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Significance of the mechanical environment during regeneration of the intervertebral disc

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Abstract The prevalence of low back pain is high, and the intervertebral (IV) disc is regarded as one of the major causes. Various approaches have been reported to either slow down disc degeneration or to repair/regenerate the disc. So far, the effect of the mechanical environment has not been addressed in these approaches, although several investigations have shown its influence on other mesenchymal tissues. In this paper, we propose that the biophysical stimuli from the mechanical environment can directly influence cell type, as well as their metabolic activity during repair/regeneration of the IV disc. To demonstrate the potential of this idea, data from the literature, as well as explorative experimental results, are presented.

Keywords Intervertebral disc · Regeneration · Mechanobiology · Tissue differentiation

Background

Low back pain is a prevalent problem in developed countries, and disc degeneration is commonly regarded as an underlying cause [20]. Currently, spinal fusion is the ultimate treatment modality after conservative therapies have failed. Although many patients become pain free, fusion increases the demand on adjacent spinal motion segments [18] with possible further degeneration and failure [6, 27]. Hence, alternative treatments are being developed and investigated [16]. Intervertebral disc prostheses of various designs have been developed [29] and some have been tested clinically. However, the optimal design has yet to be found. Furthermore, as low back pain is becoming more prevalent in young patients [30], the life span required of an artificial disc may be a limiting factor. Biological solutions with good long-term viability are thus appealing. Two concepts have been proposed, either slowing down disc degeneration or repair/regeneration of the disc. In both concepts, most investigations to date have focused on controlling cellular anabolic or catabolic activities by introducing molecules [31] and genes [22] in both laboratory and functional tissue engineering approaches [1]. More recently, cell-based therapies with both mature and immature cells [26] have been investigated. However, most of the work has concentrated on combining the cells with a carrier matrix and bioactive molecules. So far, the mechano-biological effect in these approaches has not been addressed. We propose that the biophysical stimuli from the mechanical environment can directly stimulate regeneration of the IVD by stimulating proliferation of cells, as well as their phenotype and metabolic activity.

Data from literature

In other mesenchymal tissues, such as cartilage, the effect of the mechanical environment on the biological response of chondrocytes is well documented. It has been demonstrated in vitro that static loads decrease matrix synthesis rates in a dose-dependent manner [7, 14], whereas dynamic cyclic loads of 0.01–1 Hz increase protein and proteoglycan (PG) synthesis rates [25]. On IV cells, hydrostatic pressure has both stimulatory and inhibitory effects on PG synthesis depending on the load magnitude [8, 10, 12]. Static loads in vitro increased expression of catabolic enzymes and decreased PG production [8, 12] and in vivo application of such static load have led to degeneration of the disc [11, 19]. In contrast, dynamic cyclic load of higher frequency and amplitude have been demonstrated to up-regulate the production of collagen [13]. Furthermore, these mechano-regulatory effects have not only been demonstrated in the activity of the cell to model its extracellular matrix, but have also been reported to effect cell and tissue differentiation. When cyclic hydrostatic pressure of 5 MPa at 1 Hz was applied to bone marrow stems cells in the presence of TGFβ, the matrix to cell ratio increased as well as the concentration of PGs and collagens in comparison to cells cultured in TGFβ alone [2]. It even appears that the mechano-regulation of cell differentiation is so influential that it may influence cell phenotypes in the absence of specific conditioning media. For example, cyclic hydrostatic pressure of 10 MPa at 1 Hz alone up-regulated the expression of SOX9, aggrecan and collagen type II by 300%, 240% and 470%, respectively in bone marrow stem cells [21].

Nevertheless, what is the underlying concept behind these observations? In 1960, Pauwels already tried to define some basic regulation rules. He proposed that deviatoric stress stimulates the formation of fibrous connective tissue, whereas hydrostatic compressive stress stimulates cartilage, and that both type of tissues would eventually go on to ossify once the soft tissue had stabilized the mechanical environment enough to reduce the amplitude of these two stimuli [23]. This hypothesis was further developed by Carter and his colleagues where they demonstrated using finite element analysis of healing fractures that high compressive hydrostatic stress was correlated with chondrogenesis while low hydrostatic stress was correlated with osteogenesis and that high strains were correlated with fibrous tissue formation [3, 4]. More recently, Prendergast et al [9, 24] suggested that it was not so much the hydrostatic pressure but rather the combined magnitude of interstitial fluid flow within the connective tissues and the deformational strains which regulated the cell phenotype. This algorithm was incorporated into numerical models which accurately simulated tissue differentiation at implant/bone interfaces [24] as well as tissue differentiation during fracture healing [17]. Applied to cell-based therapies for disc repair and regeneration, we believe that tissue differentiation within the IV disc should also be governed by similar mechano-regulation rules.

Explorative experimental data

The rat-tail disc model of Iatridis [11] and Stokes [28] was modified and used in skeletally mature Wistar rats. An Ilizarov type fixator with carbon-fiber composite rings and Kirschner wires were used to span the caudal 7–8 disc (C7–8). After fixation, the nucleus was aspirated with a needle (nucleotomy) and its volume controlled visually. Through the incision created by the syringe, both endplates were perforated using a micro burr until bony chips were visible. After bleeding into the nuclear space from the endplates was confirmed, the skin incision was closed with sutures. For the 8 weeks observation period, all rats were allowed unrestricted activity. They were divided into two groups, and starting the day after surgery, either compressive IV strain was applied or rats were only anesthetized for the same duration. A computer controlled servo-hydraulic actuator (Fig. 1) compressed the C7–8 disc (30% strain w.r.t. disc height on the immediate postoperative radiograph, at 0.1 Hz sinus wave-form, 60 cycles per day). After 8 weeks the animals were sacrificed and the spinal motion segments of C7–8 and C9–10 (internal control) discs were har-

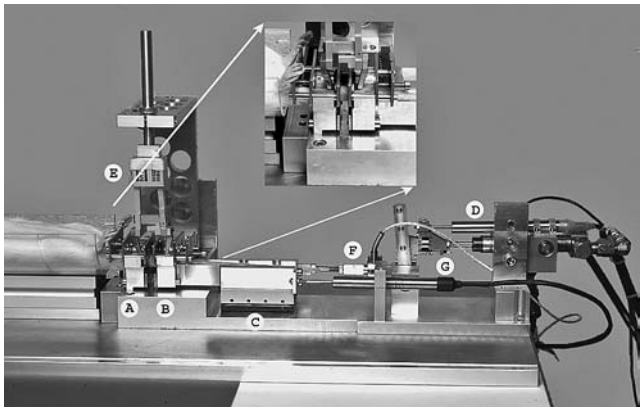

Fig. 1 Application of IV strain across C7–8 rat tail disc. The rat is supine (left) and the proximal fixator ring (**A**) is held stationary. The distal ring (**B**) is mounted to a linear stage (**C**) driven by a micro hydraulic actuator (**D**). The extensometer (**E**) is attached to the K-wires at the wire/skin interface and provides measurement and feedback for the controller. Forces and displacement are measured by a load cell (**F**) and a linear variable displacement transducer (LVDT) (**G**) respectively. Overhead view shows close up of the extensiometer attached to the K-wires at the wire/skin interface

vested and histologically prepared. All procedures were carried out under permission from the Animal Experimentation Commission of the Veterinarian Office of Canton Graubünden, Switzerland.

Although uniform endplate defects were intended, the surgical procedure was quite demanding and two different endplate defects were evident. In some rats there was full perforation through the physes (F = full) and in others, only through the epiphyses (P = partial). Seven rats in the unstimulated group had full defects, whereas only five in the stimulated group had full defects and two partial defects. All intact control discs (level C9–10) appeared normal with characteristic shrunken appearance of nucleus pulposus due to precipitation of the PGs during aldehyde fixation. The inter-lamellar spaces of the inner annulus stained lightly for PGs, whereas the outer annulus stained only for collagen (Fig. 2). In all cases, the haematoma within the IV disc space, which had formed after the nucleotomy and endplate perforation, was replaced by a more mature tissue at the end of the healing period. The specific type of tissue and the synthetic activity (matrix volume) were distinct between the applied IVD displacement and the endplate defect type. In addition, when analyzed within the context of mechano-regulation theories [4, 24], our results are interestingly consistent.

Mechano-biological theories of tissue differentiation all postulate that in the absence of high volumetric stresses or strains, fibrous connective tissue or bone would develop depending on the magnitude of the deviatoric strains. Hence, it would have been expected that bone may have formed resulting in IV fusion in the uncompressed discs as the newly forming tissue should have been protected from high strains by the fixator. However, in the center of the uncompressed discs, where the nucleus had been removed, loose fibrous connective tissue formed. This tissue also had small amounts of a material within the matrix consistent with haemosiderin. The inner and outer annulus was not much different from the intact control discs, without much staining for PGs (Fig. 3). One possible explanation for this is excessive strain. The animals were unrestricted in their activities and the tail muscles and ligaments were left intact. Although stiff carbon fiber rings were used in the external fixator, the vertebral bodies were fixed to the rings by K wires (0.8 mm). This stabilization may have been too compliant allowing activity-induced motions resulting in strain levels exceeding that required to allow formation of bone. Another possibility is that insufficient neo-vascularization within the nuclear space might have inhibited bone formation. Although we did not analyze vascularization or perfusion, the matrix deposits consistent with haemosiderin indicated that the newly formed tissue in this group was not very active and was not well perfused.

In contrast to bone and fibrous connective tissue, development of cartilage is predicted to occur by mechano-regulation theories of tissue differentiation, when the magnitude of volumetric tissue stress or strain is sufficiently high. In all stimulated discs, regardless of endplate perforation type, the inner annulus, positioned

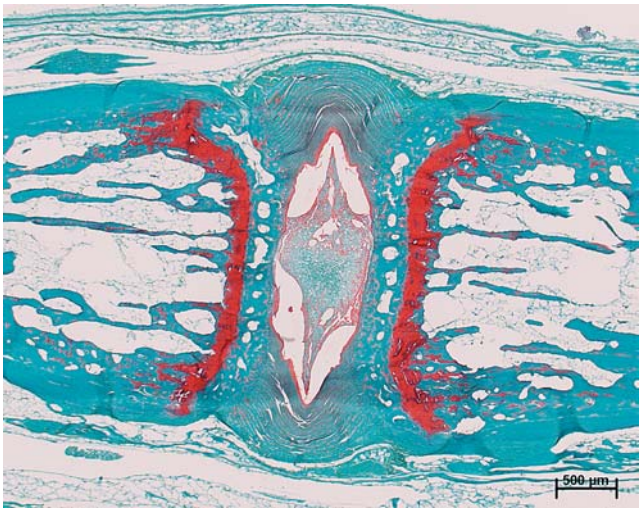

Fig. 2 Mid-longitudinal section of control adjacent rat-tail C9–10 IV disc. The decalcified paraplast embedded 6 µm sections are stained with haematoxylin, fast green and safranin-O (bone and collagen fibers green, PGs–red). The nucleus pulposus was shrunken due to precipitation of the PGs during fixation. The inner and outer annulus fibrosus lacked staining for PGs

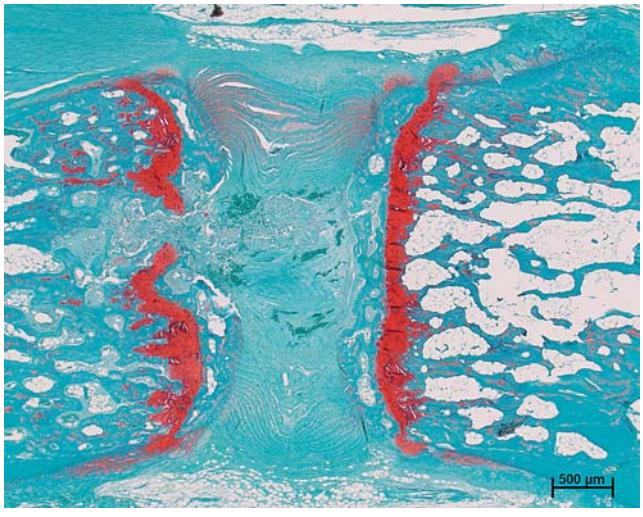

Fig. 3 Mid-longitudinal section of unstrained rat tail C7–8 IV disc. The decalcified paraplast embedded 6 µm sections are stained with haematoxylin, fast green and safranin-O (bone and collagen fibers green, PGs–red). In the center of the disc, connective tissue developed and there were matrix deposits consistent with haemosiderin. The endplate defect is only visible on one vertebral in this section plane. Both endplate defects were not always visible on same section plane

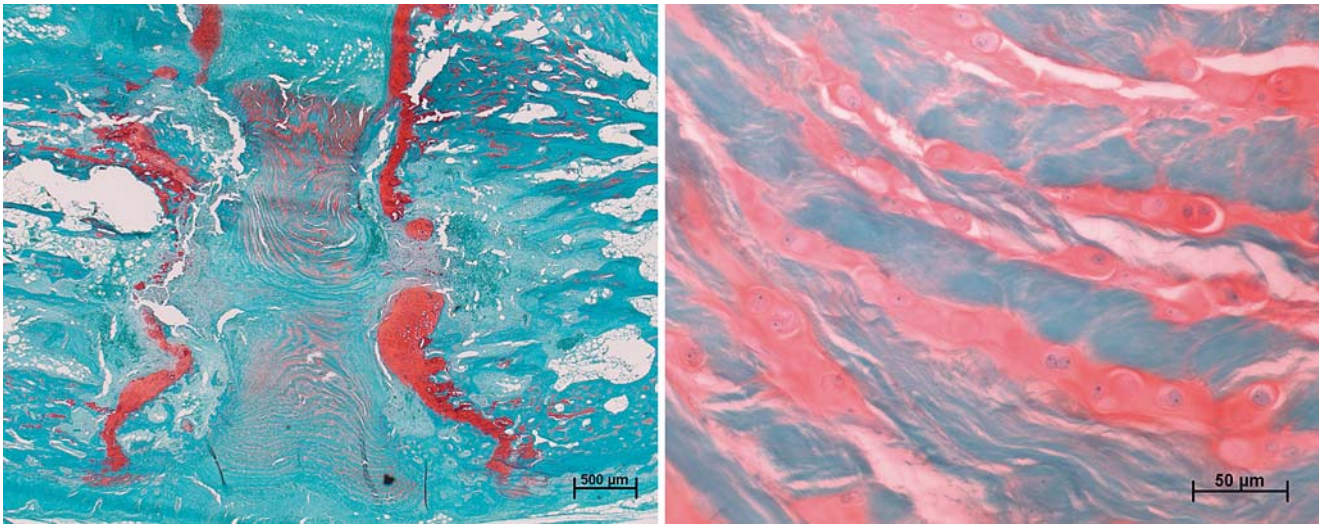

Fig. 4 Mid-longitudinal section of compressed rat tail IV discs with large endplate perforation; overview (i) and close up (ii) of the inner annulus region. The decalcified paraplast embedded 6 µm sections are stained with haematoxylin, fast green and safranin-O (bone and collagen fibers green, PGs–red). The region between the endplate perforations contained fibrous tissue, but the adjacent inner annulus stained intensely for PGs. The cells in the inter-lamellar tissue appeared chondrocytic

between two bony endplates, was compressed daily. Because of the lack of a significantly stiff nuclear tissue, the inter-lamellar tissue probably experienced compressive hydrostatic stresses. Accordingly, the cells in these regions became chondrocytic in appearance and its surrounding matrix increased in volume and became rich in PGs (Figs. 4i, ii, 5) This is also consistent with findings that hydrostatic pressure was found to increase PG synthesis of chondrocytes and tendon fibrocartilage cells in vitro [15, 25]. In those discs with partial endplate perforation, the presence of intact bone within the epiphyses allowed application of compressive volumetric stress, when they were stimulated. The tissue which developed in the center of these discs, appears like cartilage from many morphological perspectives and were so rich in PGs that they also precipitated during histological preparation (Fig. 5).

Under excessive strain levels tissue differentiation is governed towards fibrous tissue according to mechanoregulation rules. In the center of the discs, which were compressed and had full endplate perforations, the tissue was probably extruded out of the endplate perfora-

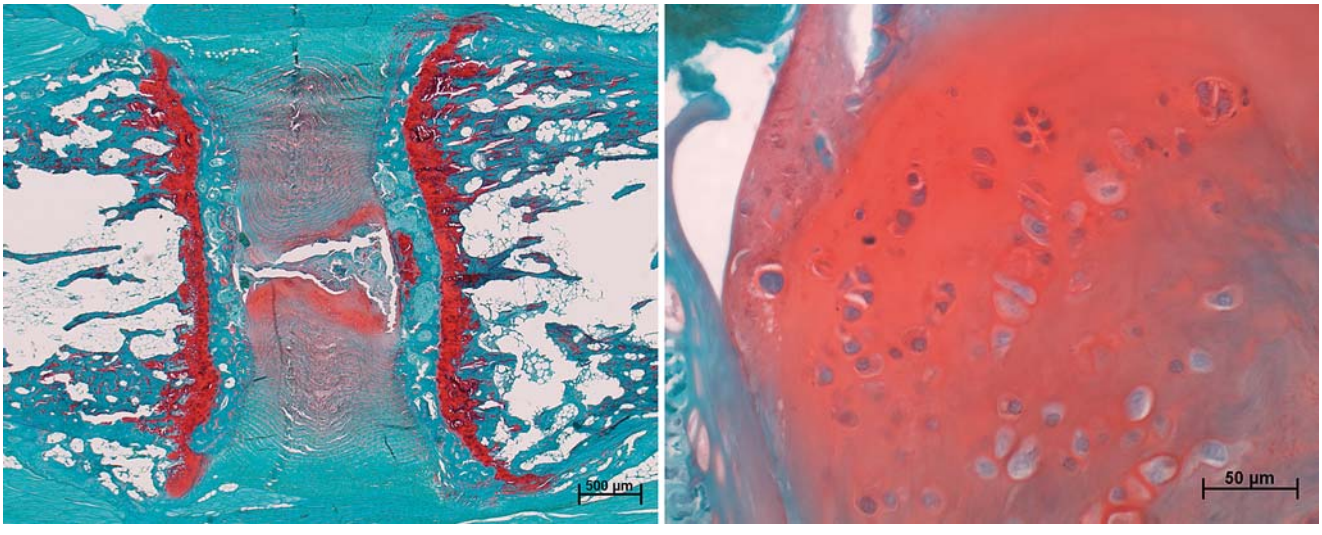

Fig. 5 Mid-longitudinal section of compressed rat tail IV discs with partial endplate perforation; overview (i) and close up (ii) of the center, where the nucleus pulposus had been. The decalcified paraplast embedded 6 µm sections are stained with haematoxylin, fast green and safranin-O (bone and collagen fibers green, PGs–red). Cartilaginous tissue staining for PGs, partially shrunk during histological preparation, formed within the nuclectomy space and the adjacent inner annulus also became well stained for PGs

tion into the vertebral bodies as compressive IVD strains were applied, leading to high tensile strains. The connective tissue which developed was of a more fibrous and denser nature (Fig. 4i) than that of the nuclear region in unstrained nuclectomized discs. As a result of such tensile strains, the collagen fibers became aligned with the longitudinal direction of principal tensile strain as would be expected from collagen network development theories [5].

Discussion

The experimental data should be interpreted to be explorative. The defect, which was intended for migration of precursor cells from the cancellous bone into the nuclear space, affected the mechanical environment depending on perforation type. The rat-tail model is technically challenging, especially the perforation of the endplate. Also, the use of natural precursor cells did not allow discerning the origin of the newly formed tissue. Although the amount of aspirated nuclear tissue was consistent between discs, the regenerated tissue could have developed from small amount of remaining nuclear tissue. However, this would still not account for the robust response of the inner annulus. Finally, the exact mechanical environment within the healing tissue was not measured per se. Its gross nature was simply assumed from the structure of the nuclectomized discs and the applied IVD strain. Further investigations will have to address these aspects, that is, by using tissue engineering methods and exogenous labeled stem cells combined with more in-depth analysis, for example, immunohistochemistry, in situ hybridization or RT-PCR. Due to the restricted number of animals, no quantitative or conclusive results, even with more exhaustively analysis, are possible. Nevertheless, all resulting situations could be correlated to their mechanical environment.

In summary, the results of the explorative experiment combined with data from the literature about other mesenchymal tissues support the potential of our idea that the mechanical environment influences cell type and biological activity of repair tissue within IV disc according to general mechano-regulatory concepts. However, much is still required to understand and prove this concept. It may well be that this effect of the mechanical environment is only minor with respect to other factors or that its greatest effect is synergistic in combination with them. In any case, it broadens our horizon for alternative methods of IV disc regeneration.

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