viability of cells present in surgical samples of degenerate intervertebral disc tissue.
Methods: The technique for live/dead differentiation of cells used in this study utilises the fluorescent probes chloromethyl fluorescein diacetate (CMFDA) and ethidium homodimer (EH). Samples of herniated discs were obtained from patients undergoing routine discectomy and processed within 2 hours of excision. A small piece of each sample, approximately 5 mm^2, was incubated in 1 ml of 25 μM CMFDA/2mM EH solution in the dark for 2 hours at 37°C. The tissue was then blotted dry and snap frozen. Cryosections were taken at various points throughout the thickness of the tissue sample, mounted with Vectashield containing DAPI and visualised with a fluorescent microscope. Cell viability was analysed by counting at least 200 cells from a minimum of five fields of view.
Results: To date 21 surgical disc samples from patients, aged 17–57 years, have been examined. Preliminary results show that greater than 80% of cells are alive after excision. Cells in these discs commonly occur in clusters (approximately 54% of disc cells are clustered). Clustered cells exhibited a higher proportion of live cells compared to single cells within the same tissue section, e.g. 93% of clustered cells were viable compared to 82% of single cells. Cell viability appeared consistent across all locations of the disc samples. Dead cells, when they did occur, were predominantly seen around the periphery of the disc sections which may be derived from trauma during dissection.
Conclusions: In contrast to reports in the literature, these results indicate that the viability of cells from the herniated disc may be adequate to consider for utilising in biological therapies. Interestingly, a greater proportion of clustered cells appear to be viable compared to single cells. Additionally we plan to investigate whether there is any relationship between cell viability and the pathology, duration of symptoms or age of patient, as this may serve as an indicator of the suitability of degenerate discs for cell therapy.

464
INTRADISCAL INJECTIONS OF ORTHOKINE-DERIVED AUTOLOGOUS CONDITIONED SERUM (ACS) FOR LUMBAR DISC DEGENERATION

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Purpose: Biology offers several strategies for restoring the degenerating disc, including the use of natural proteins that increase matrix accumulation and assembly, enhance the number of disc cells, or in other ways lead to restoration of the native healthy disc. This is the basis for administering Orthokine-derived Autologous Conditioned Serum (ACS) a new treatment method that not only supplies with a pharmaceutical monosubstance but with nutrients, anti-inflammatory cytokines and several growth factors.
When peripheral blood is withdrawn and incubated with etched glass beads, leukocytes within the aspirate enrich the plasma with anti-inflammatory cytokines, such as IL-1Ra, as well as growth factors, including PDGF, FGF-2, TGFβ, IGF and HGF. After centrifuging and filtering, the ACS is returned locally to the affected region. It has been used successfully, by way of local injection, for the treatment of human and equine osteoarthritis, after ACL-reconstructive surgery and radiculopathy (see references).
Methods: A non-blinded, prospective study was conducted to evaluate feasibility and efficacy of ACS injections in patients suffering from lumbar disc pain (verified by anamnesis and clinics, MRI scan and distension tests, no relieve after 8 weeks of well conducted conservative treatment, pain reduction less than 50% VAS, after periradicular infiltration procedures). 19 patients had a discography and three intradiscal injections of ACS once per week for three consecutive weeks and were followed for six months. Outcome was measured by patient administered outcome instruments (VAS, ODI).
Results: Patients with showed a clinically remarkable and significant reduction in pain and disability after the ACS injection series. Mean improvement was 58% in VAS. 11 out of 19 patients reported at least 50% pain improvement. No serious side effects occurred. There were no infections in this series.
Conclusions: Although, these results must be confirmed in larger clinical trails and with objective assessments like quantitative MRI scans, the use of ACS in the intervertebral disc could be worthy of consideration given its impressive safety record and rich mixture of growth factors, cytokine antagonists, and, possibly, additional helpful agents.

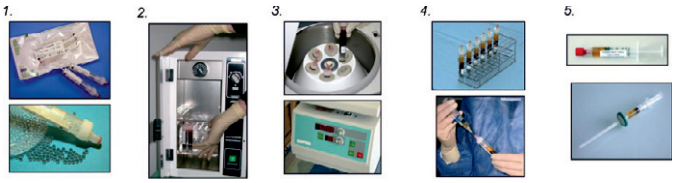

Fig. 1. ACS Production **1.1**–**1.5.** For the ACS preparation whole blood is drawn using special syringes (Orthokine EOT Syringe, Orthogen, Duesseldorf, Germany) with increased inner surface area (1). While the blood is incubated for six to nine hours (*2*), peripheral blood leukocytes reproducibly produce elevated amounts of endogenous anti-inflammatory cytokines. Following centrifugation and extraction (*3+4*), autologous conditioned cell free serum (ACS) is then portioned (5) and either stored until needed at minus 18° Celsius or directly injected into the affected disc, joint or spinal region of the patient.

Table 1. Cytokines and growth factors present in autologous conditioned serum (ACS) produced using the 10 ml Orthokine system.

	Mean
IL-1Ra	2015 pg
IL-6	28.7 pg
IL-10	33.4 pg
FGF β	27 pg
VEGF	509 pg
HGF	1,339 pg
IGF1	117,209 pg
PDGG AB	39,026 ng
TFG β	97,939 ng

Abbr.: IL-1Ra = interleukin-1 receptor antagonist; IL-6 = interleukin-6; IL-10 = interleukin-10; FGFβ = fibroblast growth factor-β; VEGF = vascular endothelial growth factor, HGF = hepatocyte growth factor; IGF1= insulin-likegrowth-factor-1; PDGF = platelet derived growth factor, TGF-β = transforming growth-factor-β. There is no noticeable induction of the proinflammatory IL-1β or TNF-α. Measurements from ELISA kits (R&D Systems).

Stem Cells, Tissue Engineering & Repair

465
MESENCHYMAL STEM CELLS IN TYPE I COLLAGEN VEHICLES AS AN ADDITIONAL TREATMENT FOR ROTATOR CUFF TEARS: A RAT EXPERIMENTAL MODEL

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Introduction: Supraspinatus tendon (SE) is an essential structure for the proper function of rotator cuff and it is the most frequently tendon affected in the case of rotator cuff tears. Common surgical procedures to repair SE lesions are often unsatisfactory due to the inadequate recovery. Regenerative medicine is one of the most promising techniques to improve these treatments by taking advantage of the regenerative potential of mesenchymal stem cells (MSCs).
The aim of this study was to evaluate, in an experimental rat model, the effectiveness of recovery after surgical treatment using MSCs embedded in collagen type I vehicles.
Methodology: A chronic rotator cuff tear model was developed detaching at the insertion site the SE of adult Sprague-Dawley rats (9 months old), n = 4.
One month post-injury, the tendon was sutured with a type I collagen vehicle with or without MSCs. One million cells, previously obtained from the femoral channel of age matched rats were embedded in type I collagen gel (n = 4) or attached to type I collagen membranes (n = 4). In parallel, a control group was treated only with the vehicle type I collagen gel (n = 4) or with type I collagen membrane (n = 4), free of cells.
Recovery was evaluated for each specimen three months after injury determining the biomechanical strength (Newtons, N) of the tendon-humerus interface (Instron). The contralateral shoulder was used as a