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Animal Models for Disc Degeneration-an Update

Li Jin, Gary Balian, and Xudong Joshua Li*

Department of Orthopaedic Surgery, University of Virginia, Charlottesville, VA 22908

Abstract

Intervertebral disc degeneration is considered a major cause of back pain that places a heavy burden on society, both because of its effect on the physiology of individuals and its consequences on the world economy. During the past few decades, research findings in the pre-clinical setting have led to a significant increase in the understanding of intervertebral disc degeneration, although many aspects of the disease remain unclear. The goal of this review is to summarize existing animal models for disc degeneration studies and the difficulties that are associated with the use of such models. A firm understanding of the cellular and molecular events that ensue as a result of injuries, as well as environmental factors, could be instrumental in the development of targeted therapies for the treatment of intervertebral disc degeneration.

Keywords

disc degeneration; animal model; intervertebral disc; annulus injury; low back pain

Low back and leg pain is a common musculoskeletal disorder, and affects 70–85% of people at some time in their life (Andersson, 1999; Vos et al., 2012). Intervertebral disc degeneration (IVD) is the main cause of back pain (Adams and Hutton, 1983; Vos et al., 2012). Due to aging of the population, disc degeneration is increasing exponentially (Urban et al., 2003). Despite tremendous efforts in disc research, current treatment strategies are limited and focus on alleviating pain and delaying the time to surgery. The main reason is the complicated nature of the disease: a wide range of etiology, vague definition, unclear pathology, lack of a good animal model, and unclear underlying cellular mechanisms. Furthermore, the anatomical differences between the disc of humans and of laboratory animals further hinder progress in identifying an ideal target for the treatment of disc degeneration. Although animal models help us understand disc biology, disease progression and the quest for therapeutic remedy, the interpretation of information obtained from animal studies should be cautious in extrapolating to humans, as there are many differences between species (detailed information please refer to excellent reviews (Lotz and Ulrich, 2006; Alini et al., 2008; Daly et al., 2016)). The intent of this review is to update knowledge of existing animal models and discuss some of their limitations, but not a metadata analysis.

*Corresponding author: Dr. Xudong Li, Orthopaedic Surgery Laboratory, University of Virginia, Charlottesville, VA 22908, USA, lispine@icloud.com, Tel: 434-982-4135, Fax: 434-922-1691.

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1. Intervertebral disc anatomy

The IVD is composed of a gelatinous nucleus pulposus (NP) in the center, surrounded by the fibrocartilaginous annulus fibrosus (AF), and inferior and superior cartilage endplates (Fig. 1). The NP is highly hydrated and rich in proteoglycans that resist compressive force. The AF is a concentric lamellar structure that resists tensile strain. The endplate functions as a center for the transfer of nutrients and waste through the vasculature of the vertebral bone (Nachemson et al., 1970). Each compartment of the IVD is comprised of its own cells to maintain disc homeostasis: NP cells are chondrocyte-like, present at a low density and interspersed in a proteoglycan-rich matrix, making up 50% of the wet weight, and because of that, 80% of the wet weight of the NP is water (Maroudas et al., 1975). Cells in the AF are fibroblast-like and are aligned parallel to the collagen fibers; while cells in the inner AF are more oval compared to the outer AF. The cartilage of the endplates is a thin layer (<1mm) of hyaline cartilage (Sah et al., 1989).

The general view of a healthy disc is an avascular and aneural structure with few blood vessels, and with some sensory and sympathetic perivascular nerve fibers in the outer lamellae of the annulus (Ashton et al., 1994; Virri et al., 1996; Palmgren et al., 1996)). The small vessels are located in the canals of endplates and in the outer layer of the AF. The aneural nature of the inner disc may be due to the matrix of dense collagen that prevents vessels from passing through the annulus (Gruber et al., 2005).

2. Intervertebral disc degeneration

During aging and early disc degeneration, the most significant change in the disc is loss of proteoglycan leading to diminished hydration. The boundary between NP and AF is blurred. With less proteoglycan, NP becomes more fibrotic with irregular bifurcating and interdigitating lamellae. Clefts and fissures are often found in the discs, especially in the nucleus. Nerve fibers and vessels increase with the degeneration (Urban et al., 2003). Apoptotic and necrotic cells are seen in the inner part of the disc (Gruber et al., 1998). The biochemical activity of catabolic enzymes, including cathepsins and MMPs increases in degenerated discs (Yang and Li, 2009; Jin et al., 2013, Vo et al., 2013a). In addition, the distribution of collagen types shows significant changes. Eventually, disc degeneration leads to a loss of load bearing function, a diminution in disc height that together lead to a tendency towards bulging and disc herniation (Fig. 2).

The most common condition in patients seen by a spine surgeon is a posteriorly or posterolaterally herniated or prolapsed IVD that is pressing on the nerve roots in the spinal canal (Fig. 3). The herniated nucleus exacerbates the symptoms further by inducing local sterile inflammation that sensitizes the nerve root thereby causing pain. The mechanisms that are the basis for disc degeneration and the connections between disc degeneration and painful symptoms are still a mystery, therefore animal models can serve as important tools for further investigation. However, most existing animal models may not cause nerve compression or neuropathic pain.

3. Animal models of the disease

Animal models are important pre-clinical tools for biomedical research. Ideally, the most appropriate animal model for a specific disease is to artificially develop a condition that replicates the human condition. Due to the complicated etiology and pathogenesis of disc degeneration, developing a suitable animal model is challenging because it should 1) share features that are similar to disc degeneration in humans; 2) be reliable, and reproducible, as well as cost and labor efficient. There are 4 major categories among the animal models that are available (Table 1): genetic predisposition, mechanical loading, structural disruption including annulus/nucleus injury, chemical digestion, and endplate injury, and radicular pain.

1) Genetic predisposition

The animal model that exhibits age dependent changes in the disc shares many features with human IVD disease. The sand rat is a well characterized spontaneous disc degeneration model. Gruber and coworkers (Gruber et al., 2002; 2009; 2011; 2014; Tapp et al., 2008) found significant age related degenerative changes. Radiographic signs of degeneration were evident in the animals at the age of 2 months; wedging, narrowing, irregular disc margins, cell death, and endplate calcification were the most common degenerative changes in the older animals. The males of this species showed a higher incidence of wedging and endplate calcification than the females at 2–6 months, however, such differences between males and females disappeared by 12 months of age.

A spontaneous mutation of the BDL strain of mice leads to the Kyphoscoliotic (KY) condition; the mice show postural muscle atrophy during post-natal growth (Mason and Palfrey, 1984; Blanco et al., 2001). A hereditary form of the KY BDL strain exhibited changes in thickness of the thoracic-lumbar vertebrae and structural changes in the cervical discs. In newborn ky/ky mice, both the vertebrae and the IVDs are normal, however, small changes in the shape of the disc and the ratio of NP/AF occur 9 days after birth. In the adult mice, disc degeneration, including disc herniation could be seen both anteriorly and posteriorly, with destruction of the vertebral endplate and cystic lesions affecting the spinal cord.

A mutation in GDF5 mice showed signs of disc degeneration (Li et al., 2004) and GDF5 overexpression rescued the disease (Liang et al., 2010). Mutations of GDF5 protein lead to skeletal malformations in human, such as brachydactyly type A2, brachydactyly type C, Du Pan Syndrome, and Grebe type chondrodysplasia (Jin and Li, 2013).

Advances in gene knockout and knockin technology provide important opportunities for the addition of new animal models for the investigation of a host of diseases. Select transgenic mouse strains have been shown to be valuable for studying disc degeneration (Vo et al., 2013a).

Millecamps (Millecamps et al., 2011; 2012; 2015; Miyagi et al., 2014) described a SPARC (secreted protein, acidic, rich in cysteine) transgenic mouse strain that developed age-dependent disc degeneration with increasing severity. These mice exhibited low tolerance to axial stretching, hind paw hypersensitivity to cold, and impaired motor activity, as well as

increased innervation of discs accompanied with upregulation of CGRP and neuropeptide-Y in the dorsal root ganglia (DRG) and the spinal cord dorsal horn.

Bedore et al (Bedore et al., 2013) reported a decrease in aggrecan and collagen II, but an increase in collagen I, in the NP of a notochord specific CCN2 knockout mouse, which, also showed a reduction of CCN1 and CCN3 expression. I IL-1 receptor antagonist (IL-1rn) knockout mice showed alterations in catabolic and anabolic metabolism, including the loss of proteoglycan and collagen structure that were accompanied by an increase in the expression of matrix degrading enzymes (Phillips et al., 2013). This finding is consistent with the role of IL-1 in the progression of disc degeneration and suggests that IL-1 is a possible target for the therapeutic treatment of disc degeneration.

The β -catenin conditional activation mice (Wang et al., 2012a), breeding Col2a1-CreER(T2) and β -catenin(fx(Ex3)/fx(Ex3)) transgenic mice, showed extensive osteophyte formation, upregulation of mmp13, adamts4, and adamts5 genes. Furthermore, deletion of mmp13 or adamts5 expression in these animals rescued them from disc degeneration suggesting that β -catenin signaling may be involved in the pathogenesis of degenerative disease.

GDF8, a member of the transforming growth factor β superfamily, functions as a negative regulator of skeletal muscle growth. The muscle mass of GDF8 knockout mouse is double that of the WT mouse. Hamrick found that GDF8 deficient mice showed signs of disc degeneration, and staining with Toluidine blue showed loss of proteoglycan in the endplate and inner AF areas that were associated with endplate ossification (McPherron and Lee, 1997). Such observations suggest that muscle mass and bone mass are associated with disc degeneration.

Large animals can also serve as experimental models for the study of spontaneous disc degeneration. Bergknut et al (Bergknut et al., 2012) compared the discs of chondrodystrophic and non-chondrodystrophic (with notochordal cells) dogs. Signs of disc degeneration were seen in the younger (<1-year-old) chondrodystrophic and the 5–7-year-old nonchondrodystrophic dogs. Cho et al (Cho et al., 2011) observed that the number of disc cells and anabolic metabolism decreased during the aging of pig, and these changes were associated with an increase in MMP-1 expression. The results suggest that changes in IVD with age in dogs and pigs are similar to the changes seen with age in humans.

Recent studies (Valentine et al., 2006; Stolworthy et al., 2015) have proposed the use of alpacas as a potential large animal model for further studies of disc degeneration. These animals not only share similar spinal posture, disc size, and biomechanical flexibility to humans but also develop features of disc degeneration as they age: the incidence rate and severity of lower cervical discs increases in the older animals. These studies suggest that alpacas, and closely related animal species, may serve as a large animal model for future investigations on disc degeneration of the cervical spine.

Other factors contribute to disc degeneration also. Wang (Wang et al., 2012b) reported degenerative changes of the disc in mice with chronic tobacco smoke. Smoke induced cellular senescence, reduction in proteoglycan synthesis as well as total proteoglycan content, with an associated elevation of MMP activity, increased vertebral endplate porosity

and bone loss. Finally, changes in cell metabolism and ossification in the endplate appear in the mouse model of atherosclerosis (ApoE mice) (Zhang et al., 2013) as well as the diabetic mouse model (Fields et al., 2015).

2) Mechanical force

Biomechanics plays a crucial role in disc homeostasis. Mechanical loading is one of the contributing factors for disc degeneration; notably, the risk of disc degeneration is increased in manual laborers such as carpenters and drivers of machinery (Luoma et al., 2000). Exercises, such as walking and jogging have some beneficial effects on maintaining a healthy disc (Iatridis et al., 1999a; Saamanen et al., 1993). Altering the mechanical environment of the spine causes morphologic and biochemical changes in the disc; these changes are similar to early disc disease in human IVD (Cassidy et al., 1989; Puustjarvi et al., 1993).

Kroeber et al (Kroeber et al., 2002) applied axial load to the lumbar spine of rabbits using a custom made external loading device. After 14 and 28 days of loading the discs exhibited a typical degenerative phenotype: narrowed disc space, disorganized annulus, an increase in dead cells both in the annulus and endplates. These changes were irreversible after 28 days of unloading. In a rat tail disc model, dynamic loading for two weeks at a physiological level led to a decrease in the height and angular compliance of the disc, and increased angular laxity (Ching et al., 2003). The cellular response to dynamic loading was different between the AF and the NP. Kim et al observed changes in COMP and type II collagen gene expression following one hour of dynamic compression of the rat tail disc (Kim et al., 2011). MacLean *et al* observed elevated anabolic gene expression in the nucleus at 0.01 Hz but an increase in catabolic genes at 1 Hz. However, all loading frequencies caused a significant upregulation of the mRNA for catabolic factors (Maclean et al., 2004).

Static compression induced degenerative changes in disc composition and mechanical properties, as well as a decrease in the number of cells in proportion to the loading frequency and magnitude (Lotz et al., 1998; 2000; Iatridis et al., 1999b; Hutton et al., 2000). After static compression in a rat tail disc model, MMP-3 was upregulated at 7 days and increased with the loading duration. The same group later showed that the number of NP and AF cells decreased as the time of loading increased. Starting at day 7, notochordal cells decreased and this was accompanied by an increase in apoptotic cells. Upregulation of all MMPs and ADAMT-4 was observed in this static compression model together with a decrease in aggrecan and type II collagen (Hirata et al., 2014; Yurube et al., 2010; 2012; 2014). These and other observations (Vo et al., 2013b; Kuo et al., 2014) suggest that static compression induces cell death and mild disc degeneration. In a rat tail disc model, Yan et al. reported that in a rat tail disc model, static compression changed the mRNA expression of integrins as well as their downstream signaling. In addition, compression on 1-level disc induced severe disc degeneration and remodeling (Yan et al., 2016).

Inflammation was also induced in the compression model. Comparing the expression of inflammatory mediators and neuropeptide in DRGs between rat disc dynamic compression and injury models, Miyagi (Miyagi et al., 2011) demonstrated that inflammatory mediators

(TNF α , IL-1, IL6) and the neuropeptides were elevated only transiently in the injury group but persisted in the compression group up to 8 weeks.

More recently, Sakai et al studied the effect of constant compression in a GFP bone marrow chimeric mouse by looping the tail and aspirating NP tissue, and found a limited number of GFP positive cells in the IVD (Sakai et al., 2015). This study suggested that the bone marrow cells are recruited during the degenerative process in this compression model. However, it is not clear whether the bone marrow cells migrate to the IVD after the compression has induced structural changes in the disc.

Mechanical loading also induces disc degeneration in large animals. Hutton (Hutton et al., 2000) attached coil springs to the dog lumbar IVDs for 16 and 27 weeks. The most pronounced alteration was seen in the matrix of the NP but not in AF; the matrix showed an increase in collagen type I accompanied with a decrease in proteoglycan and collagen type II.

Results from mechanical loading in all of the above mentioned animal models lead to changes in disc matrix constituents, enzyme activity, the fate of cells, and gene expression after both static and dynamic loading, the degree of degeneration is dependent on the magnitude, duration, and frequency of loading.

3) Structural disruption

surgical procedure and enzyme digestion have been widely used to induce disc degeneration in rodents and large animals. This mainly includes injuries to NP, AF, and endplates,

Nucleus or Annular injury—Many different disc injury techniques have been investigated to induce disc degeneration; these include nucleotomy, full and partial disc puncture through a ventral approach, and percutaneous puncture of the annulus.

Varying degrees of nucleotomy have been performed to induce disc degeneration. Omlor et al (Omlor et al., 2009) removed 10% of NP volume with a 16G biopsy cannula in a minipig disc degeneration model. Changes in disc height and matrix components were seen 3 weeks after nucleotomy. Kim et al (Kim et al. 2011) removed the NP from rat discs with a microsurgical drill leading to the loss of proteoglycan and disc height at 9 weeks, which was associated with sustained hyperalgesia. A comparison of needle aspiration with a diode laser (Lucas et al., 2012) demonstrated that histologically, laser induced disc degeneration is similar to the spontaneous and progressive disc degeneration of needle approach.

Puncturing the annulus with a needle has become a preferred method for disc degeneration studies because it is straightforward and relatively reproducible. Masuda (Masuda et al., 2005) compared the annular stab to a needle puncture model in rabbits. Following the stab, disc height shrank at 2 weeks with no further progression. Ulrich (Ulrich et al., 2007) compared the inflammatory response to single and triple-stab disc injury. In discs stabbed multiple times, the NP was replaced with collagen and elevated pro-inflammatory cytokines, while the inflammation in a single stab was transient and localized to the wound. This study suggested that repeated injury accelerated disc degeneration and was associated with an

inflammatory response. Cunha et al (Cunha et al., 2017) compared two different gauge needles in a rat tail disc injury model. Both radiographical and histological observations showed that a 21G needle induced more severe disc degeneration compared to a 25G needle. The incidence of disc herniation is also proportional to needle size, moreover, the number of macrophages that infiltrate the disc as well as apoptotic cells are commensurate to the volume of the herniated tissue. After needle puncture, the mouse tail disc showed a decrease in GAG content and mRNA expression of aggrecan at 12 weeks, this was accompanied by an increase in fibronectin and collagen I (Yang et al., 2009). Similar to the rabbit annular puncture model, the fibrocartilaginous phenotype was observed in the degenerated discs. Moss et al has reported (Moss et al., 2013) details of surgical techniques used in an annular puncture model of rabbits.

In addition to changes in the structure and biochemical composition, the biomechanics of disc is also impacted by needle size as well as the depth of the injury. Michalek et al (Michalek et al., 2010) reported that the torsional properties were directly related to the disruption of fibers, while the compression stiffness was not impacted by needle size or loading. Elliott (Elliott et al., 2008) evaluated disc mechanics after injection of PBS with a 27G or 33G needle in rats, or, a 27G needle in sheep. Disc biomechanics changed after injection compared to the pre-injury with a large diameter needle, while the small needles had no impact on the mechanics. The severity of disc changes depended on the needle to disc height ratio: a ratio over 40% induced disc changes in all the studies.

To minimize the variation in technical parameters, percutaneous annular injury under fluoroscopic or CT guidance was investigated in several laboratories. After surgery, gradual progression of disc degeneration, including changes in the structure and matrix components, was confirmed by MRI, histology, biochemistry, and gene expression profiling (Kwon, 2013; Li et al., 2014; Kim et al., 2015). In one study, Issy (Issy et al., 2015) inserted the needle tip crossing the NP to the contralateral AF, and then rotated it 360° twice. Radiology and histology confirmed disc degeneration between 7 and 30 days after injury. Zhou et al (Zhou et al., 2013) created a rabbit disc degeneration model guided by CT. Keorochana (Keorochana et al., 2010) compared disc degeneration induced by three different sized needles inserted percutaneously into rat caudal discs. The severity of disc degeneration increased with increasing needle size. Proteoglycan and aggrecan decreased over time. By contrast to other studies, Sox-9 and collagen II positive cells were increased in the pericellular area within the NP and at the junction between NP and AF.

The minimally invasive procedure was also tested in large animal models. Xi et al (Xi et al., 2013) found progressive and mild disc degeneration in Rhesus monkeys by percutaneous puncture of the disc. Similarly, Yoon et al (Yoon et al., 2008) punctured the lumbar spine of mini pigs under fluoroscopy. Using the uninjured discs as controls, early degeneration of the injured discs was seen at 5 weeks as MRI and histology confirmed progressive degeneration following injury to the annulus.

This method offers a minimally invasive approach for the study of disc degeneration, however injury to blood vessels and nerves may be difficult to control at the site and depth of entry especially in the small rodents.

Chemical induction—Biochemical reagents have been used to induce animal models for disc degeneration. Sugimura (Sugimura et al., 1996) compared different enzymes on the IVD of monkeys. The study showed that both chondroitinase ABC and chymopapain induced degenerative changes but the former was less toxic than chymopapain to the non-cartilage tissue. Norcross et al (Norcross et al., 2003) injected chondroitinase ABC into tail discs of rat, and observed significant loss of disc height, as well as proteoglycan and NP cells. However, stiffness of the NP was increased in the chondroitinase ABC group compared to the control. Another group (Boxberger et al., 2008) reported similar results confirming that enzymatic digestion with chondroitinase ABC induced early signs of disc degeneration in a rat lumbar spine. In a goat model (Hoogendoorn et al., 2008), injection of Chondroitinase ABC induced long-term, mild disc degeneration within 18 weeks. The degeneration did not recover during the 26 week follow up period.

Injection of other reagents has also been used to induce disc degeneration. Anderson et al (Anderson et al., 2005) demonstrated that addition of the N-terminal fibronectin fragment to rabbit disc led to osteophyte formation, a progressive loss of normal disc structure, and a reduction in anabolic metabolism. Later, the same group reported similar findings in NP explant cultures and NP cells in alginate culture. Oegema et al showed that the fibronectin fragments increased in the degenerated human discs, and may further enhance the degradation of matrix (Oegema et al., 2000). Lee et al (Lee et al., 2009) evaluated disc degeneration following the injection of incomplete Freund's adjuvant (CFA) into rat IVD. The CFA rat showed an increase in hind paw withdrawal response, progressive disc degeneration, and expression of specific pain-related transmitters and mediators. Zhou et al (Zhou et al., 2007) assessed disc degeneration by percutaneously injecting 5-bromodeoxyuridine (BrdU) into the IVD of sheep and found progressive structural changes in the disc up to 14 weeks.

Chemically induced disc degeneration involves the injection of materials into the disc space, consequently, the results may be confounded by the process of inserting the needle that is used to deliver the enzyme, reagent or growth factor, therefore, needle size and depth of injury need to be controlled carefully. Peeters et al (Peeters et al., 2015) used a slow release system for goat disc regeneration by conjugating BMP2/BMP7 to a fibrin/hyaluronic acid hydrogel.

Endplate injury—The endplate is an important constituent that maintains disc structure. Yuan et al (Yuan et al., 2015) reported a rat ischemic sub-endplate induced disc degeneration model developed by injecting absolute alcohol, intradiscally, into the rat tail. Changes were seen in disc height and bone sclerosis. NP cells first changed from a vacuolar cell type to chondrocyte-like cells and then to fibrocartilaginous cells. Disorganized lamellae appeared first followed by fibrosis and rupture of the AF. The growth plate in the endplate regressed and eventually disappeared. Wei et al (Wei et al., 2015a; 2015b,) reported percutaneous injection of pingyangmycin (in rabbit) or bleomycin (in rhesus monkey) into the subchondral bone adjacent to the disc using CT guidance. After surgery, progressive narrowing of the disc space and loss of MRI signal were observed accompanied with osteogenesis in the endplate. An elevation in catabolic and reduction in anabolic gene expression were also detected.

In a dog model, disc degeneration was induced by perforating the NP via the vertebral endplate. The ensuing degeneration was confirmed by MRI, histology and biochemistry (Hutton et al., 2004). In mature dogs, the endplate channels were sealed by placing bone cement by way of the vertebrae, and, subsequent to this treatment, there was no detectable difference between the condition of the control and experimental discs with respect to disc space, gross morphology and an increase in proteoglycan content. In contrast, Kang et al (Kang et al., 2014) detected severe disc degeneration in immature pigs when the endplate was blocked with bone cement, which represents a more severe model than the stab-induced AF injury controls. Not surprisingly, MRI showed abnormal nutritional diffusion patterns in the discs after the endplate had been blocked.

While the studies that involve injuring the endplate showed promising results, details of structural changes to the endplate and consequent effects on nutrient transport between the vertebrae and discs need to be explored further.

4) Radicular pain

when disc herniation occurs, the herniated NP not only compresses the nerve roots but also induces inflammatory responses, which may play a crucial role in the radicular pain. Researchers have applied autologous NP to spinal nerve roots in rodents to mimic radicular pain in human (Kallakuri et al., 2005; Omarker and Myers, 1998; Omarker, 2011; Kim et al., 2011; de Souza Grava et al., 2012; Cuellar et al., 2013). However, this procedure is technically demanding, predisposes to iatrogenic neural deficit, and uses a posterior laminectomy approach generating confounding bony pain.

4. Conclusion and perspective

Due to the unique position and complexity of the IVD and the consequent degenerative changes with age, it has been proven difficult to create an ideal animal model for disc degeneration that replicates the disease in humans. In addition, differences in anatomy between animal and human posture, disc size, cell type, and loading further complicate the quest for an ideal animal model. Notochord cells disappear with maturity at 10 years old in humans, representing an important step of disc degeneration. By contrast to the human disc, notochord cells are retained in the majority of animal species (Daly et al., 2016), which manifests as a difference between human and animal models. Animals with spontaneous disc degeneration as well as transgenic mice are good candidates for helping us understand the disease despite their limitations. Chemical induction may be an adequate approach for mild disc degeneration, although dosage of reagents and the depth of injection need to be optimized. The mechanical force model has been shown to be suitable for progressive disc degeneration but loading time and frequency need to be standardized. Needle puncture has become a popular means for modeling disc degeneration due to reproducibility and the relatively short time for induction of degenerative changes, but it is not an appropriate model for early intervention. It is noteworthy that most of these studies have focused on the changes in disc structure and biochemistry but neglected the natural progressive process of disc degeneration and symptomatic radiculopathy. Moreover, a posterior or posterolateral approach to the disc space is not suitable for open surgery because the neural components

and bony structures are located posterior to the disc. Thus, a posterior approach has a higher chance of injuring a neural component than an anterior approach. A new animal model is in needed to reflect the disease in human: disc degeneration and the associated pain.

A recent study compared the hyperalgesia response in rat between annular puncture and inflammatory cytokine injection. The authors (Lai et al., 2016) showed that the rate and severity of disc degeneration are proportional to the degree of injury to the annulus, and the ensuing behavioral response to pain is commensurate with the loss of disc height and the inflammatory state of the disc. Annular puncture with injection of an inflammatory cytokine might be a potential model for disc degeneration. In another study, disc degeneration was induced in rat L4/5 discs by puncturing with a 0.4 mm needle anteriorly or posteriorly. Inflammatory cytokines were detected in the NP cells. Mechanical allodynia was observed with the posterior approach from day 1 up to 21 days but not in the anterior approach (Li et al., 2014; Liu et al., 2014). These studies suggest that the inflammation and disc herniation cause behavioral changes in response to pain. Future investigations may benefit from the development of models in large animals, combined with studies using models that exhibit spontaneous mutations as well as transgenic animal models.

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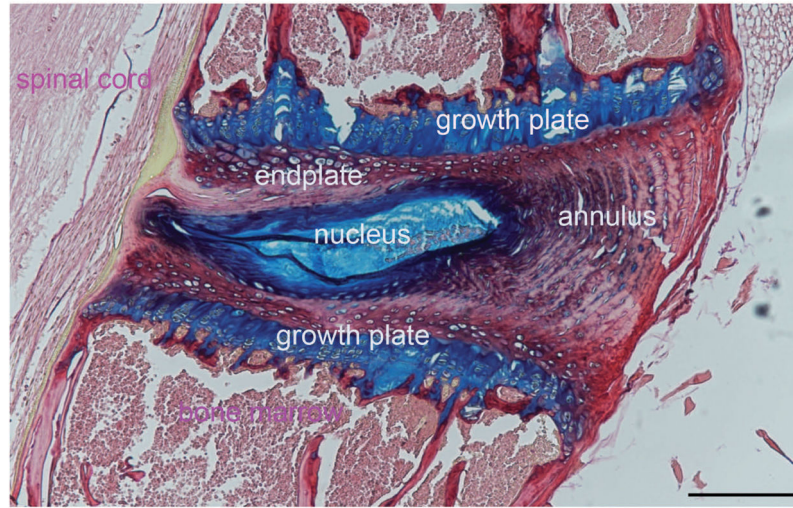


Figure 1.

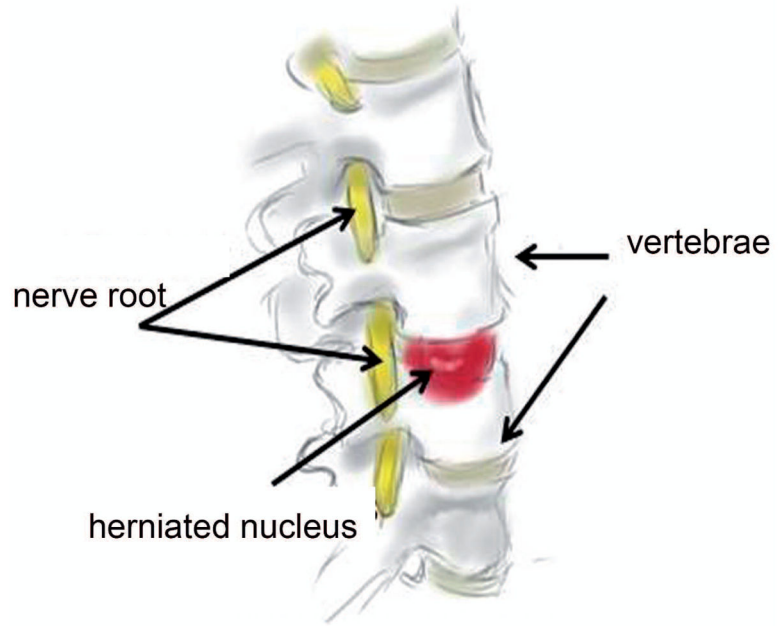


Figure 2.

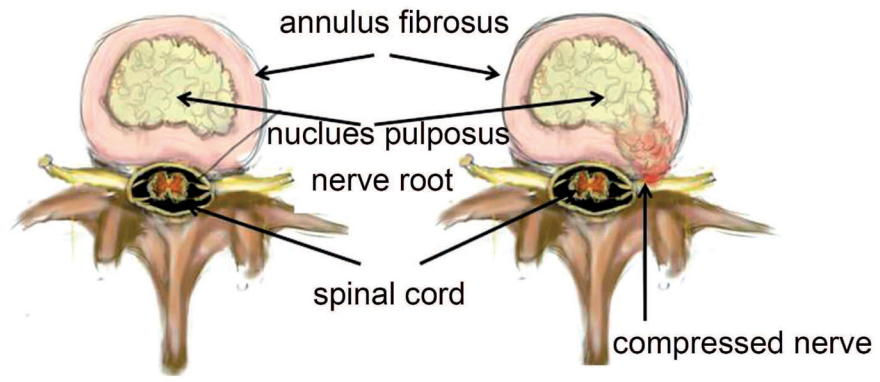


Figure 3.

Table 1

current existing animal model for disc degeneration.

Injury Category	Animal Species	References
Genetic predisposition	BDL strain of mouse	Mason and Palfrey, 1984; Blanco et al., 2001
	SPARC transgenic mouse strain	Millecamps et al., 2011; 2012; 2015; Miyagi et al., 2014
	Notochord specific CCN2 knockout mouse	Bedore et al., 2013
	IL-1 receptor antagonist knockout mouse	Phillips et al., 2013
	β -catenin conditional activation mouse	Wang et al., 2012a
	GDF8, GDF5 mice	Mcpheeron et al., 1997; Li et al., 2004; Liang et al., 2010
	Sand rat	Gruber et al., 2002; 2009; 2011; 2014; Tapp et al., 2008
	Dog	Bergknut et al., 2012
	Pig	Cho et al., 2011
	Alpacas	Valentine et al., 2006; Stolworthy et al., 2015
	Chronic disease disorder mouse	Wang et al., 2012b; Zhang et al., 2013; Fields et al., 2015
Mechanical force	Mouse tail	Lotz et al., 1998; Sakai et al., 2015
	Rat tail	Iatridis et al., 1999b; Ching et al., 2003; Maclean et al., 2004; Kim et al., 2011; Hirata et al., 2014; Yurube et al., 2010; 2012; 2014; Kuo et al., 2014; Yan et al., 2016
	Rat lumbar	Miyagi et al., 2011
	Rabbit lumbar	Kroeber et al., 2002
	Dog lumbar	Saamanen et al., 1993
Nucleus or annular injury	Mouse tail	Yang et al., 2009
	Rat tail	Ulrich et al., 2007; Issy et al., 2015; Cunha et al., 2017
	Rat lumbar	Elliott et al., 2008; Kim et al., 2011; Li et al., 2014; Lai et al., 2016
	Rabbit lumbar	Masuda et al., 2005; Lucas et al., 2012; Kwon, 2013; Moss et al., 2013
	Sheep lumbar	Elliott et al., 2008
	Porcine lumbar	Yoon et al., 2008; Omlor et al., 2009;
	Monkey lumbar	Xi et al., 2013
	Percutaneous rat tail	Keorochana et al., 2010
	Percutaneous rabbit lumbar	Zhou et al., 2013; Kim et al., 2015
	Percutaneous rhesus monkey	Xi et al., 2013
Chemical induction	Rat tail	Norcross et al., 2003
	Rat lumbar	Boxberger et al., 2008; Lee et al., 2009
	Rabbit lumbar	Anderson et al., 2005
	Goat lumbar	Zhou et al., 2007; Hoogendoorn et al., 2008; Peeters et al., 2015
	Monkey lumbar	Sugimura et al., 1996
Endplate injury	Rat tail	Yuan et al., 2015
	Rabbit lumbar	Wei et al., 2015a
	Dog lumbar	Hutton et al., 2004; Kang et al., 2014

Injury Category	Animal Species	References
	Rhesus monkey	Wei et al., 2015b
Radicular pain	Autologous NP implant to exposed rat DRG	Kallakuri et al., 2005; Kim et al., 2011; Olmarker et al., 2011; de Souza et al., 2012; Cuellar et al., 2013;

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