In Vivo Models for Spinal Disc Regeneration

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Abstract: Intervertebral disc (IVD) degeneration is one of the common health conditions related to pain in lower back, representing one of the major reasons for disabilities from musculoskeletal problems. Spinal disc replacement and regeneration is a recognised approach in clinical approaches, with several commercially available implants. However, better fitted implants and customised solutions are still needed. This paper presents a short review of the most important aspects for appropriate selection and application of different *in vivo* models for intervertebral disc (IVD) degeneration. Techniques for inducing disc degeneration in models are elaborated, including needle puncture models, enzymatic or chemical induction, genetic and age-related models and mechanical loading and instability models. Different assessment and evaluation methods in *in vivo* studies are analysed, such as imaging techniques, histological analysis, biomechanical testing and molecular and cellular assays. Current strategies for disc regeneration that have been tested *in vivo* are presented, including biomaterial scaffolds and hydrogels, cell-based therapies and translational considerations.

Keywords: Intervertebral disc, *In vivo* models, Bioimaging, Organ-on-chip

1. Introduction

Intervertebral disc (IVD) degeneration is a leading contributor to musculoskeletal disability. Spinal disc replacement and regeneration is an established clinical approach, but customised solutions are still needed [1]. *In vivo* models are used to test new implants, designs, and materials [2]. This article reviewed *in vivo* animal models and explored future directions for replacing them with advanced technologies.

2. Intervertebral disc (IVD) degeneration

The intervertebral disc (IVD) is a fibrocartilaginous structure located between the bodies of adjacent vertebrae in the spinal column. These discs play a crucial role in enabling the elasticity of the spine, absorbing mechanical loads, and maintaining the

stability and mobility of the spinal column [1]. Disc degeneration is a cell-mediated process triggered by structural damage and inflammation, leading to matrix breakdown, loss of disc height, increased mobility, and pain due to an imbalance favoring catabolic activity. Regenerative strategies aim to restore disc structure and function by reducing inflammation, preserving endplate integrity, and promoting matrix synthesis through cell therapies, biomaterials, and growth factor-based molecular treatments [1].

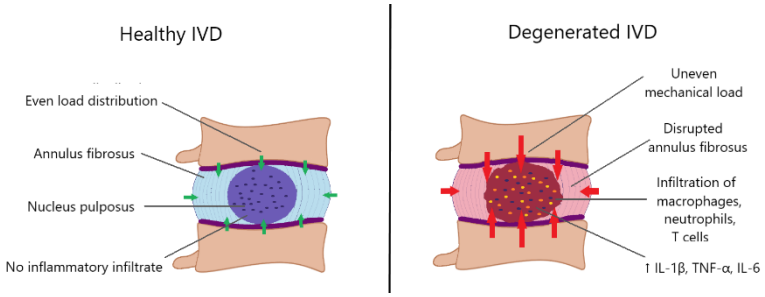

Figure 1. Comparison between healthy and degenerated intervertebral disc (IVD)

A healthy IVD has an intact annulus fibrosus and balanced internal environment, while a degenerated IVD shows disrupted structure, uneven mechanical stress, immune cell infiltration, and elevated pro-inflammatory cytokines, driving inflammation and tissue breakdown, as shown in Figure 1.

3. *In vivo* animal models of spinal disc degeneration

Animal models are essential for studying intervertebral disc degeneration and therapies, with small models offering genetic tools and fast results, while large models better replicate human disc anatomy and biomechanics for testing treatments [2]. Small models are more suitable for basic research due to the rapid development of degeneration and the possibility of genetic manipulation, while large models allow for more reliable translation of results to clinical practice due to anatomical and biomechanical similarities with humans.

4. Techniques for inducing disc degeneration in models

In vivo models of disc degeneration include needle puncture, biochemical, genetic or age-related, and mechanical loading models. The needle puncture model is a simple, reliable method that mimics key disease features by mechanically damaging the annulus fibrosus, but lacks the chronic progression observed in humans [3]. Biochemical models use enzymes like chymopapain and chondroitinase ABC to degrade extracellular matrix components, enabling controlled *in vitro* studies of disc degeneration and therapeutic testing. Genetic and age-related models, such as transgenic mice with type IX collagen mutations, reveal how hereditary factors and aging accelerate disc degeneration, suggesting a link to degenerative spine diseases. *In vivo* mechanical loading and instability models induce intervertebral disc degeneration by altering spinal biomechanics, simulating conditions such as abnormal motion or compressive stress to study disease progression and test therapies.

4.1 Assessment and evaluation methods in in vivo studies

Common methods include imaging, histology, biomechanical testing, and molecular assays, with bioimaging techniques like Magnetic Resonance Imaging (MRI), micro Computed Tomography (micro CT), Positron Emission Tomography (PET), and advanced microscopy enabling real-time, non-invasive monitoring of disc structure and biochemical changes [4]. In histological analysis, degeneration is assessed using four validated histological grading systems. The most common IVD biomechanical tests include compression, torsion, bending, tension, and cyclic loading to assess their mechanical strength, stiffness, and viscoelastic properties. Molecular and cellular assays, such as qRT-PCR, Western blot, immunohistochemistry, and ELISA, are used to analyse gene and protein expression, localization, and quantification in IVD tissues. These tests help reveal biological processes like inflammation, tissue degradation, and regeneration, providing insights into the molecular mechanisms underlying disc health and disease.

5. Current strategies for disc regeneration tested *in vivo*

Current *in vivo* strategies for disc regeneration include biomaterial scaffolds, hydrogels, and cell-based therapies, with translational efforts focused on aligning models with human pathology while addressing challenges, ethical concerns, and regulatory requirements [5]. Scaffolds can be produced in various customised forms, especially considering additive manufacturing possibilities. Hydrogels mimic the extracellular matrix and support IVD regeneration with biocompatible, biodegradable designs, while cell-based therapies using MSCs promote tissue repair mainly through paracrine effects; however, translating these approaches to humans faces challenges due to anatomical differences, unclear disease mechanisms, variable protocols, and ethical and regulatory requirements.

6. Future directions and emerging technologies

Future research on replacing animal models for IVD regeneration focuses on 3D tissue engineering, organoids, and organ-on-chip systems [6], computational and in silico platforms [7]. Advances in tissue engineering and bioprinted artificial scaffolds fully support feasibility of *in vivo* tissue engineering platforms, especially considering emerging *in vivo* bioreactor concepts.

7. Conclusions

This paper outlines key factors in intervertebral disc degeneration and highlights how small and large animal models replicate tissue damage to support the development of effective healing strategies. Biomaterial scaffolds, hydrogels, and cell-based therapies represent advanced methodologies for studying various factors, while simultaneously offering potential for healing and tissue regeneration. Future research aims to replace

animal models with artificial biomaterial platforms that can fully replicate all conditions, including the live organism environment, thereby enabling a complete shift toward human-relevant and ethical alternatives.

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