Tissue and Cell 46 (2014) 505–513

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Augmentation and repair tissue formation of the nucleus pulposus after partial nucleotomy in a rabbit model

M. Endres[a,b], M.L. Zenclussen[c], P.A. Casalis[c], U. Freymann[a], S. Gil Garcia[a], J.P. Krueger[a,b], U.-W. Thomale[d], C. Woiciechowsky[c,e], C. Kaps[a,*]

[a] TransTissue Technologies GmbH, Berlin, Germany
[b] Charité-Universitätsmedizin Berlin, Department of Rheumatology, Tissue Engineering Laboratory, Berlin, Germany
[c] Charité-Universitätsmedizin Berlin, Department of Neurosurgery, Berlin, Germany
[d] Charité-Universitätsmedizin Berlin, Department of Pediatric Neurosurgery, Berlin, Germany
[e] Spine Centre Berlin, Germany

ARTICLE INFO

Article history:
Received 21 July 2014
Received in revised form
10 September 2014
Accepted 10 September 2014
Available online 19 September 2014

Keywords:
Intervertebral disc degeneration
Polyglycolic acid
Hyaluronan
Cell-free implant
Rabbit model

ABSTRACT

Disc degeneration alters disc height and mechanics of the spinal column and is associated with lower back pain. In preclinical studies gel-like materials or resorbable polymer-based implants are frequently used to rebuild the nucleus pulposus, aiming at tissue regeneration and restoration of tissue function. To compare the outcome of tissue repair, freeze-dried resorbable polyglycolic acid–hyaluronan (PGA/HA) implants without any bioactive components or bioactivated fibrin (fibrin-serum) was used in a degenerated disc disease model in New Zealand white rabbits. Animals with partial nucleotomy only served as controls. The T2-weighted/fat suppression sequence signal intensity in the nuclear region of operated discs as assessed by magnet resonance imaging was reduced in operated compared to healthy discs, indicating loss of water and did not change from week 1 to month 6 after surgery. Quantification of histological and immunohistochemical staining indicated that the implantation of PGA/HA leads to significantly more repair tissue compared to nucleotomy only. Type II collagen content of the repair tissue formed after PGA/HA or fibrin-serum treatment is significantly increased compared to controls with nucleotomy only. The data indicate that intervertebral disc augmentation after nucleotomy has a positive effect on repair tissue formation and type II collagen deposition as shown in the rabbit model.

1. Introduction

Back pain, a major public health problem, is strongly associated with degeneration of the intervertebral disc (Luoma et al., 2000). Disc degeneration alters disc height and the mechanics of the rest of the spinal column, which can lead in the long term to spinal stenosis (Urban and Roberts, 2003). As the current clinical approaches are limited to treat symptoms but not the biologic alterations of the disc, new strategies using biomaterials to induce intervertebral disc regeneration are being developed.

The use of biomaterials provides the opportunity to restore structure and function of the degenerated intervertebral disc, which is often associated with back pain (Luoma et al., 2000). Many experimental approaches focus on the use of cells embedded in scaffolds. In these approaches, the cells of the degenerated disc are enriched by adding cells alone or embedded in an adequate scaffold (Urban and Roberts, 2003). Some of these approaches have been shown to be successful in animal models, such as the use of autologous cultured disc-derived chondrocytes in a canine model (Ganey et al., 2003) or the use of annulus fibrosus cells in an atelocollagen scaffold (Sato et al., 2003). Even though some preliminary clinical studies using this methodology show encouraging outcomes (Meisel et al., 2006), there are still some drawbacks. Most cell-based therapies are two-step procedures where in a first step a tissue biopsy is taken and in a second step harvested and expanded cells derived from the biopsy are transplanted into the defect. These two interventions can be stressful for the patient, expensive and time-consuming, which is seen as a considerable clinical disadvantage of cell-based therapies in nucleus pulposus regeneration (Endres et al., 2010; Omlor et al., 2012). Furthermore, removal of nucleus pulposus tissue as cell source might accelerate degeneration of the host tissue (Fassett et al., 2009). Therefore, attempts are being made to make biopsies as a cell source redundant by focussing on

* Corresponding author at: TransTissue Technologies GmbH, Charitéplatz 1, 10117 Berlin, Germany. Tel.: +49 030 91540375; fax: +49 030 450 513 943.
E-mail address: christian.kaps@transtissue.com (C. Kaps).

http://dx.doi.org/10.1016/j.tice.2014.09.003
0040-8166/

techniques for cell migration and recruitment from adjacent tissues (Abbushi et al., 2008).

Currently, the use of different matrices and injectable carriers based on gels is in the focus of developments for either cell-based and cell-free nucleus substitutes which support the maintenance of disc height and support cell migration and tissue regeneration (Henriksson et al., 2011). It has been shown that the use of appropriate gel materials has the potential to suppress undesirable angiogenesis in vitro (Scholz et al., 2010). In general, major disadvantages of gels are their fluid-like behaviour with no initial stability and the potential risk of extrusion when injected into the nucleus compartment after nucleotomy (Omlor et al., 2012).

The use of a biocompatible cell-free polyglycolic acid/hyaluronan (PGA/HA) scaffold with its elastic properties might solve the problems of extrusion and handling. Therefore our aim was to compare a typical, clinically approved gel bioactivated with allogenic serum (fibrin-serum) with a PGA/HA implant without further bioactivation with serum in a regenerative approach with regard to quantity of repair tissue formation in an existing rabbit discectomy model (Abbushi et al., 2008; Endres et al., 2010).

2. Materials and methods

2.1. Preparation of cell-free disc implants

Resorbable pure polyglycolic acid (PGA) scaffolds (BioTissue AG, Switzerland) of 10 mm × 10 mm × 1.1 mm were loaded with 10 mg/ml hyaluronic acid (HA) (Ostenil, TRB Chemedica AG, Germany) as described previously (Abbushi et al., 2008; Endres et al., 2010). The implants were freeze-dried for 16 h under sterile conditions using a lyophilisator (Leybold-Heraeus, Germany) and stored in a desiccator at room temperature. Prior to implantation, cell-free implants were cut in 5 mm × 5 mm pieces and allowed to re-hydrate in physiological saline.

2.2. Surgery and MRI evaluation

Animal care and experimental procedures were followed according to institutional guidelines and conformed to the requirements of the state authority for animal research conduct (LaGeSo No. 0071/06, Berlin). In all 13 rabbits, partial nucleotomy with removal of approx. 50% of the nucleus pulposus was performed on L6/L7 or L7/S1 level using a retroperitoneal approach. Animals were divided into three groups. In one group of animals ($n = 5$), cell-free PGA/HA scaffolds rehydrated in physiological saline were implanted into the disc defect. In the second group ($n = 5$) animals were treated with 0.5 ml fibrin (Tissucol Duo S; Baxter, Germany) mixed with 10% allogeneic rabbit serum (Kraeber &Co GmbH, Germany). For this purpose, allogeneic rabbit serum was added to the fibrinogen component resulting in a final concentration of 10% (v/v) and filled into a syringe. Fibrinogen/serum and thrombin was applied to the application system provided together with the fibrin sealant components. In the third group ($n = 3$) animals with partial nucleotomy only served as controls (named sham operated group). To reduce a selection bias and to diminish possible effects from a learning curve, implant surgeries and controls were performed alternately.

One week and 6 months after surgery, the T2-weighted/fat suppression sequence signal intensity index was determined by two independent investigators using magnetic resonance imaging (MRI). MR images were taken using a 1.5 T imager (GE Twin Speed 1.ST, General Electric, Milwaukee, USA). The rabbits were positioned in prone position on a quadrature surface coil, and sagittal T2 weighted/fat suppression sequence spin echo images parallel to the lumbar spine were obtained. The signal intensity levels of

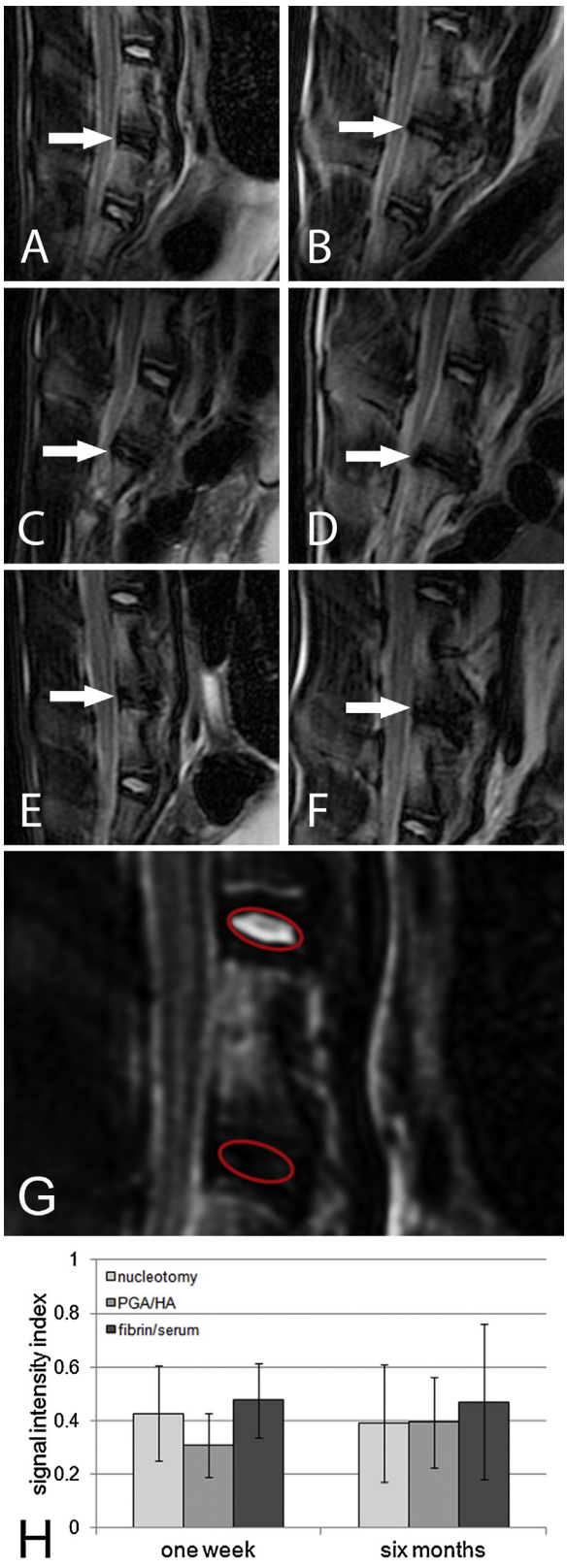

Fig. 1. Magnetic resonance images of the lumbar spine of rabbits with operated disc (white arrow) 1 week (A, C, E) and 6 months (B, D, F) after surgery. After partial nucleotomy either the PGA/HA implant (A, B) or fibrin-serum (C, D) was implanted. The sham operated group received only partial nucleotomy (E, F). Evaluation of signal intensity was performed in a defined region of interest in the centre of the disc (G, red oval). Signal intensity index revealed no significant differences between the groups (H). The mean is given and error bars represent the standard deviation (SD). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)