for gene expression, DNA, proteoglycan, and collagen contents, and immunohistochemically for matrix molecules.
Results: Serial passaging of NP cells resulted in dedifferentiation. By P1 the morphology of the cells in monolayer changed from a polygonal shape to elongated spindle shape with concomitant significantly decrease in NP associated markers *Aggrecan*, *Col2A1*, and *SRY* (*sex determining region Y*)-*box 9* (*SOX9*) and notochordal associated markers *Brachyury* (*T*), *Cytokeratin 8* (*CK8*) and 19 (*CK19).* In contrast, the dedifferentiation marker *Collagen type 1* (*Col1*) significantly increased by P1 and continued to increase with passaging. Immunostaining reveals cytokeratin expressing cells decreases with serial passage and are infrequently seen by P2 and P3. The NP tissue regeneration capacity of passaged cells decreases as indicated by diminished tissue thickness, and significantly less DNA, Col2A1, and proteoglycan content compared to tissues formed by P0 cells. TGF<$\beta 1$> and TGF<$\beta 3$> treatment equally enhanced the ability of cells at each passage to form tissue. However, neither TGF<$\beta 1$> nor TGF<$\beta 3$> was able to restore the notochordal phenotype as no keratin positive cells were seen.
Conclusions: This study demonstrated that cells harvested from NP tissue undergo dedifferentiation with serial passaging in monolayer culture with loss of NP and notochord phenotype. Treatment with TGF<$\beta 1$> or TGF<$\beta 3$> enhanced NP tissue formation by passaged NP cells but was unable to restore the notochordal cells. These results suggest that nucleus pulposus and notochord cells are regulated by different signaling mechanisms. To generate NP *in vitro* that resembles the native tissue, it will be necessary to identify the regulatory gene(s) required to induce notochordal cell differentiation.

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DESIGN AND EVALUATION OF ELECTROSPUN STRUCTURED BIOMATERIALS FOR *ANNULUS FIBROSUS* REPAIR

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Purpose: Impairment of the *annulus fibrosus (AF)* structure is believed to be a major source of discogenic low back pain. Extensive AF radial tears lead to intervertebral disc (IVD) herniation, defined as the protrusion of the central part of the disc, the *nucleus pulposus (NP)*, outside the IVD, causing a compression of the nerve root in the spinal canal and leading to radicular pain. Current surgical strategies are limited to symptomatic treatment of pain and are mainly focused on the repair of the NP, at the expense of the structural integrity of the AF. Annular tears have been shown to persist in time due to the limited intrinsic healing capacity of the AF. Unrepaired defects in the AF are also associated with up to 20% of postoperative reherniation and an increase by 20% of the IVD degeneration prevalence when compared to the general population. For these reasons, in the last decade, engineering strategies for IVD regeneration have increasingly focused on AF closure and repair. Thus, the aim of this study is to design an electrospun implant composed of polycaprolactone (PCL) that mimics the oriented and multi-lamellar fibrous structure of the native AF and to assess its ability to properly close an AF defect, maintain normal IVD biomechanics and prevent further disc degeneration.
Methods: Oriented and non-oriented PCL (Mn 80,000 Da) mats were produced by electrospinning technology. Fibrous mats were characterized by scanning electron microscopy (SEM) and uniaxial tensile mechanical analysis. Spontaneous colonization of PCL mats by AF native cells was assessed *in vitro* by close apposition of an ovine annular explant to the scaffold. Mats were observed either at day 14 and 28 with SEM and confocal microscopy after YOYO-1 and phalloïdin-alexa fluor® 568 staining, to evaluate cell colonization. Extracellular matrix deposition and cell phenotype were characterized using histological (hematoxylin and eosin, alcian bleu, picrosirius red) and immunofluorescent staining (type I and II collagen, aggrecan).
Results: Electrospinning apparatus allowed the synthesis of oriented and non-oriented fibers with an average diameter of 1.115 ± 0.419 nm and 1.027 ± 0.363 nm, respectively. Uniaxial tensile tests confirmed the reproducibility of the fiber production, and oriented fibrous mats presented a significantly higher young modulus than the non-oriented ones (50 ± 1 MPa *vs* 14 ± 2 MPa, respectively). SEM and confocal observation showed colonization of both mats surfaces after 14 days of *in vitro* contact with AF explants. Moreover, phalloïdin and YOYO-1 staining, which allow visualization of actin filaments and nuclei, respectively, showed that AF cells on the mat surface aligned themselves differently according to the orientation of the underlying fibers. Ovine AF cells on oriented PLC mats adopted an elongated morphology and aligned in a parallel manner to the underlying scaffold while a disorganized actin filament organization was found for cells on non-oriented mats. Immunofluorescent staining showed a typical fibroblast-like cell phenotype and a deposition of extracellular aligned type I collagen fibers on the surface of oriented PCL biomaterials.
Conclusions: In this study, we confirmed the feasibility of using the electrospinning technology to synthesize oriented and non-oriented fibrous scaffold with similar fiber diameters. We validated the spontaneous colonization of mat surfaces by ovine annular cells, suggesting that *in vivo* cells from surrounding tissue could colonize the implant. Moreover, oriented fibers promoted the alignment of cells and the deposition of an aligned ECM, suggesting that this type of mats could promote the synthesis of a highly organized repair tissue. The potential application of both biomaterials will be further investigated *in vivo* by implantation in an ovine model of surgically induced AF defect.

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AN OPEN LABEL STUDY TO EVALUATE SAFETY AND EFFICACY OF A NOVEL HYDROGEL (HYALODISC) IN PATIENTS WITH DEGENERATIVE DISC DISEASE

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Purpose: Degenerative Disc Disease (DDD) is a complex pathological process including narrowing of the disc space due to the decreasing amount of water contained in intervertebral discs. This study was planned to investigate the effectiveness of a HYALODISC, an hydrogel based on hyaluronic acid (HA) amide derivative, in reducing low back pain due to disc degeneration.
Methods: Twenty-three patients with chronic low back pain (LBP) for at least 3 months, presenting up to three lumbar black discs (Pfirrmann grade III or IV), LBP of at least 40 mm on Visual Analogue Scale (VAS) and a Roland-Morris Disability Questionnaire (RMDQ) score of at least 9 (mean age 42.9 years [min.33-max.58; 36.4% female; mean BMI 23.6 kg/m^2]) were enrolled in this multi-centre, open-label, 6-month follow-up, clinical study. At inclusion, mean VAS at baseline was 67.1. Patients received a single dose (1, 5 mg) of intradiscal injection of HYALODISC, supplied in a pre-filled syringe. The primary efficacy variable of the study was the change from baseline in pain measured by VAS at week 4, 12 and 24. Secondary objectives of the study were to evaluate: the efficacy of one single guided intradiscal injection of HYALODISC on: Black disc hydration, evaluated as reduction from baseline of at least one Pfirrmann grade by MRI; Patient's response to therapy according to (RMDQ); Quality of life assessed by changes from baseline in the Euro-Qol-5 Dimension (EQ-5D) Index; Patient's and investigator's global assessment of patient's health status, safety and local tolerability.
Results: The mean and median VAS for pain markedly decreased from baseline to any post baseline time point. The decrease was statistically significant at any time point and in the overall study period ($P < 0.0001$ at any time. Regarding secondary endpoints: the reduction from baseline of at least one Pfirrmann grade by MRI was statistically significant at both Week 4 ($P = 0.0039$) and Week 24 ($P = 0.0010$); The decrease of RMDQ score from baseline was statistically significant at any time point and in the overall study period ($P < 0.0001$ at any time); the increase from baseline to Week 24 of EQ-5D index was statistically significant ($P = 0.0001$); the mean VAS values for PGTA and COGA markedly decreased from baseline to any post baseline time point ($P < 0.0001$ at any time, except for $P = 0.0017$ at Week 4; and $P < 0.0001$ at any time, except for $P = 0.0002$ at Week 4) respectively. Finally, the intradiscal injections of HYALODISC were safe and well tolerated in terms of adverse events occurred during the study.
Conclusions: The study confirmed that HYALODISC intradiscal injection is a well-tolerated treatment, associated with a marked decrease of low back pain intensity from baseline to any post baseline time point (Week 4, 12 and 24). Consistently with positive primary efficacy outcome, all secondary endpoints had a positive result with a statistically significant

improvement from baseline. Positive results of one single guided intradiscal injection of HYALODISC on black disc hydration, evaluated by MRI could be related to the well-known HA properties in recalling water. The rehydration of the disc could explain the low back pain relief after HYALODISC injection. These study findings must be confirmed by a larger randomized controlled trial.

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DEVELOPMENT OF CONTROLLED RELEASE SYSTEMS OF BIOLOGICAL FACTORS FOR THE REGENERATION OF INTERVERTEBRAL DISC

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Purpose: The degeneration of intervertebral disc (IVD), and notably of its central part the nucleus pulposus (NP), is responsible for 40% of chronic low back pain (LBP). Recent studies have reported the existence of endogenous regenerative cells in the IVD vicinity and within the IVD. These endogenous cells have been characterized as mesenchymal stem cell-like progenitors residing in specific niches. In response to chemokines (stromal derived factor-1 (SDF-1)) these cells can be recruited and migrate to the site of injury and thus contribute to the endogenous repair process. In this context, the development of microbeads-based local delivery systems of factors involved in progenitors recruitment has recently been contemplated for IVD regenerative medicine. Because of their physicochemical and biological properties, microbeads of pullulans (PMBs) have long been investigated as drug carriers. In addition, we recently demonstrated that transforming growth factor-β1 (TGF-β1) and growth differentiation factor5 (GDF5) are potent stimulators of the differentiation of mesenchymal stem cells (MSC) into NP-like cells. In this context, the aim of this work was to develop an intradiscal pullulan microbeads-based delivery system for the controlled release of SDF-1, TGF-β1 and GDF5. This drug delivery system would be able to sequentially contribute to 1) the recruitment and mobilization of resident progenitors, 2) the differentiation of the mobilized progenitors and 3) the subsequent regeneration of NP.
Methods: Chemotaxis assays were performed to determine the *in vitro* cell migration. Human MSCs (1250 cells/μl) were incubated with or without SDF-1 (250 ng/ml) in Transwells for 4h, migratory cells were stained by crystal violet then quantified by spectrophotometry. In parallel, PMBs were prepared by a simultaneous crosslinking protocol coupled to a water-in-oil emulsification process. Freeze-dried PMBs were incubated with GDF-5 and TGF-β1 separately (25 mg of PMBs at final concentration of 1, 2 and 4 μg/mL and in a final volume of 500 μL of PBS) for 24 h at 4°C under rotary stirring at 24 rpm. GDF-5 and TGF-β1 release assays were performed in PBS at 37°C for 21 days and concentrations were measured by ELISA.
Results: SDF-1 has improved the *in vitro* migration of hMSCs, increasing by more than twice the number of migratory cells. GDF-5 and TGF-β1 were successfully adsorbed on PMBs with a 100% efficiency. Release experiments showed a burst release within the 1st h, at 604 ng/h and 50 ng/h for GDF-5 and TGF-β1 respectively, then the release rate decreased during 21 days with 0.6 ng/h and 0.15 ng/h during the last 7 days for GDF-5 and TGF-β1, respectively. At day 21, GDF-5 was entirely released, whereas only 40% of TGF-β1 was released. This different release profiles could be explained by the difference of molecular weight (13 kDa for GDF-5 and 25 kDa for TGF-β1).
Conclusions: We have confirmed that SDF-1 improved hMSCs *in vitro* migration, and that PMBs are suitable microcarriers for the loading and release of GDF-5 and TGFβ-1. The loading and release capability of SDF-1 by PMBs, as well as SDF-1 bioactivity after release will be analyzed, to obtain a fast and massive recruitment of resident progenitors *in vivo*. Then, we will study the action of GDF-5 and TGFβ1 released from PMBs on in vitro NP differentiation, by using a 3D matrix model to mimic the NP microenvironment. Nucleopulpogenic differentiation will be evaluated by analysis of specific extracellular matrix production and gene expression markers.

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ACUPOTOMY THERAPY FOR JOINT PAIN RELIEF OF LUMBAR DISC HERNIATION-SYSTEMATIC REVIEW AND META-ANALYSIS

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Purpose: Lumbar disc herniation (LDH) is a major public health problem and a serious affect on work and life. Few effective medical treatments for the disease currently exist. Acupotomy therapy as an acceptful minimally-invasive surgical treatment has been used for LDH pain. It combines tradition Chinese medicine and micro-surgery that separates neurolemma and muscle of patients' back, resulting in pain relief. A comprehensive literature review is a key link to understand its benefits and for guiding treatment for LDH pain relief. We conducted a systematic review and meta-analysis to evaluate the efficacy of acupotomy for pain associated with LDH.
Methods: We performed a search on Cochrane Library, PubMed, EMBASE, 3 universal Chinese databases (CNKI, Wan Fang and VIP), and reference lists of published articles through Oct 2017. We include randomized controlled trials using acupotomy therapy for LDH patients who met the LDH of State Administration of Traditional Chinese Medicine (TCM diasease diagnosis and efficacy standards). The effect of acupotomy on pain relief was measured with Visual Analogue Scale (VAS) and Japanese Orthopaedic Association (JOA). Study quality was evaluated with Jadad criteria assessing randomization, single blinding, and dropout rates for each study. The differences between treatment groups were reported as mean change (*P*-value).
Results: After screening 513 abstracts, 9 studies met eligibility criteria and were conducted between 2008 and 2017. A total of 1,118 LDH patients (39% female, mean age = 48years, mean symptom duration = 48 months) were included. Table 1 summarizes the trials evaluating the effect of acupotomy therapy on pain relief of LDH. The typical treatment was once a week, for 1–4 weeks. Nine studies used all acupuncture .The overall quality of trials was modest (mean Jadad score = 2.5). All studies reported an effect of acupotomy on pain relief compared to controls. Figure 1 shows a meta-analysis comparing effects of acupotomy therapy with controls on pain relief. A meta-analysis comparing acupotomy with other controls is not reported due to variation in outcomes assessed. *Adverse events were not reported.*
Conclusions: Acupotomy treatment may improve pain which is connected with LDH. Further rigorously designed and well-controlled RCTs with long-term follow-up are warranted.

Table 1
Nine RCTs of acupotomy therapy on Lumbar disc herniation

Author Year	N[a](Age)[b]	Acupotomy therapy	Controls	Duration - (weeks)	Pain Mean Difference[c] (P-value)-[1]
Peng 2014	200 (ND)	Release soft tissue adhesion (once/5day;3times)	Acupuncture therapy (30 min,1time/1day, 7days)	2	VAS[d] score: ↓0.76 (*P < 0.01*) JOA score: ↑4.24 (P < 0.01)
Meng 2012	120(48y)	Release soft tissueadhesion (once/wk;4times)	Acupuncture therapy (30 min,3times/1wk,4wks)	4	JOA[e] score: ↑0.6 (*P < 0.01*)
Liu 2015	196 (46y)	Release soft tissue adhesion (once/3days;5times)	Acupuncture therapy (30 min, 1time/1day,15days)	2	VAS score: ↓1.2 (*P < 0.01*)
Zhang 2013	90 (42y)	Release soft tissue adhesion (once/3-5days;2wks)	Acupuncture therapy (30 min,1time/1day,2wks)	2	VAS score: ↓0.76 (*P < 0.05*)
Shi 2008	120 (48y)	Release soft tissue adhesion (once/5-7days;6times)	Acupuncture therapy (30 min, 1times/1day, 4wks)	4	VAS score: ↓1.33 (*P < 0.01*)
Wu 2012	100 (57y)	Release soft tissue adhesion (once/wk,3times)	Acupuncture therapy (30 min, 1times/1day, 3wks)	3	VAS score: ↓1.82 (*P < 0.05*)
Li 2017	130 (ND)	Release soft tissue adhesion (once/wk;4times)	Acupuncture therapy (30 min, 1times/1day, 4wks)	4	JOA score: ↑2.02 (*P < 0.01*)
Hao 2015	80 (ND)	Release tissue under points of pain (once/wk,4times)	Acupuncture therapy (30 min, 1times/1wk, 4wks)	4	VAS score: ↓1.72 (*P < 0.05*)
Wang 2013	82 (53y)	Release soft tissue adhesion (once/3days;5times)	Acupuncture therapy (30 min, 1times/1day, 15days)	2	JOA score: ↑9.72 (*P < 0.01*)

[a] N= number of patients included;[b] Mean age reported in years; [c]Mean difference was calculated between group comparisons; [d]VAS; Visual Analogue Scale (range 0-10, lower score = better outcome);[e] JOA: Japanese Orthopedic Association (higher score = better outcome).