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Review of *in vitro* mechanical testing for intervertebral disc injectable biomaterials

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ABSTRACT

Many early stage interventions for intervertebral disc degeneration are under development involving injection of a biomaterial into the affected tissue. Due to the complex mechanical behaviour of the intervertebral disc, there are challenges in comprehensively evaluating the performance of these injectable biomaterials *in vitro*. The aim of this review was to examine the different methods that have been developed to mechanically test injectable intervertebral disc biomaterials in an *in vitro* disc model. Testing methods were examined with emphasis on overall protocol, artificial degeneration method, mechanical testing regimes and injection delivery. Specifically, the effects of these factors on the evaluation of different aspects of device performance was assessed. Broad testing protocols varied between studies and enabled evaluation of different aspects of an injectable treatment. Studies employed artificial degeneration methodologies which were either on a macro scale through mechanical means or on a microscale with biochemical means. Mechanical loading regimes differed greatly across studies, with load being either held constant, ramped to failure, or applied cyclically, with large variability on all loading parameters. Evaluation of the risk of herniation was possible by utilising ramped loading, whereas cyclic loading enabled the examination of the restoration of mechanical behaviour for initial screening of biomaterials and surgical technique optimisation studies. However, there are large variations in the duration or tests, and further work is needed to define an appropriate number of cycles to standardise this type of testing. Biomaterial delivery was controlled by set volume or haptic feedback, and future investigations should generate evidence applying physiological loading during injection and normalisation of injection parameters based on disc size. Based on the reviewed articles and considering clinical risks, a series of recommendations have been made for future intervertebral disc mechanical testing.

1. Introduction

Lower back pain is a prolific condition affecting over 500 million people worldwide with estimated costs up to and above $100 billion per year in western countries (Maniadakis and Gray, 2000; Katz, 2006; Lambeek et al., 2011; Hartvigsen et al., 2018). Whilst the exact causes of lower back pain are often multifactorial, an important contributor is intervertebral disc degeneration (Luoma et al., 2000; Urban and Roberts, 2003). Where disc degeneration is implicated, treatments are generally focused on pain management. In the early stages, these treatments are usually conservative, and involve physical therapy or anti-inflammatory drugs. Late-stage treatments rely on surgeries such as intervertebral disc replacement or spinal fusion. These surgical interventions are highly invasive and expensive procedures which present mixed outcomes (Brox et al., 2010; Anderson et al., 2015; Dhillon, 2016). Consequently, there is a strong clinical need to develop successful minimally invasive techniques to treat intervertebral disc degeneration at an earlier stage, preventing or delaying the need for invasive surgery.

The main components of the intervertebral disc are the central gelatinous nucleus pulposus, the surrounding concentric fibrous layers of the annulus fibrosus, and the cartilaginous endplates which separate the disc from the vertebrae caudally and cranially. The nucleus and annulus are two different viscoelastic, hydration-dependent tissues primarily made up of water, collagen, and proteoglycans (Raj, 2008; Nikkhoo et al., 2017). The presence of the proteoglycans causes a swelling pressure in the nucleus pulposus. When compressed, the nucleus pressure increases, causing hoop stresses in the annulus which are carried by the collagen fibres in tension as shown in Fig. 1 (Iatridis and Ap Gwynn, 2004; Newell et al., 2017). Disc degeneration has been associated with a loss of proteoglycans, disorganisation of the extracellular matrix, tears

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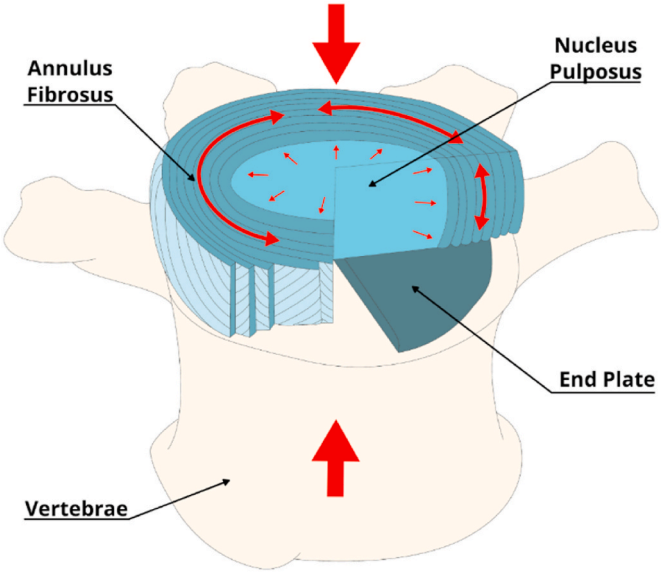

Fig. 1. - An illustration of a non-degenerate intervertebral disc under a compressive load where the nucleus pulposus distributes the loading into the annular layers resulting in annular layers tensioning.

in the annulus, and loss of disc height. In particular, the loss of proteoglycans causes a loss of water content which impacts the individual properties of each component, resulting in changes to the overall mechanical behaviour of the disc (Gower and Pedrini, 1969; Urban and McMullin, 1988; Urban and Roberts, 2003; Vergroesen et al., 2016).

There are several promising early-stage minimally invasive injectable treatments for intervertebral disc degeneration currently in development. These aim to restore the mechanical or biological properties of the disc through the injection of a biomaterial into the degenerated nucleus. A biomaterial can either be cellular or acellular depending on the intended use. While a small number of products have reached clinical trial, a greater proportion are still in the development phase (Showalter et al., 2015; Thorpe et al., 2016; Schmocker et al., 2016; Pelletier et al., 2016; Miles et al., 2016; Varma et al., 2018). *In vitro* mechanical testing is a key step in development of new injectable biomaterials for intervertebral disc degeneration, as it allows evaluation of the efficacy and effects of different clinical variables (Schmitz et al., 2020). By improving *in vitro* testing, risks associated with *in vivo* trials can be reduced by eliminating inappropriate materials or techniques earlier (Törnqvist et al., 2014). However, such testing can be challenging due to the interactions between the injected biomaterial and surrounding natural tissue, with its inherent variation. This increases the variance in the test outcomes compared to devices that can be tested in isolation; it also puts a cap on the duration of testing due to the need to maintain the mechanical integrity of the tissue components. In addition to this, comparison across treatments is difficult as each biomaterial will interact differently with the components of the disc.

Despite these challenges, a number of research groups have developed methods to examine the mechanical performance of injectable biomaterials for intervertebral disc degeneration. The aim of this review is to evaluate the biomechanical test methodologies adopted in these *in vitro* studies, identify current best practice and potential areas for future development.

2. Methods & inclusion criteria

Literature was reviewed for articles which reported the evaluation of an injectable intervertebral disc biomaterial where the testing involved the application of a load to an *in vitro* intervertebral disc model. A broad literature search was completed in PUBMED for studies prior to September 2020 with the following key search terms: intervertebral disc, [*in vitro,* OR *ex vivo*], inject, and generic terms for different treatments (e.g. hydrogel, cell scaffold). The search returned 143 studies which was reduced to 34 studies based on the inclusion criteria that it must report (i) injection of a biomaterial into a disc and (ii) application of some form of mechanical loading to the disc in an *in vitro* environment. Three additional studies were added to the review from references found within these 34 publications. Across these publications, a wide variety of testing methods were reported. This review focused on the factors that are of specific interest for the development of injectable biomaterials, namely, the testing approach, the technique used for artificial degeneration, the mechanical loading regime, and the method of biomaterial delivery. There are many other factors that could affect the outcomes of the test, including the tissue choice, specimen preparation, test environment, and preloading which are not covered in detail here because they have been examined and reported in previous reviews (O'Connell et al., 2007; Beckstein et al., 2008; Neidlinger-Wilke et al., 2014; Dreischarf et al., 2016; Schmidt et al., 2016; Newell et al., 2017).

3. Results

All tests reported in this review were undertaken on individual intervertebral discs, with different levels of bony attachment, taken from cadaveric human or animal spines.

3.1. Testing approach

In order to assess the efficacy of a given biomaterial, studies have employed different approaches to test and compare discs across various states. These states broadly fit into three categories: (1) native, where the discs are tested directly following dissection, (2) degenerate, where the discs are tested following an artificial process to cause degeneration, or (3) treated, where the discs are tested following injection of a biomaterial. Two approaches were identified in this review: single state testing and sequential state testing. In general, in single state testing, discs are placed into groups based on a state (native, degenerate, treated, or control procedure) and a given mechanical test protocol is applied. Alternatively, in the sequential state approach, a protocol is applied where an individual disc is tested through multiple states, usually native, degenerate, and treated. Forms of the sequential approach have been applied to groups of specimens to accommodate sham procedures and control specimens. Across the two approaches, specimen group sizes between four (Likhitpanichkul et al., 2014) and 12 (Lin et al., 2019) were identified. A summary of testing approaches applied by each study is shown in Table 1 and a flow chart with representative protocol for the two approaches is shown in Fig. 2.

Table 1
Testing approach identified in studies.

Testing Approach	Study
Single state	Boyd and Carter (2006); Tsantrizos et al. (2008); Freemont and Saunders (2008); Chan et al. (2010); Leckie et al. (2012); Milani et al. (2012); Balkovec et al. (2013); Likhitpanichkul et al. (2014); Zhou et al. (2014); Smith et al. (2014); Murab et al. (2015); Khalaf et al. (2015); Malonzo et al. (2015); Thorpe et al. (2016); Miles et al. (2016); Teixeira et al. (2016); Growney Kalaf et al., 2017; Gullbrand et al. (2017); Peroglio et al. (2017); Lin et al. (2019)
Sequential state	Arthur et al. (2010); Malhotra et al. (2012); Chan et al. (2013); Cannella, Jessica L Isaacs et al., 2014; Balkovec et al. (2016a); Balkovec et al. (2016b); Pelletier et al. (2016); Schmocker et al. (2016); Dupré et al. (2016); Sloan et al. (2017); Varma et al. (2018); Hom et al. (2019)

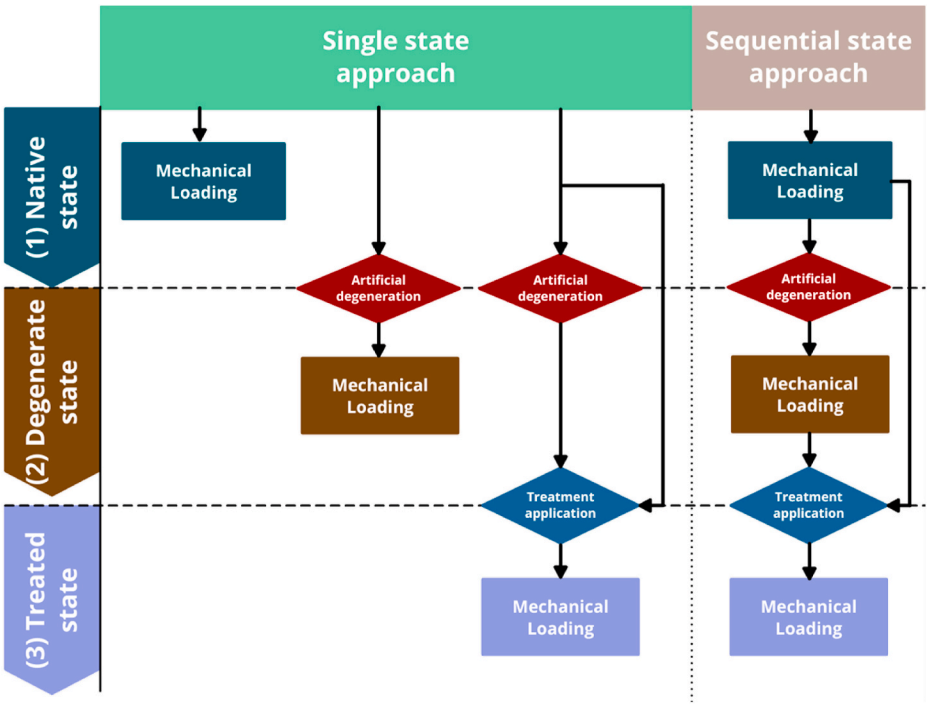

Fig. 2. Flow chart of representative approaches for single state and sequential state testing. Each testing section shows a point where mechanical testing data is gathered. The bypassing arrows indicate testing where the native state is treated directly, for example *in vivo* degenerate discs treated without any prior intervention.

3.2. Artificial degeneration techniques

Artificial degeneration was identified as a common method to induce degenerative changes where the native state does not present any measurable degeneration (e.g. food-chain animal tissue). Two types of methods were identified to induce artificial degeneration: biochemical and mechanical. Eleven studies used biochemical degeneration where one of four different enzymes were used: collagenase, chondroitinase-ABC, papain, and trypsin. Culture times varied across studies and were between 2 h (Thorpe et al., 2017) and 12 weeks (Gullbrand et al., 2017). Alternatively, 20 studies used a form of mechanical degeneration consisting of two broad methodologies: annular damage only or nucleotomy (e.g. trans-endplate, trans-annular). A summary of these methods, including max reported incubation times, needle puncture sizes, and a broad overview of nucleotomy techniques used by each study is shown in Table 2.

3.3. Mechanical loading

3.3.1. Constant/ramped loading

Across all studies, a mechanical testing regime was applied in which the disc specimens were loaded in a materials testing machine. For this review, constant/ramped loading was considered to be where the load was applied to the disc either constantly for a period of time or increasing over time at a given rate. The studies which employed this approach used a range of loading rates and maximum loads or strains, as shown in Table 3. The maximum and minimum loads applied were 18.6 kN (16.9 MPa) (Hom et al., 2019) and 0.09 kN (0.1 MPa) (Malonzo et al., 2015) respectively. Of the 18 studies identified with this loading, seven increased the load to cause a catastrophic failure (see Table 3). Three studies also applied 5° flexion during the loading to represent the physiological axis of loading (Cruz et al., 2018; Hom et al., 2019; Lin et al., 2019).

3.3.2. Cyclic loading

Two different types of cyclic loading were identified in this review: one where the discs were tested under uniaxial compression, and the other where loads were applied over a range of motion. A summary of the uniaxial compressive loading is shown in Table 4. The loading range varied from study to study, with the highest maximum being 1.85 kN (0.96 MPa) (Smith et al., 2014) and the lowest minimum being a tension of 0.275 kN (0.28 MPa) (Varma et al., 2018; Hom et al., 2019). The frequency applied was between 10 Hz (Peroglio et al., 2017) and 0.1 Hz (Cannella et al., 2014; Peroglio et al., 2017; Varma et al., 2018; Hom et al., 2019), and the number of cycles applied ranged from 100,800 cycles (Chan et al., 2010) to three (Dupré et al., 2016).

In studies where a range of motion loading was applied, flexion/extension, axial rotation and/or lateral bending were used. The type and magnitude of loading is summarised in Table 5. Loading was applied as a torque or an angular displacement where the maximum and minimum values were 7.5 Nm (Tsantrizos et al., 2008) or 4° (Hom et al., 2019), and −7.5 Nm (Arthur et al., 2010) or −4° (Hom et al., 2019). Similar to the uniaxial loading, the maximum and minimum frequencies were 2 Hz (Chan et al., 2013) and 0.1 Hz (Hom et al., 2019) respectively. The number of cycles ranged from three (Dupré et al., 2016) to 8000 (Balkovec et al., 2013).

Several methods for measurement analysis were identified, examples include: analysing the data extracted on the last cycle only (Leckie et al., 2012; Malhotra et al., 2012; Likhitpanichkul et al., 2014; Cannella et al., 2014; Dupré et al., 2016; Schmocker et al., 2016; Gullbrand et al., 2017; Varma et al., 2018; Hom et al., 2019), on all cycles (Milani et al., 2012; Balkovec et al., 2013, 2016b; Thorpe et al., 2016), or on a select group of cycles (e.g.: last 10 cycles) (Chan et al., 2010, 2013; Arthur et al., 2010; Murab et al., 2015; Pelletier et al., 2016); or applying a separate measurement protocol after the main testing protocol (Khalaf et al., 2015; Showalter et al., 2015; Balkovec et al., 2016b).

3.4. Biomaterial delivery

As shown in Table 6, the biomaterials were injected either to a set volume (11 studies) or by using haptic feedback on the syringe (12 studies). Two studies used alternative methods to control delivery, Arthur et al. (2010) controlled the injection based on pressure of an implanted balloon and Cannella et al. (2014) controlled injection based

Table 2
Artificial degeneration techniques applied across different studies, highlighting max reported incubation times, annular damage, and nucleotomy details. Note: Chan et al. (2010); Balkovec et al. (2013); Dupré et al. (2016); and Miles et al. (2016) did not apply any artifical degeneration.

Publication	Tissue	Artificial degeneration				
		Biochemical			Mechanical	
		Collagenase	Chondroitinase	Papain/ Trypsin	Annular damage	Nucleotomy
Chan et al. (2010)	Bovine	–	–	–	–	–
Chan et al. (2013)	Bovine	–	–	10 days	25G	–
Cruz et al. (2018)	Bovine	–	–	–	4 mm biopsy punch	up to 0.12 g removed with rongeur
Freemont and Saunders (2008)	Bovine	18 h	–	–	–	–
Hom et al. (2019)	Bovine	–	–	–	4 mm biopsy punch	0.188 ± 0.025 g removed with rongeur
Growney Kalaf et al. (2014)	Bovine	20 h	–	–	100 × 22G punctures	–
Growney Kalaf et al. (2017)	Bovine	22 h	–	–	21G	–
Likhitpanichkul et al. (2014)	Bovine	–	–	–	Box cut 4.5 mm by 4.5 mm	–
Lin et al. (2019)	Bovine	–	–	–	Cruciate incision	up to 0.2 g removed with rongeur
Malonzo et al. (2015)	Bovine	–	–	7 days	22G	–
Milani et al. (2012)	Bovine	18 h			–	–
Miles et al. (2016)	Bovine	–	–	–	–	–
Murab et al. (2015)	Bovine	18 h			–	–
Peroglio et al. (2017)	Bovine	–	–	–	–	Transpedicular nucleotomy
Saunders et al. (2007)	Bovine	18 h	–	–	–	–
Schmocker et al. (2016)	Bovine	–		6 days	–	–
Teixeira et al. (2016)	Bovine	–	–	–	21G needle	–
Thorpe et al. (2016)	Bovine	2 h		–	–	–
Varma et al. (2018)	Bovine	–	–	–	Cruciate incision	up to 0.2 g removed with rongeur
Gullbrand et al. (2017)	Caprine	–	12 weeks	–	–	–
Arthur et al. (2010)	Human	–	–		3.25 mm hole	Tissue removed with nucleotome for 20 min
Boyd and Carter (2006)	Human	–	–	–	–	Nucleotomy completed - no data
Cannella et al. (2014)	Human	–	–	–	–	up to 0.24 g removed with nucleotome for 20 min
Dupré et al. (2016)	Human	–	–	–	–	–
Showalter et al. (2014)	Human	–	–	–	4 × 4mm incision	up to 2.06 g removed with rongeur
Smith et al. (2014)	Human	–	–	–	4 mm incision	up to 2.17 g removed with rongeur
Tsantrizos et al. (2008)	Human	–	–	–	–	Nucleotomy completed - no data
Borde et al. (2015)	Murine	–	–	–	21G	–
Sloan et al. (2017)	Murine	–	–	–	1 mm window	Compression to herniation
Malhotra et al. (2012)	Ovine	–	–	–	2.5 mm incision	up to 0.02 g removed with rongeur
Pelletier et al. (2016)	Ovine	–	–	–	18G	–
Tsujimoto et al. (2018)	Ovine				5 × 3 mm window	0.2 g removed
Balkovec et al. (2013)	Porcine	–	–	–	–	–
Balkovec et al. (2016a)	Porcine	–	–	–	12G	–
Balkovec et al. (2016b)	Porcine		–	–	–	Compressive fracture
Khalaf et al. (2015)	Porcine	–	–	7 days	–	–
Leckie et al. (2012)	Porcine	–	4 weeks (*in vivo*)	–	-	–
Zhou et al. (2014)	Porcine	–		–	–	Trans end plate nucleotomy

on change in disc height. Loading applied during injection was identified in the form of a 50 N compressive load (Arthur et al., 2010), a 50 N traction force (Balkovec et al., 2013), and injection was completed *in vivo* with post sacrifice *in vitro* testing (Leckie et al., 2012). The maximum and minimum volumes injected were 2.3 ml (Dupré et al., 2016) and 0.1 ml (Chan et al., 2010, 2013; Borde et al., 2015) respectively.

4. Discussion

This review focused on specific factors relating to the *in vitro* mechanical testing of injectable biomaterials for intervertebral disc degeneration, namely the testing approach, artificial degeneration method, mechanical loading regime, and biomaterial delivery. In each of these areas, a number of different techniques were employed, and their respective advantages and limitations are discussed in more detail below.

4.1. Testing approach

In a single state testing approach, each specimen is only mechanically tested once (in the native, degenerated or treated state). By utilising this approach, comparison of destructive testing was possible for each group, for example: destructive biological analysis or mechanical failure. As comparison is completed across groups, careful consideration must be given to the size of each group to allow appropriate statistical analysis to be carried out. In the reviewed work, samples sizes of four to twelve were used and the median group size was six. Due to natural tissue variation, statistical power calculations are difficult to complete ahead of testing. Instead statistical significance was evaluated post-test, where groups generally used analysis of variance testing with post hoc statistical corrections (e.g. Bonferroni or Tukey) or a Kruskal-Wallis test for non-parametric data. Although small samples sizes have been used, statistically significant differences between specimens in different states have been observed in some mechanical properties but not often in all properties. For example, samples sizes of six to eight were able to find significant differences in disc height (Arthur et al., 2010; Pelletier et al., 2016; Balkovec et al., 2016a), compressive stiffness or modulus (Varma et al., 2018; Showalter et al., 2015), or flexion-extension parameters (Varma et al., 2018, Pelletier et al., 2019; Cannella et al., 2014, Dupré et al., 2016). When testing, a sufficient sample size to enable robust statistical testing between control groups is needed to identify any measurable effects of degeneration or treatment.

Alternatively, in a sequential testing approach, data collection occurs in each individual state. As a direct result of this, comparison of destructive testing cannot be completed with a sequential state

Table 3
Constant load review table.

Publication	Tissue	Max Compressive Load (MPa/kN)	Rate	To failure?
Chan et al. (2010)	Bovine	0.60/*0.66**	–	N
Cruz et al. (2018)	Bovine	-	2 mm/min	Y
Freemont and Saunders (2008)	Bovine	*0.60***/1.00	–	N
Hom et al. (2019)	Bovine	12.5 0/*13.70**	2 mm/min	Y
Likhitpanichkul et al. (2014)	Bovine	0.20/*0.20**	–	N
Lin et al. (2019)	Bovine	16.90/*18.60**	2 mm/min	Y
Malonzo et al. (2015)	Bovine	0.10/*0.09**	–	N
Miles et al. (2016)	Bovine	*5.40***/9.00	1 mm/min	Y
Teixeira et al. (2016)	Bovine	0.46/*0.50**	–	N
Varma et al. (2018)	Bovine	*1.00***/0.17	1 N/s	N
Gullbrand et al. (2017)	Caprine	*3.80***/0.23	–	N
Boyd and Carter (2006)	Human	*1.20***/3.50	–	Y
Tsantrizos et al. (2008)	Human	*0.41***/1.20	–	N
Borde et al. (2015)	Murine	20% initial disc height	–	N
Sloan et al. (2017)	Murine	40% strain	–	Y
Balkovec et al. (2016b)	Porcine	*10.75***/14.20	–	Y
Khalaf et al. (2015)	Porcine	0.80/*0.70**	60 s	N
Zhou et al. (2014)	Porcine	*1.70***/*0.15*	90 N/s	N

**Value reported as a force and converted to MPa using data from* Beckstein et al. (2008).
*** Value reported in MPa and converted to a force using data from* Beckstein et al. (2008); Paul et al. (2012); Dreischarf et al. (2016)*, all converted values are italicised.*

approach. However, a disc acts as its own control and the different states for a single disc can be compared directly. Using the disc as its own control is useful for optimising treatments by comparing properties between states. Although direct comparison of the native state to the treated state is necessary for optimisation, a direct comparison alone does not consider any procedure or time dependent changes to the tissue. Consequently, several groups applied sham procedures or utilised treated state controls. Additionally, groups attempted to reduce natural tissue degradation with protease inhibitors or antibiotics in order to better identify the procedure-dependent tissue changes (Chan et al., 2010; Likhitpanichkul et al., 2014; Khalaf et al., 2015; Lin et al., 2019).

Each approach fundamentally enables assessment of different requirements. The single state approach is subject to natural tissue variability between groups. This approach is necessary to investigate if the procedure adversely affects the disc, for example due to increasing herniation risk or biological reaction, which is represented *in vitro* with destructive testing. A sequential state approach is better suited for examining multiple variables associated with the procedure and find optimum values, because the sample sizes can be smaller, allowing more variables to be considered. These methods need to be used in conjunction with each other to fully assess a new injectable biomaterial. Both methods are important in bringing a new treatment to market, providing both regulatory evidence and understanding of the clinical variables to take forward to clinical trial.

4.2. Artificial degeneration

In vivo intervertebral disc degeneration is a multifactorial complex process influenced by the biological and mechanical behaviour, as well as the level of degeneration present (Vergroesen et al., 2015). Replicating degeneration *in vitro* is challenging, and a wide range of biochemical and mechanical methodologies have been developed. In the case of the *in vitro* tests considered in this review, where the mechanical performance of the procedure is being investigated rather than changes in the biological properties, it is important that the simulated degeneration represents the mechanical state of the degenerated disc. This creates a control state for optimisation and treatment development. Two categories for degeneration methods were identified: biochemical and mechanical.

In this review, artificial biochemical degeneration refers to using an enzyme to either selectively or non-selectively break down the biochemical makeup of the tissue. Two families of enzyme were utilised: proteases (papain, collagenase), which break down the amide bonds within protein structures and carbohydrases (chondroitinase ABC), which break down the glycosidic bonds within polysaccharide structures.

Papain and trypsin are non-selective proteases which break down various amino-acid linkages in the proteins (Amri and Mamboya, 2012; Rawlings and Salvesen, 2013), while collagenase selectively causes the breakdown of collagen (Nagase, 2001). Chondroitianse ABC results in the breakdown of the proteoglycan and glycosaminoglycans in the disc (Prabhakar et al., 2005). Both families of enzymes cause the degeneration of the disc to occur by destabilising the connective tissue. This then causes measurable mechanical changes in the disc that aim to represent natural tissue degeneration.

As shown in Table 2, seven of the 13 studies that applied biological degeneration used collagenase, whilst papain or trypsin was used in four studies and chrondroitinsae ABC was used in two studies. From the evidence presented, no major benefit from one type of enzyme over another has been identified.

It is important to note that to actually deliver the enzymes a needle puncture is required. This is a form of mechanical degeneration and depending on the bore size may contribute towards observed degeneration (Elliott et al., 2008). When looking to only introduce biochemical degeneration, use of small gauge size needles would be required which is reflected in Table 2 where needle sizes between 21 and 25 gauge were used to deliver enzymes. Another important aspect of using this method of degeneration, which is under-reported, is in controlling the level of degeneration. The level of degeneration is related to many variables (such as incubation time, incubation temperature, and enzyme concentration) and must be controlled to ensure that the degeneration methodology is reproducible, and the achieved degeneration level is similar between samples. Most enzymes are only denatured either through high temperatures or by chemically altering the enzyme structure, consequently at physiological temperatures, tissues will continue to degenerate *in vitro*. Without control measures, such as the use of an inhibitor, continued degeneration would add further variability to samples (Nikawa et al., 1994).

Mechanical degeneration disrupts how the disc distributes loads, by either directly damaging the annulus or by undertaking a nucleotomy. As shown in Table 2, five studies reported a method of directly damaging the annulus only, where in four studies needle punctures were used (12–21 gauge needle size), and in one study a 4.5 mm^2 box cut was completed. In both techniques, the annular fibres are torn, compromising its ability to distribute loads and creating a potential increased risk of herniation. The more common method of mechanical degeneration, used by 16 studies, was a full or partial nucleotomy. By removing the nucleus, the mechanics of the disc change drastically, as the nucleus has reduced ability to tension the annulus. Twelve of the 16 studies completed the nucleotomy through the annulus, which again would also cause annular damage degeneration effects. Whilst a trans-annular approach may represent a clinical setting for the use of these identified biomaterials (e.g.: disc restoration post herniation), the influence of annular damage on the disc may compromise restorative effects. As shown in Table 2, the nucleus was accessed with two tools to remove nuclear material, a rongeur or a nucleotome. In using these tools either a set mass (for the rongeur) or a set time (for nucleotome) was employed to control the level of degeneration. An alternative trans-annular

Table 4
Uniaxial cyclic loading table.

Publication	Tissue	Max compressive load (MPa/kN)	Min compressive load (MPa/kN)	Frequency (Hz)	Max number of cycles/ test stage	Temperature	Test Environment
Chan et al. (2010)	Bovine	0.20/*0.22**	−0.20/*-0.22**	1	100,800	37 °C	Bioreactor
Chan et al. (2013)	Bovine	10% strain	0/0	0.5 to 2	10	37 °C	Bioreactor
Hom et al. (2019)	Bovine	0.50/*0.55**	- 0.25/*-0.28**	0.1	20	–	–
Growney Kalaf et al., 2014	Bovine	*0.09***/0.15	*0.03***/0.05	2	30,000	–	PBS Spray
Growney Kalaf et al., 2017	Bovine	*0.09***/0.15	*0.03***/0.05	2	10,000	–	PBS Spray
Likhitpanichkul et al. (2014)	Bovine	0.40/*0.44**	0/0	0.1	~14,000	37 °C	Bioreactor
Lin et al. (2019)	Bovine	*0.18* **/0.30	*0.03***/0.05	1	To failure (max: 21,000)	Room	PBS Bath
Milani et al. (2012)	Bovine	30% strain	0/0	0.167	5	–	–
Murab et al. (2015)	Bovine	0.3% strain	0/0	0.167	5	–	–
Peroglio et al. (2017)	Bovine	0.08/*0.08**	0.02/*0.02**	0.1 to 10	756,000	37 °C	Bioreactor
Saunders et al. (2007)	Bovine	*0.60***/1.00	0/0	1 mm/min	5	Room	PBS Bath
Schmocker et al. (2016)	Bovine	0.20/*0.22**	0.05/*0.06**	0.2 to 1	500,000	37 °C	Bioreactor
Thorpe et al. (2016)	Bovine	0.65/*0.72**	0.53/*0.58**	2	200	37 °C	Bioreactor
Varma et al. (2018)	Bovine	0.50/*0.55**	- 0.25/*-0.28**	0.1	25	Room	PBS Bath
Gullbrand et al. (2017)	Caprine	*0.38***/0.23	*−0.19***/−0.12	0.5	20	37 °C	PBS Bath
Arthur et al. (2010)	Human	*0.34***/1.00	*−0.05***/−0.15	0.1 to 1	50	–	–
Cannella et al. (2014)	Human	*0.51***/1.50	*−0.05***/−0.15	0.1	5	–	–
Dupré et al. (2016)	Human	*0.09***/0.25	0/0	–	3	–	–
Showalter et al. (2015)	Human	0.96/*1.85**	0.12/*0.23**	2	10,000	Room	PBS Bath
Smith et al. (2014)	Human	0.96/*1.85**	0.12/*0.23**	2	10,000	37 °C	PBS Bath
Malhotra et al. (2012)	Ovine	*0.39***/0.30	*−0.39***/−0.30	1	20	Room	PBS Bath
Pelletier et al. (2016)	Ovine	*1.29***/1.00	*−0.13***/−0.1	100 N/s	4	37 °C	Humidity Chamber
Tsujimoto et al. (2018)	Ovine	*0.30***/0.3	*−0.39***/−0.30	1	1000	–	–
Balkovec et al. (2016b)	Porcine	30% estimated strength	0/0	0.5	1000	37 °C	Water Bath
Leckie et al. (2012)	Porcine	*0.30***/0.40	0/0	60 mm/min	5	–	–
Zhou et al. (2014)	Porcine	*0.15***/0.20	*0.08***/0.10	0.5 to 5.5	2640	Room	Humidity Chamber

**Values reported in MPa and converted to a force using data from* Beckstein et al. (2008).
*** Values reported as a force have been converted to MPa using data from* Beckstein et al. (2008); Paul et al. (2012); Dreischarf et al. (2016), *all converted values are italicised. One study applied uniaxial cyclic loading to failure of the disc whilst applying a* 6 Nm *flexion throughout (Lin* et al., *2019).*

Table 5
Range of motion cyclic loading.

Publication	Tissue	Flexion/ extension	Axial rotation	Lateral bending	Loading	Frequency	Max number of cycles/ test stage	Temperature	Test Environment
Chan et al. (2013)	Bovine	N	Y	N	±2°	0.5, 1, 2Hz	10	37 °C	Bioreactor
Hom et al. (2019)	Bovine	N	Y	N	±4°	0.1 Hz	20	–	–
Arthur et al. (2010)	Human	Y	Y	Y	±7.5 Nm	0.1 Hz	–	–	–
Dupré et al. (2016)	Human	Y	Y	Y	±5 Nm	–	3	–	–
Tsantrizos et al. (2008)	Human	Y	Y	Y	0 to 7.5 Nm	–	5	37 °C	PBS Bath
Pelletier et al. (2016)	Ovine	Y	Y	Y	±5 Nm	1 Nm/s	4	37 °C	Humidity Chamber
Tsujimoto et al. (2018)	Ovine	Y	Y	Y	±6Nm	–	1	–	–
Balkovec et al. (2013)	Porcine	Y	N	N	–	–	8000	37 °C	Water Bath
Balkovec et al. (2016a)	Porcine	Y	N	N	Unique/ specimen	–	10	37 °C	Water Bath
Balkovec et al. (2016b)	Porcine	Y	N	N	–	–	10	37 °C	Humidity Chamber
Leckie et al. (2012)	Porcine	Y	Y	Y	±5 Nm	2°/s	5	–	–

method was used in two studies where compressive overload was also used to generate a form of nucleotomy. This technique is promising, however, required prior annular damage to ensure herniation rather than end plate fracture. Two alternative techniques identified were nucleotomy via endplates (Zhou et al., 2014) or through the pedicles (Peroglio et al., 2017). These approaches maintain the annulus and may reduce overall disruption to the disc compared to a trans-annular approach. However, the trans-endplate or trans-pedicular approaches are more complex to implement as repair of the bone may be required. The trans-endplate approach is completed *in vitro* and accesses the disc in the cranial-caudal axis, going through the superior end of the prepared bone-disc-bone unit. The trans-pedicular approach is completed *in vivo* and access to the disc is accomplished via a posterior lateral approach though the pedicles. Both techniques access the nuclear material via a hole in the endplate, which was smaller using the trans-pedicular approach. Overall, trans-annular nucleotomy with rongeurs is the most prevalent mechanical method for inducing artificial degeneration and may be a suitable *in vitro* representation of a herniated disc patient undergoing surgery.

While biochemical and mechanical methods have advantages and

Table 6
- Biomaterial delivery table, note: two studies used different control methods for injection (Arthur et al., 2010), used pressure, and Cannella et al. (2014) used percent increase in disc height.

Publication	Tissue	Biomaterial	Delivery Method		
			By volume	By haptic feedback	Max volume injected (ml)
Chan et al. (2010)	Bovine	Cellular hydrogel	Y	N	0.1
Chan et al. (2013)	Bovine	Stem cells	Y	N	0.1
Cruz et al. (2018)	Bovine	Hydrogel	–	–	–
Freemont and Saunders (2008)	Bovine	Microgel	Y	N	0.5
Hom et al. (2019)	Bovine	Hydrogel	N	Y	–
Growney Kalaf et al., 2014	Bovine	biomaterial	Y	N	1
Growney Kalaf et al., 2017	Bovine	Hydrogel	–	–	–
Likhitpanichkul et al. (2014)	Bovine	Hydrogel	–	–	–
Lin et al. (2019)	Bovine	Hydrogel	–	–	–
Malonzo et al. (2015)	Bovine	Cellular hydrogel	N	Y	0.15
Milani et al. (2012)	Bovine	Hydrogel	–	–	–
Miles et al. (2016)	Bovine	Hydrogel	Y	N	0.125
Murab et al. (2015)	Bovine	Hydrogel	–	–	–
Peroglio et al. (2017)	Bovine	Hydrogel	Y	N	0.15
Saunders et al. (2007)	Bovine	Hydrogel	Y	N	0.5
Schmocker et al. (2016)	Bovine	Hydrogel	N	Y	0.2
Teixeira et al. (2016)	Bovine	Biomaterial	Y	N	0.5
Thorpe et al. (2016)	Bovine	Hydrogel	N	Y	0.2
Varma et al. (2018)	Bovine	Hydrogel	N	Y	0.75
Gullbrand et al. (2017)	Caprine	Hydrogel	N	Y	–
Arthur et al. (2010)	Human	Polymer	–	–	–
Boyd and Carter (2006)	Human	Hydrogel	–	–	–
Cannella et al. (2014)	Human	Hydrogel	N	N	–
Dupré et al. (2016)	Human	Hydrogel	N	Y	2.3
Showalter et al. (2014)	Human	Hydrogel	Y	N	0.5
Smith et al. (2014)	Human	Cellular hydrogel	Y	N	0.5
Tsantrizos et al. (2008)	Human	Biomaterial	–	–	–
Borde et al. (2015)	Murine	Hydrogel	N	Y	0.1
Sloan et al. (2017)	Murine	Hydrogel	–	–	–
Malhotra et al. (2012)	Ovine	Hydrogel	N	Y	0.35
Pelletier et al. (2016)	Ovine	Water-in-oil emulsion	N	Y	–
Tsujimoto et al.	Ovine	Hydrogel	N	Y	0.25
Balkovec et al. (2013)	Porcine	Hydrogel	N	Y	1.4
Balkovec et al. (2016a)	Porcine	Hydrogel	–	–	–
Balkovec et al. (2016b)	Porcine	Hydrogel	N	Y	–
Khalaf et al. (2015)	Porcine	Biomaterial	Y	N	1
Leckie et al. (2012)	Porcine	Hydrogel	Y	N	0.25
Zhou et al. (2014)	Porcine	Hydrogel	Y	N	1

disadvantages, an important consideration for early stage degeneration models is the level of annular damage caused by the approach. For example, in studies where the effect of the treatment injection is of interest, methods that preserve an intact annulus would be necessary because any pre-exiting damage due to the simulated degeneration could mask the outcomes of interest. Both categories of degeneration are relative to the scale of the intended measurement and observation. In the biochemical degenerate models, the changes are caused to the microstructure of the disc tissue. In contrast, mechanical models cause changes on the bulk or macrostructure. Due to the differences in the scales of these respective changes, the suite of experimental methods that can be employed to investigate and evaluate the changes are broad. Future work would likely require both mechanical and biochemical techniques individually to fully evaluate treatments.

4.3. Mechanical loading

4.3.1. Constant/ramped loading

Two protocols can be identified from the constant/ramped loading: ramping a load to failure and applying a single load over a length of time. Failure testing is vital for the development of injectable intervertebral disc biomaterials, as when completed in conjunction with native state controls, the mechanical safety of the treatment can be assessed. In the literature reviewed, failure was consistently defined as either nucleus herniation or endplate fracture which reflects the clinical risks associated with injectable treatments (Boyd and Carter, 2006; Sloan et al., 2017; Hom et al., 2019; Lin et al., 2019). Rate of loading is known to affect the mechanics of the disc (Newell et al., 2019), however, as shown in Table 3, no widely accepted rate was identified. Studies that provided a loading rate generally had limited justification or referred to another biomechanical testing investigation. Two other experimental factors were identified as potential influencers of the failure load: state of the disc, and additional non-uniaxial loading. In general, a lower failure load was identified for degenerated and treated specimens when compared to native state controls (Sloan et al., 2017; Hom et al., 2019; Lin et al., 2019). However, one key factor affecting this was that these studies applied a trans-annular nucleotomy which increased failure risk through the created annular damage. The studies which applied 5° bending did not show a reduced load to failure compared to others (See Table 3). This bending was used to better mimic physiological loading and to create a more consistent location for failure.

The studies that applied a single load over a length of time were not aiming to create failure, and instead were used to evaluate creep or relaxation properties (Zhou et al., 2014; Borde et al., 2015; Khalaf et al., 2015; Gullbrand et al., 2017; Varma et al., 2018). Generally, the purpose of this form of loading was to act as a supplement towards other mechanical behaviour. For example, in studies that used cell culture, low static loading contributed to wider mechanotransduction effects (Chan et al., 2010; Likhitpanichkul et al., 2014; Malonzo et al., 2015; Teixeira et al., 2016), more specifically the loading was used as a supplement of cyclic tests where it acted to represent the diurnal behaviour of the disc (Chan et al., 2010; Likhitpanichkul et al., 2014). A further important use of this loading was to act as a pre-load, which aimed to balance the

osmotic equilibrium of the disc prior to the wider protocol (Growney Kalaf et al., 2017; Hom et al., 2019). In general, these lower loads were an important supplement to encompass or control other effects as part of a wider investigation.

Testing to failure has not been widely reported, and there is not yet consistency in the methods used. However, it is an important aspect of the evaluation of injectable disc biomaterials and can identify risks that would not be seen in non-destructive testing.

4.3.2. Cyclic loading

Three important parameters were identified regarding cyclic loading: the magnitude of the loading peaks, the frequency, and the length of the test or number of cycles applied.

For uniaxial compressive loading, the load magnitude was generally associated with activities identified in historic intradiscal pressure measurements studies (Nachemson, 1963; Wilke et al., 1999; Sato et al., 1999). Although this is considered a suitable method to generate representative loading, as shown in Table 3, there was little consistency when comparing across research groups, even when taking into account differences in the specimens used. This variation comes from groups selecting different representative activities, and further variation is introduced when converting from intradiscal pressure to force, which is generally done by multiplying by a factor related to the disc cross sectional area (Nachemson, 1963; Dreischarf et al., 2016). By using the cross sectional area, even more variability is introduced due to different techniques for area measurement. An important restriction of cyclic uniaxial compressive loading is that it can only represent basic activities with minimal bending. Although this limits the range of physiological activities that are represented, it does mean that analysis is simpler and easier to compare. Therefore, uniaxial compressive loading provides a useful first stage-gate to eliminate biomaterials that cannot provide sufficient mechanical support, and also enables comparison of different biomaterial properties and surgical variables.

Studies that examined physiological ranges of motion in cyclic testing of interventions employed at least one of the following: flexion/extension, axial rotation, or lateral bending (see Table 5). For ease of testing and analysis, protocols generally applied each type of motion independently of each other. As with the uniaxial loading, these values were based on historic data and recommendations (Panjabi et al., 1986; Wilke et al., 1998) to represent a medium to high range of motion for the disc (White and Panjabi, 1978). Alongside the bending, the majority of studies applied a form of axial compression (50–300 N) as a preload either before or throughout the test. By applying this type of loading the experiment set up is more complex, however, it better simulates physiological loading. In terms of demonstrating the effectiveness or optimising the application of a treatment, applying a range of motion builds on the data provided by uniaxial compressive loading. Specifically, more adverse loading could aid in examining whether the biomaterial migrates or is expulsed. However, as it is a more complex test set up, uniaxial compressive loading seems more appropriate for initial evidence generation and optimisation.

The mechanical behaviour of the disc has been shown to be affected by loading rate (Costi et al., 2008; Sen et al., 2009; Newell et al., 2019), and was reflected by studies in this review which found changes in behaviour across different frequencies (Chan et al., 2013; Zhou et al., 2014). As shown in Tables 4 and 5 the most common frequencies were 0.1, 1, and 2 Hz. These frequencies represent standard activities such as resting (0.1 Hz) or walking (2 Hz). Whilst there is not a widely accepted set frequency to run tests, there is a range of frequencies that best represent general activities and are widely used.

The final key parameter considered is the duration of the test or the number of cycles. This is as an important factor for intervertebral disc testing, where studies have found that the mechanical properties of the disc change over time (Brinckmann et al., 1987; Périé et al., 2006; Showalter et al., 2014; Alsup et al., 2017). As shown in Tables 4 and 5, the number of cycles for various tests spanned over a large range (as low as three to over 100,000). The maintenance in tissue hydration is important in these tests, especially where the injected biomaterial is intended to have an osmotic effect. In general, studies that applied over 10,000 cycles across a single test stage were carried out in cell culture and focused on mechanotransduction effects, whereas shorter cycle studies were used for investigating mechanical behaviour. For example, Peroglio et al. (2017) examined gene expression and cell viability, whilst from a mechanical perspective only examined change in disc height. Although not directly reviewed in this article, the test environment (for example: fluid bath, saline soaked gauze, air) is likely to influence the mechanical properties throughout the duration of a cyclic test (Pflaster et al., 1997; Costi et al., 2002; Bezci et al., 2015). Overall, there is no clear ideal number of cycles. Too few cycles present the risk of not fully capturing the mechanical behaviour of the disc, as initial levels of hydration, which are influenced by the tissue preparation and handling, will dominate. Alternatively, too many cycles risk causing mechanical deterioration of the disc resulting in skewed data. It is likely that less than a few hundred cycles would not be sufficient to detect changes due to alterations in the biomaterial behaviour and hydration, but tests lasting days risk degrading the disc unrelated to the injected biomaterial. Since it is not feasible to test to millions of cycles *in vitro* due to tissue degradation, some form of accelerated simulation is required. One potential avenue is to exacerbate and accelerate potential issues from biomaterial injection using higher loads to generate extremes of physiologically relevant disc pressure (above 1 MPa) over a lower number of cycles (i.e. thousands rather than millions). A testing approach where fewer cycles are used to predict the longer term behaviour may also be possible. In both cases, further work is necessary to build evidence of appropriate loads and durations.

When analysing an injected biomaterial, the purposes of the cyclic loading tests should also be to examine any deterioration in the performance due to biomaterial extrusion, dispersion into the surrounding annulus, or fatigue of the material itself. All of these variables affecting deterioration may be dependent on the properties of the individual biomaterial. As yet, no studies have focused on this behaviour, and it is recommended that future work should attempt to determine the length of test necessary to identify time-dependent changes in the performance.

4.3.3. Other forms of mechanical testing

Although not directly reviewed in this study, mechanical testing can also be performed *in silico*, where a computational model representative of an intervertebral disc treated with an injectable biomaterial can be developed and the parameters of interest assessed systematically. *In silico* models have the advantage that more tests can be conducted non-destructively once models have been validated against experimental data obtained for the biomaterial of interest. There is a large body of computational work on intervertebral discs biomechanics (as reviewed by Schmidt et al., 2013 and Baumgartner et al., 2021 for models aimed at predicting regeneration), however *in silico* testing of injected biomaterial in the disc usually assumes a generic disc behaviour and anatomy (Khalaf et al., 2015; Baumgartner et al., 2021). This type of model has the disadvantage that it does not capture the inter-specimen variation which is due to anatomical differences and variability in the state of the tissue, both of which having an important effect on the outcome of a model (Mengoni et al., 2017; Sikora et al., 2018; Stadelmann et al., 2018).

4.4. Biomaterial delivery

Two main methods were identified regarding the delivery of the biomaterial, either utilising syringe haptic feedback or injecting to a set volume. Haptic feedback has a history of being used in clinics for procedures such as discography (Kapural and Goyle, 2007) or enzyme induced nucleotomy (Fraser, 1984). This type of method is difficult to practically transfer to *in vitro* testing as there is a host of variables that will affect the feedback. Firstly, it is a qualitative measure that will be

dependent on the user. This user specific feedback while injecting will be further impacted by the variation in natural properties of the disc (e.g. size, degeneration level). On top of this, the properties of the chosen biomaterial (e.g. viscosity) and the delivery needle (e.g. needle bore size, needle length) are experimental factors that will further compound to distort the user's response of the injection. Alternatively, using a set volume, can be readily reproducible within and across studies, but does not as effectively reflect historical clinical practice. As with haptic feedback, the individual specimen size will heavily influence any mechanical outcomes observed from using a set volume method. Whilst set volume is more readily reproduced *in vitro*, when transferring to clinic it would likely require specific information on the properties of the disc being treated.

Therefore, to reduce this inherent variation that will occur from injection, some level of normalisation to disc size could be adopted. This may allow better comparison between specimens and evaluation of the most suitable volumes to inject.

The main objective of analysing the delivery should be to optimise the restorative effects of a treatment, preventing under- or over-filling of the disc. In doing so, surgical risk can be further mitigated preventing unnecessary surgery by under-filling or an increased herniation risk by over-filling. One study, where the injection was based on changes in the disc rather than haptic feedback or set volume, analysed the restorative effects of the selected injection variable on the relative increase in disc height (Cannella et al., 2014). Although this study used a different technique for controlling injection compared to other work, it demonstrates an effective way to perform analysis enabling treatment optimisation. This analysis could readily be applied to other injection control techniques. An understanding of the restorative effects based on the injection variables is likely vital for the development of injectable biomaterials. On top of this, this understanding will be necessary to provide evidence for guidelines on use at eventual clinical trial.

Loading on the disc during injection will affect the intradiscal pressure and annular tensioning, which will in turn affect the quantity of biomaterial that can be injected into the disc. Importantly, compressive loading during injection will directly affect studies with injection control based on haptic feedback. One study applied a small traction load to the disc to better facilitate injection (Balkovec et al., 2013). This appeared to improve injection achieving the desired effects for the study, however applying a tractive load cannot be as readily translated to clinic as there may be a requirement for additional equipment or increased invasiveness of the surgery. Two studies (Arthur et al., 2010; Cannella et al., 2014) applied a low compressive load during injection, aiming to represent lying supine reflecting potential surgical practice. The inclusion of an applied load during injection is likely to affect the volume and distribution of the biomaterial, so a representation of physiological loading in clinic is important to include.

5. Conclusion and recommendations

This review demonstrates that there is a range of protocols and techniques used to assess key factors relating to injectable therapies for intervertebral disc degeneration. No widely accepted standard test methodology was identified, and the differences in approaches used currently makes comparison of *in vitro* biomaterial performance between studies problematic. As few nucleus augmentation devices have been used clinically, there is a lack of evidence of potentially major clinical risks, and evidence from previous nucleus replacement devices is also limited (Bertagnoli and Schönmayr, 2002; Lindley et al., 2010; Iatridis et al., 2013; Akgun et al., 2014). Any kind of testing standardisation and future recommendations should be based on this limited evidence and scientific judgement to assess other potentially routes for clinical failure.

A clear clinical risk is herniation or biomaterial expulsion (Iatridis et al., 2013; Akgun et al., 2014). This risk can be assessed using a single state approach alongside a ramped load to catastrophic failure protocol. Currently only low loading rates (1–2 mm/min) which were based on previous literature have been utilised. Utilising a high loading rate changes the mechanical behaviour of the disc (Newell et al., 2019) and may provide more physiologically relevant representation of traumatic conditions for treatment assessment. A more realistic representation of clinical herniation can be achieved by applying extreme ranges of motion (flexion/extension, lateral bending axial rotation) in combination with each other and the ramped force to failure. As only nucleotomy has been reported in the reviewed literature for this load to failure testing, introducing testing which uses other forms of artificial degeneration with reduced annular damage may provide better insight into the performance of biomaterials.

Another clinical adverse outcome is subsidence due to the mechanical properties of the biomaterial not matching the tissue (Lindley et al., 2010; Rosales-Olivares et al., 2007). Therefore, evidence that a biomaterial has successfully restored the native bulk mechanical properties from a degenerate state is required. To do this, a sequential state approach using a degenerate model that can generate a statistically significant difference between the native and degenerate states is recommended. When assessing the subsidence risk, a cyclic loading protocol would be necessary. Based on the evidence to date, loads above 1 MPa intradiscal pressure applied over a high number of cycles (>10, 000) may be necessary. However further evidence is needed to refine these testing parameters or to use modelling to predict progressive subsidence from fewer cycles.

These tests could be supplemented with further testing to develop the broad understanding of how a biomaterial integrates, diffuses, or breaks down within the disc over time. Currently, this has not been focused on in the literature and presents a challenge to any form of standardised testing for injectable biomaterials for intervertebral discs. A predominantly qualitative test, which uses non-destructive imaging and applies a simple load after injection of a suitable contrast agent into the disc, could provide some initial insight into this integration behaviour. Further analysis assessing the test environment for biomaterial components may provide insight regarding possible leakage or breakdown. Additionally, a high load, high cycle testing regime would demonstrate changes in mechanical properties over the duration of the test, which may show integration, diffusion, or breakdown of a biomaterial. Alternatively, once mechanical testing is completed, evaluating the changes in combined rheological properties of the biomaterial when integrated into the tissue may be informative.

Finally, biomaterial delivery is a vital factor across all the testing. Although potentially less clinically relevant, for initial *in vitro* testing a set volume approach is recommended. A set volume acts as a simple control and can remove unnecessary variability. However, it is important to note that a set volume method is best suited where there is a narrow range of tissue variability e.g. for controlled animal models. Consequently, when other techniques are used it is recommended that additional thought is given to normalising the data based on the geometry of the disc.

In summary, although some further assessment of methods is needed, several important clinical questions can be answered by utilising a combination of the reviewed methods to assess the restorative mechanical behaviour of injectable disc therapies.

Declaration of competing interest

Dixon A.R.: No conflict of interest to declare.
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