VAS), and Ranawat grade scale and complications. Radiographic parameters including atlas-dens interval (ADI), O-C2 angle, C2-C7 Cobb angle, and C2-C7 sagittal vertical axis (SVA) were measured.

Results: The mean follow-up time was 41 months. Eight men and 2 women with a mean age of 58 years were enrolled. Nine of 10 patients showed improvement on the Ranawat grading scale, but 1 patient who required a Halo-vest due to aggravated instability after surgery remained unchanged. The statistical results of the preoperative and postoperative radiographic measurements were not significant. There was no perioperative complication.

Conclusion: Surgical intervention remains the most effective therapy for CVJ stenosis with myelopathy. In this study, patients showed improvement in clinical and neurological outcomes after, even when presenting with preoperative atlanto-axial instability, after undergoing decompressive C1 laminectomy without posterior stabilization. In select patients with certain indications, decompressive C1 laminectomy could be a viable option, especially in the elderly, patients with comorbidity.
Introduction: Diffuse idiopathic skeletal hyperostosis (DISH) is characterized by the formation of bone along the anterior spine. Further ossification of the outer intervertebral discs (IVD) can be observed. However, the nucleus pulposus (NP) remains unaffected. We investigated the phenotype of disc cells of DISH patients and why the IVD resists ossification.

Methods: Cells were isolated from IVD tissue of DISH and control patients. TGFβ BMP signaling pathway genes were compared by qPCR. IVDs of three DISH patients were tested against three control patients (same disc level and similar age). IVD of two donors could be separated in NP, annulus fibrosus (AF) and cartilaginous endplate (CEP), one donor was investigated without discriminated IVD tissue.

Results: In six of the seven comparisons a mean up-regulation of Interleukin 6 (IL-6) was detected (mean ± SEM of all comparisons: 88.8 ± 79.4-fold in DISH-IVD compared to controls). Early Growth Response 2 (EGR2) and Insulin-like Growth Factor 1 (IGF1) up-regulated in DISH-IVD donors (i.e., 20.5 ± 12.4-fold and 19.0 ± 19.5-fold, respectively). The two Growth and Differentiation Factors 5 and 6 (GDF5 and 6) were down-regulated in two of the three DISH-IVDs (i.e., -21.9 ± 16.2-fold and -8.2 ± 4.2-fold, respectively).

Conclusion: Most interestingly, the DISH-IVD cells showed a considerable change in IGF1 and IL-6. IGF1 was already determined as a serum marker for rheumatic diseases, such as DISH. These results are unexpected considering the fact that the ossification occurs in the neighboring ligaments and enthesis leaving the inner part of the IVD macroscopically unaffected.
P12
A COMPARISON OF ACCESSORY RODS CONSTRUCTS TO TWO RODS CONSTRUCTS AFTER CORRECTIVE FUSION SURGERY INCLUDING SACROILIAC FIXATION FOR ADULT SPINAL DEFORMITY DOES IT PREVENT OR AGGRAVATE COMPLICATIONS

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Introduction: The rate of rod fracture is higher after three-column osteotomy and if the magnitude of correction compared to previous deformity is high. It has been reported that the use of accessory rods constructs (ARC) could prevent rod fractures after adult spinal deformity surgery because of their greater strength and resistance to fatigue as compared to the two rods constructs (TRC) system. We investigated the influence of the ARC on proximal junctional kyphosis (PJK) and rod fracture compared to that of the TRC.

Methods: We reviewed data from 59 patients who had undergone adult spinal deformity surgery with sacropelvic fixation at a single institution between June 2011 and May 2017. Preoperative demographic data were reviewed and radiographic parameters were. Kaplan-Meier analysis was used for evaluating the timing and incidence of PJK and rod fracture between groups.

Results: There were no significant differences between both groups in the demographics and preoperative radiological parameters. However, the rate of rod fracture was higher in the TRC group than in the ARC although the rate of PJK was not significantly different between groups. The rate of PJK development was much faster in the ARC group, according to survival analysis.

Conclusion: The use of ARC is a simple and effective way to increase the stability of the surgical site and to prevent problems such as rod fractures, instead of using TRC. However, continuous monitoring is vital after ARC, because it can affect the prevalence and occurrence of PJK by rod construct stiffness.
P13
ANGULAR LOSS COULD HAPPEN DURING EARLY PERIOD OF FOLLOW UP AFTER CORRECTIVE SURGERY FOR ADULT SPINAL DEFORMITY WITH SPINAL SAGITTAL MALALINMENT HOW MUCH AND WHERE COULD BE THE LOCATION

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Introduction: Lumbar lordosis in the sagittal plane has become one of the most important goals of adult spinal deformity (ASD) surgery. Intraoperatively, surgeons generally depend on cross-table lateral radiographs to determine lumbar lordosis. The use of intraoperative radiographs is limited in predicting lumbar and pelvic measurements obtained with the standing whole spine radiographs, owing to differences in patient positioning - prone versus standing position. The aim of this study to determine the relationship between preoperative, intraoperative, and postoperative lumbar lordosis after ASD with sacropelvic fixation.

Methods: We reviewed patients who had undergone ASD surgery with sacropelvic fixation between June 2011 and May 2017. All patients had preoperative, intraoperative, and postoperative measurements of lumbar lordosis. The intraoperative cross-table lateral radiograph was collected with the patient in a prone position on the operative table. Pelvic parameters were measured using preoperative and postoperative standard standing whole spine radiographs.

Results: A total of 59 patients were included in the study with sacropelvic fixation. All patients had a significant improvement in sagittal vertical axis, lumbar lordosis (LL), and pelvic incidence (PI) - LL after corrective surgery. However, LL showed a significant loss of 4.1° from the intraoperative prone position to the immediate postoperative standard standing position (p < 0.0001).

Conclusion: Lumbar lordosis is an important factor in preoperative planning of global sagittal alignment while performing corrective surgery. According to this study, the LL requires careful evaluation of the radiograph in the prone position during surgery, as it is overestimated compared to postoperative standard standing whole spine radiographs.
PERCUTANEOUS ENDOSCOPIC LUMBAR DISCECTOMY AND PLATELET RICH PLASMA BIOLOGICAL THERAPY FOR THE TREATMENT OF LUMBAR DISC HERNIATION

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Introduction: Percutaneous lumbar endoscopic discectomy (PELD) is a minimally invasive surgical procedure that can be performed in awake patients through an incision less than 1 cm. Platelet rich plasma (PRP), which is comprised of autologous growth factors and cytokines, has been widely used in the clinical setting for tissue regeneration and repair. This study describes our experience and technical note with PELD combined with PRP for the treatment of lumbar disc herniation.

Methods: A total of 11 lumbar disc herniation patients who underwent PELD surgery combined with autologous PRP epidural injection treatment after PELD between February 2018 and September 2018 were retrospectively evaluated. Exclusion criteria for this study included previous surgery, tumor, infection and spondylolisthesis at the lumbar level. Both pre- and postoperative visual analog scale (VAS) scores (lower limb pain) and Oswestry Disability Index (ODI) scores were used for clinical assessment. All assessments were completed 1 day before surgery, 1 week after surgery, 1 months, 2 months after surgery, and at final follow-up after surgery. Data were analyzed from 11 patients (6 men and 5 women; mean age, 34.6 years). The average follow-up period was 5 months. Following treatment, no patient experienced adverse events. No neurologic deficit or surgical site infection occurred.

Results: The mean preoperative lower limb pain VAS scores decreased from 7.81 ± 0.83 to 1.44 ± 0.58 at 1 week after surgery, 1.38 ± 0.51 at 1 months after surgery, 1.02 ± 0.58 at final follow-up after surgery. The mean preoperative ODI scores decreased from 75.2 ± 13.3 to 25.4 ± 10.8 at 1 week after surgery, 10.2 ± 3.2 at 1 months after surgery, 70.2 ± 1.2 at final follow-up after surgery at the final follow-up after surgery (p <0.01).

Conclusion: This serial therapy yielded good results. We demonstrated that minimally invasive PELD surgery for the treatment of lumbar disc herniation can preserve the motional segment, we also combination with epidural injection of autologous PRP after PELD surgery. Epidural injection of autologous PRP is a safe and a possibly effective treatment for disc regeneration. Future randomized controlled clinical studies should be performed to systematically evaluate the effects of this therapy.
L5/S1 Percutaneous lumbar endoscopic discectomy

L5/S1 Percutaneous epidural autologous platelet rich plasma injection
P15
INTRADISCAL AND EPIDURAL PLATELET-RICH PLASMA INJECTION FOR THE TREATMENT OF CHRONIC DISCOGENIC LOW BACK PAIN
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Introduction: Low back pain is a very common cause of pain and disability caused by intervertebral disc degeneration, which included degradation of the extracellular matrix, with loss of proteoglycan and water content in the nucleus pulposus and collagen degeneration in the annulus fibrosus. Platelet-rich plasma (PRP) has been found to be effective for a variety of musculoskeletal conditions. The treatment of discogenic pain with PRP is under investigation.

Methods: Patients were diagnosed with discogenic low back pain by clinical means, imaging. Inclusion criteria for this study included chronic low back pain without leg pain for more than 3 months; one or more lumbar discs degeneration, as indicated via magnetic resonance imaging (MRI); and at least one symptomatic disc, confirmed using standardized provocative discography. and exclusion of other structures included discitis and tumor. Patients underwent treatment of intradiscal and epidural injection of PRP at one or multiple levels. Outcome measures included the use of a visual analog scale (VAS) and Oswestry Disability Index (ODI) scores.

Results: Data were analyzed from 7 patients (3 men and 4 women; mean age, 37.8 years). The average follow-up period was 3 months. Following treatment, no patient experienced adverse events or significant narrowing of disc height. The mean pain scores before treatment (VAS, 7.1 ± 1.1; ODI, 63.2 ± 11.3) were significantly decreased at one month (VAS, 2.1 ± 1.1; ODI, 35.2 ± 16.5), and this was generally sustained throughout the observation period (3 months after treatment: VAS, 1.2 ± 1.4; ODI, 19.2 ± 11.5; p < 0.01).

Conclusion: This study demonstrated that intradiscal and epidural injection of autologous PRP in patients with low back pain was safe, with no adverse events observed during follow-up. Intradiscal and epidural PRP injection is a safe and a possibly effective treatment for discogenic low back pain. Randomized placebo controlled trials are needed to further evaluate the efficacy of this treatment.

Key words: intervertebral disc degeneration - chronic discogenic low back pain - autologous platelet rich plasma
L5/S1 intervertebral disc degeneration (High Intensity Zone)

L5/S1 Percutaneous intradiscal and epidural autologous platelet rich plasma injection

L5/S1 Percutaneous intradiscal and epidural autologous platelet rich plasma injection
(3565) Growth factors in spinal fusion and disc regeneration
P16
STIMULATION OF INTERVERTEBRAL DISC CELLS IN ALGINATE BEAD CULTURE WITH BONE MORPHOGENETIC PROTEIN 2 AND/OR L51P
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Introduction: In clinics, Bone Morphogenetic Protein 2 (BMP2) was applied to support spinal fusion. Further BMP2 was tested in IVD models and showed potential for IVD regeneration. The aim of this study is the investigation of BMP2 and L51P on different cell types of the human IVD in 3D alginate beads, particularly their plasticity to undergo bone formation.

Methods: Human nucleus pulposus (NPC), annulus fibrosus (AFC) and cartilaginous endplate cells (CEPC) were each encapsulated in 1.2% alginate at a density of 4 Mio/mL. NPC, AFC, and CEPC beads were then cultured in α-MEM or osteogenic medium (OM) supplemented with 10% FBS and 100 ng/mL BMP2 or L51P for seven days. After four days, medium supplemented with cytokines was refreshed. Beads were then snap frozen with liquid nitrogen and analyzed by qPCR and Alcian Blue staining.

Results: Aggrecan (ACAN) expression was the highest up-regulated in IVD cells stimulated with OM and 100 ng/ml BMP2 and L51P compared to negative control (basal medium) in NPC, AFC and CEPC (mean ± SEM NP: 18.95 ± 15.65). The same was true for Collagen type 2 (COL2) expression (NP: 72.47 ± 62.95). COL1 remained unaffected (N=2).

Conclusion: Recent studies confirmed the anabolic effect of BMP2 on IVD. Also, we showed the trend of an increase in ACAN and COL2 gene expression in stimulated cells. Like in previous studies collagen type 1 (COL1) expression in stimulated IVD cells was unaffected. Interestingly showed the co-treatment of BMP2 and L51P a cumulative effect towards an increased ECM production.
P17
PRELIMINARY EXPLORATION OF EALIF (ENDOSCOPY ASSISTANT LUMBAR INTERBODY FUSION) COMBINE WITH PERCUTANEOUS PEDICLE SCREW FIXATION FOR ADULT ENERATIVE SCOLIOSIS

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Introduction: Adult degenerative scoliosis (ADS) is very common in aged people. Decompression of neural structures and correction of the spine deformity is recommended for some severe cases. However, traditional open surgery always means huge trauma. The aim of this retrospective study was to evaluate the efficacy and safety of E-ALIF combined with percutaneous pedicle screw fixation for patients with adult degenerative scoliosis.

Methods: 2 patients with symptomatic ADS (Lenke-Silva IV-V) underwent E-ALIF combined with percutaneous pedicle screw fixation were enrolled in this study. 2 patients both presented severe mechanical low back pain with radicular pain in low extremes. First, both 2 patients were performed endoscopic decompression of the neural structures and endoscopy-assistant lumbar interbody fusion in 3 and 4 levels under local anaesthesia with intravenous sedation. And then, percutaneous pedicle screws fixation and correction of the deformity were performed under general anaesthesia.

Results: We successfully performed E-ALIF combine with percutaneous pedicle screw fixation for 2 patients. The average operation time is 8.5h. Mechanical low back pain and radicular pain of the 2 patients were eased obviously. No blood transfusion and severe complications were observed during perioperative period. Postoperative X-ray shows that the sagittal and coronal deformity was definitely corrected.

Conclusions: E-ALIF combine with percutaneous pedicle screw fixation could correct moderate sagittal and coronal deformity for patient with ADS safely and effectively. However, long-term effectiveness need further follow-up.
P18
TRANSFORAMINAL ENDOSCOPIC APPROACH TO REMOVAL A BROKEN EPIDURAL CATHETER FRAGMENT
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Introduction: In continuous epidural anesthesia, the breakage of an epidural catheter is a rare complication. Here, we reported a case of removing the breakage of an epidural catheter by transforaminal endoscopic approach. In this case, the epidural catheter was fractured during its removal, and the remained breakage of epidural catheter was about 8 cm. The diameter of the fractured catheter was about 1 mm, and the breakage of epidural catheter is not visible on the skin surface. Intraoperative X-ray did not accurately determine the location of the retained catheter. Being unable to accurately locate the position, it is indeed difficult to retrieve the epidural catheter, even leading to the failure of surgery.

Methods: We attempted to use transforaminal endoscopic to remove the breakage of an epidural catheter.

Results: We eventually successfully remove the breakage of an epidural catheter; the general anesthesia was smooth, and patient had no significant discomfort after surgery.

Conclusion: In our case, we successfully used transforaminal endoscopic to remove the breakage of an epidural catheter, further demonstrating that it has a wider clinical application. In China, to remove foreign bodies in the spinal canal, transforaminal endoscopic can be an appropriate surgical method for clinical practice.
POSTER SESSION Minimally invasive spinal surgery (MISS)

P19  
BICANNULAR PERCUTANEOUS ENDOSCOPIC DECOMPRESSION FOR THE TREATMENT OF TWO LEVEL LUMBAR STENOSIS: TECHNICAL NOTE
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Introduction: Lumbar spinal stenosis is a common lumbar vertebrae disease in the elderly population, can lead to leg pain and low back pain especially when walking. It is usually caused by the herniation of intervertebral disc, the gradual narrowing of the spinal canal, and the hypertrophy of facet joints. Percutaneous endoscopic transforaminal lumbar discectomy (PETLD) is considered as a safe and effective minimally invasive surgery. This study aimed to evaluate the safety and curative effect of bicannular percutaneous endoscopic transforaminal lumbar spinal canal decompression in the treatment of two level lumbar spinal stenosis.

Methods: This retrospective study recruited 6 patients with two level lumbar spinal stenosis who underwent bicannular percutaneous endoscopic lumbar spinal canal decompression via surgical approach of posterolateral intervertebral foramen. The postoperation neurological function and pain status were evaluated by the visual analog scale (VAS) score of pain and the Oswestry disability index (ODI). The data, including preoperative comorbidities, operation time, the quantity of bleeding, bed rest time, and intraoperative and postoperative complications, were recorded.

Results: The mean operation time was 160 min, the mean quantity of bleeding was 30 mL. All patients were followed-up for 4 months to 1 years. The mean preoperative VAS score was 7.3 ± 1.3, while postoperative 1 months, 3 months, and final follow-up VAS scores were 1.8 ± 0.7, 1.1 ± 0.6, and 0.8 ± 0.6, respectively (p < 0.001). The mean preoperative ODI score was 72.4 ± 1.2, while postoperative 1 months, 3 months, and final follow-up ODI scores were 28.5 ± 3.9, 22.6 ± 4.1, and 12.5 ± 3.3, respectively (p < 0.001).

Conclusion: The percutaneous endoscopic transforaminal lumbar spinal canal decompression is an easy, safe, and effective minimally invasive surgery for patients with lumbar spinal stenosis. But the bicannular endoscopic decompression technique has a “long and steep” learning curve. The surgeons’ training level of PELD surgery was an important factor for the success of bicannular technique.
L3/4 L4/5 level spinal canal stenosis (sagittal plane)

L3/4 level spinal canal stenosis (axial plane)

L4/5 level spinal canal stenosis (axial plane)
L3/4 L4/5 Bicannular percutaneous endoscopic decompression

Bony resection L3/4 and L4/5 level traversing nerve root decompression
P20
ROBOT ASSISTED SPINE SURGERY: PITFALL AND LIMITATION
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Introduction: As an new technology of spine surgery, robot assisted surgery using intraoperative CT or navigation system was started from 2002 for improving accuracy in a difficult spine surgeries such as severe scoliosis and high cervical spine surgery. But it is still argued about it's efficacy in terms od cost effectiveness and accuracy for clinical application.

Methods: We have been involved a project to develop a new robot assisting spine surgery in craniocervical junction spine area. High cervical and subaxial cervical spine were main focus of project especially for cervical pedicle screw insertion under the guidance of robot system. With presented accuracy limitation, various application were performed for cervical pedicle screw insertion.

Results: The main pitfall was how to register the actual cervical spine with 2D CT or MRI image preoperatively obtained. The registration was very difficult in highly mobile cervical spine without other fixation frame. How to fix the cervical spine and how to match the image to actual, mobile cervical spine were main limitation to make clinically applicable robot machine. The discrepancy between actual distance and arbitrary distance was also very difficult problem to solve.

Conclusion: As a new technology, robot assisted spine surgery technique is popular issue in industry partners. But it has still many hurdles to overcome and the argument about clinical application and cost- effectiveness are remained.
A PERMANENT SPINAL CORD INJURY MODEL FOR THE APPLICATION OF TISSUE CLEARING AND EXPANSION TECHNIQUE

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Introduction: A recently developed tissue clearing and expansion technique (MAP, magnified analysis of proteome) enables high-resolution identification of three-dimensional relationships. Application of MAP in a spinal cord will be helpful in understanding the pathophysiology of spinal cord injury. Here, we established a permanent cervical spinal cord injury model in mice that is applicable to MAP.

Methods: A mouse spinal cord injury at C5 was created, which included hemi-section, deletion, and insertion of hard or fluid materials. An evaluation method suitable for the permanent cervical spinal injury was developed through the behavioral evaluation. Using permanent spinal cord injury model, MAP tried and high-resolution imaging also tried.

Results: The hemi-section group had the most significant hind limb movement recovery. Although other groups showed a gradual improved in behavior, there were no significant differences between the deletion and insert groups. At 4 weeks, the deletion group showed significantly higher BMS (Basso mouse scale) scores than the insert group, and there was no difference in BMS score according to the insert material. After 2 weeks, the insert group never reached a BMS score of 5 or higher. Four-fold expanded and cleared spinal cord using MAP was made successfully.

Conclusion: Notably, the novel fluid insert method allowed cavity formation and initial damage volume measurements, which was beneficial for making a permanent spinal cord injury model. With a permanent cervical spinal cord injury mice model, high-resolution imaging of the three-dimensional spinal cord structure was observed, specifically the neurofilaments, vascular structure (below image), and neural fibers.
MICROPILLAR-ELECTRODES FOR SIGNAL RECORDING OF SPINAL CORD

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Introduction: There are lots of diseases which occur because of disorder of neuronal signal transduction, heretofore, these diseases are treated with medications. But long-term use of medications have many problems like tolerance and aftereffects. Therefore new method is necessary which is not medication and has no problem from long-term using. Recording the abnormal signal from the nerve and control with electrical stimulation can be new method to treat neuronal disorder. Technologies for neuronal signal recording are being developed to understand the neuronal signaling system and treat the neural diseases. Recently, invasive technologies such as Multi Electrode Array (MEA) receive attention, however, they can record only the ensemble signal from the nerve. For better understand of the system it is required to record the intraneural signal and analysis of the networks between neurons.

Methods: The device is composed of 25 electrodes consist of micropillar electrodes and film electrodes. Each electrodes has channel to attach the wire to connect with the recording equipment. The micropillar were fabricated by deep reactive-ion etching (DRIE) and confirmed by scanning electron microscope. The mask for DRIE was patterned with photolithography and metal was deposited by sputter. The spinal cord was extracted from male adult rat which was anesthetized and placed on the device and pressed down. Selected two channels were connected with the signal recording equipment and recorded the signal with both channels simultaneously. Penetration of micropillar could be confirmed through H&E staining of longitudinal sectioned slides.

Results: The device have 13 micropillar electrodes and 12 film electrodes with zigzag pattern. Each silicon micropillars for electrode has a diameter of 20µm and a height of 80µm. It was confirmed that the electrodes of micropillar were able to penetrate the spinal cord without device failure by the histological assessment. The recoding capability of the device was verified by acquiring spontaneous signals or signals in response to electrical stimulation. Micropillar electrodes was able to record the signal more sensitively than film electrodes.

Conclusion: In this study, the probability of understanding the neuronal signaling system with micropillar electrodes is demonstrated. Through further studies about recording signal and understanding networks between neurons, it is expected to be able to treat the neural diseases.
P24
HUMAN-INDUCED PLURIPOTENT STEM CELLS GENERATED FROM INTERVERTEBRAL DISC CELLS IMPROVE NEUROLOGIC FUNCTIONS IN SPINAL CORD INJURY

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Introduction: Induced pluripotent stem cells (iPSCs) have emerged as a promising cell source for immune-compatible cell therapy. Although a variety of somatic cells have been tried for iPSC generation, it is still of great interest to test new cell types, especially those which are hardly obtainable in a normal situation.

Methods: In this study, we generated iPSCs by using the cells originated from intervertebral disc which were removed during a spinal operation after spinal cord injury. We investigated the pluripotency of disc cell-derived iPSCs (diPSCs) and neural differentiation capability as well as therapeutic effect in spinal cord injury.

Results: The diPSCs displayed similar characteristics to human embryonic stem cells and were efficiently differentiated into neural precursor cells (NPCs) with the capability of differentiation into mature neurons in vitro. When the diPSC-derived NPCs were transplanted into mice 9 days after spinal cord injury, we detected a significant amelioration of hindlimb dysfunction during follow-up recovery periods. Histological analysis at 5 weeks after transplantation identified undifferentiated human NPCs (Nestin(+)) as well as early (Tuj1(+)) and mature (MAP2(+)) neurons derived from the transplanted NPCs. Furthermore, NPC transplantation demonstrated a preventive effect on spinal cord degeneration resulting from the secondary injury.

Conclusion: This study revealed that intervertebral discs removed during surgery for spinal stabilization after spinal cord injury, previously considered a “waste” tissue, may provide a unique opportunity to study iPSCs derived from difficult-to-access somatic cells and a useful therapeutic resource for autologous cell replacement therapy in spinal cord injury.
P25
DEVELOPMENT OF A SOFTWARE ESTIMATES SPINAL ALIGNMENT UTILIZING ARTIFICIAL INTELLIGENCE FOR SCOLIOSIS SCREENING
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Introduction: Since early detection and early treatment was considered to be beneficial for adolescents, school screening system has been adapted in many countries. While, downsides of the previous school screening system were its accuracy and its qualitative decisions. Thus, we purposed to develop a software that can automatically estimate the spinal alignment and Cobb angle from Moiré image utilizing Convolutional Neural Network (CNN) deep learning algorithm.

Methods: 10788 sets of training data consisted of Moiré image and standing whole spine radiograph with Cobb angles between 0° and 55° were used for CNN deep learning to create the software. The software was designed to output the estimated spinal alignment and the Cobb angle as the results. Additional 3372 sets were used for validation of the software. The estimated Cobb angles were compared with the measured Cobb angles by the spine surgeons. Sensitivity and false positive rate supposing clinical school screening setting were also evaluated.

Results: The mean error of estimated vertebral position was 3.6±1.5 pixel. The mean error between estimated Cobb angles and measured Cobb angles was 3.14° in patients with Cobb angle of less than 10°, 2.97° in 10°- 20°, and 2.7° in more than 20°. If threshold of scoliosis was set at 10° and more, the sensitivity was 0.98 and the false positive rate was 0.43. While, the threshold was set at 15° and more, the sensitivity was 0.81 and the false positive rate was 0.09.

Conclusion: Our developed software estimated spinal alignments and Cobb angles from Moiré images with high accuracy. The inferior error at smaller Cobb angle might be due to the observer variability in measurements. Since the false positive rate was reported as 0.13 for > 10°, and 0.61 for > 15° using previous screening system, the developed software will be beneficial for school screening to pick up the students with the Cobb angle of 10-15° and more.
P26
DIFFERENTIAL GENE EXPRESSION OF ARTICULAR CHONDROCYTES AND INTERVERTEBRAL DISC CELLS IN NORMAL GRAVITY AND SIMULATED MICROGRAVITY
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Introduction: Due to the limited self-repair capacity of articular cartilage, the surgical restoration of defective cartilage remains a major clinical challenge. The cell-based approach, known as autologous chondrocyte transplantation (ACT), has limited success, presumably because the chondrocytes acquire a fibroblast-like phenotype in monolayer culture. This unwanted dedifferentiation process is typically addressed by using three-dimensional scaffolds, pellet culture and/or the application of growth factors. Alternative mechanical unloading approaches suggested to be beneficial in preserving the chondrocyte phenotype. In this study, we examined if mechanical unloading, could be used to expand chondrocytes in vitro such that they maintain their phenotype.

Methods: We used bovine and human chondrocytes. We cultivated them for two days and eight days, respectively, in static monolayer culture, followed by six days of Random Positioning Machine (RPM) exposure; or eight days of RPM exposure without static culture. In parallel, 2 days and 8 days cultures were performed in hypoxic (1-3% O2) conditions using a hypoxia chamber, as well as pellet cultures for 8 days. Following the harvesting of the different samples, all the conditions were analyzed at the RNA and partially at a protein level, to track and assign potential differentiation stages.

Results: In static culture, chondrocytes adhered to the flask and adapted an elongated and spread-out morphology. In contrast, in RPM-exposed samples, many suspended cell clusters could be observed.

Conclusion: This and previous studies suggest a preservation of the chondrocyte phenotype of cells in suspension that aggregate to form clusters. This culture technique could become an attractive 3D culture model for preserving the chondrogenic phenotype during cell expansion. An analogous approach has been also taken using intervertebral disc cells from bovine tails.
BIOLOGICAL RATIONALE FOR THE USE OF VERTEBRAL WHOLE BONE MARROW IN SPINAL SURGERY

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Introduction: The use of spinal fusion procedures has increased over the last decades; however, failed fusion still remains an important problem. Clinician and researchers focused their attention on the therapeutic potential of bone marrow MSCs and several methods for their isolation and cultivation have been developed. However, the best source and techniques are still debated.

Methods: Mesenchymal stem cells (MSCs) derived from whole bone marrow aspirate (BMA) and MSCs derived from density-gradient centrifugation were isolated from vertebral bodies and cultured under either hypoxic or normoxic conditions to evaluate their biological characteristics and HOX and TALE signature able to improve spinal surgery procedures. MSCs morphology, surface markers, colony-forming-units and three lineage differentiation through quantitative real-time PCR were evaluated. Additionally, gene expression analysis of HOX and TALE signatures during osteogenic differentiation, were analyzed.

Results: Our study showed that MSCs derived from whole BMA were successfully isolated and when cultured under hypoxic condition presented greater proliferation, larger colonies, and differentiated onto osteogenic and chondrogenic lineage with greater ability, while adipogenic differentiation was less efficient. Results also revealed that MSCs, differently isolated and cultured, expressed different level of HOX and TALE signatures and that HOXB8 were upregulated with greater efficiency in MSCs derived from whole BMA under hypoxia.

Conclusion: Our data indicated that hypoxic preconditioning of MSCs derived from whole BMA exhibited more suitable biological characteristics and different level of HOX and TALE gene activation. We therefore concluded that vertebral body MSCs derived from whole BMA may provide alternative sources of MSCs for tissue engineering applications for spine surgery.
P28
COMPARISON BETWEEN EACH PRDM FAMILY EXPRESSION IN VARIOUS DEVELOPMENT STAGE OF MOUSE EMBRYO AND ADULT MOUSE
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Introduction: In previous study, PR/SET family members have a role in transcriptional regulation via chromatin remodeling. Some of Prdm might focused on nervous system and have essential role in formation of tumor and cancer in adult mouse. Previous study was restricted in simple image analysis such as In situ hybridization (ISH) and Immunohistochemistry (IHC) . Further study for expression pattern is required to analysis genes by advanced pathological imaging analysis.

Methods: To analysis Prdm-7, -8, -12, -13 and MYF-5 expression in each development stage of mouse embryo, we generated transparent mouse embryo by advanced passive tissue clearing technique which known as passive tissue clearing technique (PACT). To generate hydrogel-based transparent mouse embryos, it takes few days with modified PACT method. The cleared mouse embryos were immuno-stained with Prdm specific antibodies, lectin and fluorescence dyes for days. We performed three-dimensional imaging of mouse embryo with each Prdm family and blood vessel by confocal laser microscopy and IMARIS software.

Results: Several Prdm genes are expressed at process of partial central nerve system formation including brain and spinal cord in mouse embryogenesis. We demonstrate Prdm gene expression with Prdm family and blood vessel with three-dimentional imaging by confocal microscopy. Three dimensional imaging enable to analysis a single cell unit of expression of Prdm family in hind brain, spinal cord and segment of the CNS.

Conclusion: Our study reconfirmed Prdm family gene might be expected expression patterns during mouse embryo developing and adult mouse nervous system by small embryo specific passive clearing technique and three-dimensional pathological analysis.