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The Effect of Twist on the Mechanical Properties of the Intervertebral Disc

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THE EFFECT OF TWIST ON THE IVD

Abstract

Introduction: Intervertebral disc (IVD) herniation is a common injury to the IVD and a frequent source of low back pain. IVD herniation occurs when the nucleus pulposus migrates through both the inter- and intralamellar matrices of the annulus fibrosus (AF). There are a number of mechanical risk factors associated with herniation, including repetitive flexion and twist. *In vivo*, twist combined with repetitive flexion has been associated with increased experience of low back pain and herniation. Additionally, *in vitro*, when repetitive flexion is combined with twist, IVD herniation occurs more easily than repetitive flexion alone. However, the mechanisms behind this relationship are not well understood. Therefore, the purpose of the current study was to determine the effect of twist on the mechanical properties of the inter- and intralamellar matrices.

Methods: Thirty-six bovine IVDs from the caudal region were exposed to a combination of either 0° or 12° of static twist and 0N or 1000N of compression for two hours. Following mechanical loading, three samples were dissected from each disc. One sample, containing at least two adjacent layers of the AF, was mechanically delaminated to measure interlamellar matrix strength. The variables of interest from this test were adhesion peel strength and peel strength variability. The other two samples were single layers of the AF which were mechanically tested in tension until failure. The single layer samples were pulled perpendicular to the orientation of the collagen fibres in order to measure intralamellar matrix strength. The variables of interest from the single layer tensile test were the stress and strain at the end of the toe region, Young's modulus, and the yield point. Finally, one IVD per condition underwent histological analysis in order to visually assess any damage within the AF.

Results: There were significant differences between the twisted and untwisted samples on the mechanical properties of the intralamellar matrix. Specifically, lower stress at the end of the toe region ($p=0.006$), lower Young's moduli ($p=0.010$), and lower yield points ($p<0.001$) were observed in the twisted samples compared to the untwisted samples. Additionally, histological analysis demonstrated more disruption within individual lamellae of the twisted samples compared to the untwisted samples. However, there were no significant differences between the twisted and untwisted samples on the mechanical properties of the interlamellar matrix. Additionally, there was no effect of compression on the inter- or intralamellar matrices.

Discussion and Conclusion: Twist, regardless of compression exposure, negatively affected the strength of the intralamellar matrix. This may explain why, when twist is combined with repetitive flexion, the occurrence of IVD herniation is accelerated compared to repetitive flexion alone. A mechanism of IVD herniation is migration through the AF layers via clefts within the intralamellar matrix, therefore, if the intralamellar matrix is weakened, such as from twist, then this progression will occur more easily and thus herniation will occur more easily. These findings suggest that twist may put the IVD at an increased risk for injury as a result of damage to the intralamellar matrix.

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List of Abbreviations

AF	Annulus fibrosus
ANOVA	Analysis of variance
Ca	Caudal
FSU	Functional spine unit
H&E	Hematoxylin and Eosin
IAF	Inner annulus fibrosus
IVD	Intervertebral disc
LBP	Low back pain
NP	Nucleus pulposus
OAF	Outer annulus fibrosus
PBS	Phosphate buffered saline
ROM	Range of motion

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1. Introduction

Low back pain (LBP), occurring in the lumbar region of the spine, is prevalent in Canada and considered to be one of the greatest contributors to global disability (Hoy et al., 2014). LBP can have serious consequences, both physical and mental, and affects both the lifestyle of the suffering individual as well as the productivity of the employee. One common source of pain in the low back region is the intervertebral disc (IVD); the soft fibrous structure found between each adjacent vertebrae in the spinal column. The IVD contains the nucleus pulposus (NP) surrounded by the multi-layered annulus fibrosus (AF). Within the AF there are two structures which both have important adhesive properties: the interlamellar matrix and the intralamellar matrix. The interlamellar matrix is the area between adjacent AF layers and the intralamellar matrix is the area between collagen fibres of a single layer of the AF. Herniation, a common injury to the IVD, occurs when the NP breaks through the layers of the AF through both the inter- and intralamellar matrices (Gooyers, McMillan, Noguchi, Quadrilatero, & Callaghan, 2015).

One prominent risk factor that has been associated with IVD herniation and LBP is twist. When combined with flexion, twist has been shown to contribute to LBP (Kelsey et al., 1984). *In vitro*, twist combined with flexion has been shown to increase the risk of IVD herniation and damage to the AF (Drake, Aultman, McGill, & Callaghan, 2005; Marshall & McGill, 2010; Schmidt et al., 2007; Veres, Robertson, & Broom, 2010). However, the mechanisms behind the relationship between twist and this observed increased risk of IVD herniation in combined loading scenarios is poorly understood. Therefore, it is important to study twist in isolation to determine these potential mechanisms. The proceeding review of literature summarizes the

research that has focused on ascertaining the mechanisms of IVD herniation as well as the biomechanical risk factors associated with this injury. It reveals a need for further research in the area of twist as a risk factor and its influence on the IVD.

1.1 General Anatomy

The function of the spine is to provide mobility and flexibility to the body, support the weight of the body and any externally applied loads, and protect the spinal cord. The spine is composed of multiple bony vertebrae separated by IVDs. It consists of five regions: the cervical region consisting of 7 vertebrae, the thoracic region consisting of 12 vertebrae, the lumbar region consisting of 5 vertebrae, the sacrum consisting of several fused vertebrae, and the coccyx.

The lumbar spine is particularly important and susceptible to damage as it must bear most of the upper body weight and forces generated by the trunk musculature. Further, the lumbar spine permits some movement including bending and twisting (Bogduk, 2005). Within the lumbar spine, a functional spine unit (FSU) consists of two adjacent vertebrae and the IVD between them. In twist, important components of the FSU and its ability to move are the facet joints consisting of the inferior articular processes of the upper vertebrae and the superior articular processes of the lower vertebrae. The orientation of the facets in the lumbar region are more acute to the sagittal plane, with the facets facing each other, compared to those in the cervical and thoracic regions in which they are more obliquely oriented, facing more posteriorly (Adams, Bogduk, Burton, & Dolan, 2013). This more acute orientation in the lumbar region permits lateral bending but limits twisting of an FSU because the facets come in contact with one another during rotation (Bogduk, 2005; Gunzburg, Hutton, & Fraser, 1991; Pearcy & Hindle, 1991). In humans, the range of motion (ROM) of one FSU in the lumbar spine in twist is

approximately 2-5° (Gunzburg et al., 1991; Li et al, 2009). It has been hypothesized that this is largely due to the facet joints (Adams & Hutton, 1983). Despite limited ROM of a lumbar FSU in twist, certain variables can increase this range and potentially increase the risk of damage to the IVD. When the spine is in flexion, the spacing between the facets increases which can increase the FSU's ROM in twist (Drake, Dobson, & Callaghan, 2008; Hindle & Pearcy, 1988; Pearcy & Hindle 1991**)**. Additionally, Adams & Hutton (1981) concluded that thinning of the articular cartilage of the facet joints may also increase the ROM in twist.

1.1.1 Intervertebral Disc

As mentioned previously, the IVD is comprised of the inner nucleus pulposus (NP) which is surrounded by the multi-layered collagenous AF and cartilaginous vertebral endplates (Figure 1). During twist, the IVD is exposed to not only motion, but also twisting torque, potentially increasing the risk of injury.

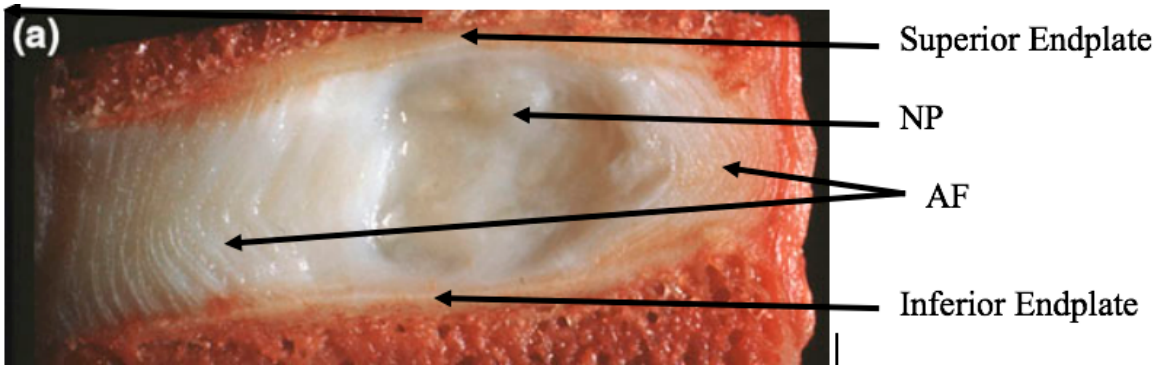

Figure 1. Healthy intervertebral disc including the nucleus pulposus, annulus fibrosus, and endplates. Image adapted from Adams et al. (2013), reproduced with permission.

1.1.1.1 Annulus Fibrosus

The AF is made up of approximately 15-25 layers of lamellae (Cassidy, Hiltner, & Baer, 1989; Marchand &Ahmed, 1990) that function to bear load applied to the IVD. Lamellae are made up of multiple collagen fibres bound tightly together that lie parallel to one another within each lamella. The AF is divided into two regions: the outer annulus fibrosus (OAF) and the inner annulus fibrosus (IAF). The collagen fibres of the OAF are primarily type I which are designed to resist tensile forces (Hayes, Benjamin, & Ralphs, 2001). The type of collagen gradually transitions from type I collagen in the OAF to primarily type II collagen in the IAF (Eyre & Muir, 1976). Unlike type I collagen, type II collagen is designed to resist compressive forces (Hayes et al., 2001). The collagen fibres within the OAF are highly organized compared to those in the IAF. The low level of organization in the IAF in addition to the high proportion of type II collagen fibres makes distinction between the IAF and the NP difficult (Yu et al., 2007).

As mentioned, the collagen fibres within a single lamellae lie parallel to one another, however, they are obliquely oriented to the transverse plane of the spine at angles between 28° and 45° (Holzapfel, Schulze-Bauer, Feigl, & Regitnig, 2005; Vergari et al 2016). Additionally, the orientation of collagen fibres in adjacent lamellae are approximately perpendicular to one another, creating a ply structure that allows the AF to resist multiple loading conditions and directions (Figure 2). In compression, the incline of the collagen fibres becomes more horizontal (Guerin & Elliot, 2006). In twist, the lamellae oriented in the direction of the applied twist are stretched and stressed, while the other half of the lamellae oriented in the opposite direction are more relaxed (Klein & Hukins, 1982; van Deursen, Snijders, Kingma, & van Dieën, 2001) (Figure 3).

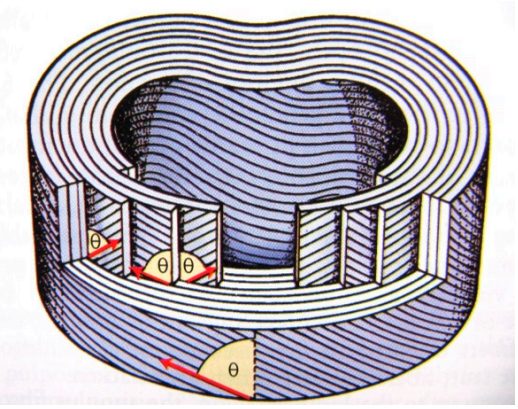

Figure 2. The orientation of the AF with alternating angles in adjacent layers. Image from Adams et al. (2013), reproduced with permission.

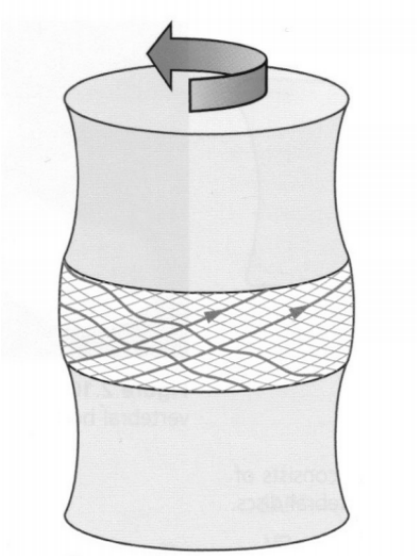

Figure 3. The orientation of the collagen fibres of the AF during twist. Image from Adams et al. (2013), reproduced with permission.

The extracellular matrix between adjacent lamellae is the interlamellar matrix. The interlamellar matrix allows for adhesion between adjacent lamellae and therefore prevents delamination, or the separation of these layers. The interlamellar matrix is composed primarily of type VI collagen, proteoglycans, and elastin (Melrose, Smith, Appleyard, & Little, 2008). Pezowicz, Robertson, and Broom (2006) demonstrated a complex collagen bridging network within the interlamellar matrix that they concluded contributes to the strong adhesion between

adjacent lamellae. A similar network was found in the intralamellar matrix, which is the extracellular matrix between adjacent collagen fibres within a single lamella, contributing to the adhesion between adjacent collagen fibres (Pezowicz, Robertson, & Broom, 2005). The intralamellar matrix is also composed primarily of type VI collagen, proteoglycans, and elastin (Melrose et al., 2008).

1.1.1.2 Nucleus Pulposus

The NP is a much more gelatinous and hydrated structure than the AF, composed primarily of type II collagen, proteoglycans, and water (Lundon & Bolton, 2001). Proteoglycans contribute to the NP's hydration and hydrostatic pressure because they are hydrophilic, meaning their chemical structure attracts water into the NP (Lundon & Bolton, 2001). This allows the NP to redistribute applied load to the AF and the vertebral endplates.

1.2 IVD Biomechanics

The IVD has unique biomechanical properties which enable it to be an important weight bearing structure and provide some mobility to the spine. One important property is that the IVD is anisotropic, in part due to the ply structure of the AF. This means that its mechanical properties are directionally dependent, allowing them to change depending on the direction of loading.

1.2.1 Stress-Strain Relationship

A common method to quantify these mechanical properties utilizes the stress-strain relationship. Stress is the force per unit area experienced within a tissue due to an externally applied load. Strain is the degree of deformation in a tissue when under an applied load. The stress-strain relationship can be represented by a normalized force-displacement curve. Force is

normalized to the cross-sectional area of the tissue to become stress (expressed in force per unit area). This allows normalization by tissue area as more force is required to deform a larger tissue sample than a smaller one. Displacement is normalized to the original length of the tissue sample to become strain because a longer sample will have greater absolute displacement at a given force than a shorter sample.

A stress-strain curve typically has three regions: an initial non-linear region termed the toe region, a linear region termed the elastic region, and a second non-linear region termed the plastic region (Figure 4). When studying the IVD, the toe region represents the uncrimping of the collagen fibres within the lamellae of the AF (Lundon, 2007). Within the elastic region, the change in stress is proportional to the change in strain. In this region, the changes in the length of the tissue are reversible once the applied stress is removed. A common property measured within the elastic region is Young's Modulus. Young's Modulus is defined as the slope of the elastic region and represents the stiffness of the tissue (Lundon, 2007). Finally, the plastic region is characterized by changes in tissue length that are no longer reversible and therefore indicates that micro-damage is occurring within the tissue (Lundon, 2007). An important property within the plastic region is the yield point which is the transition point between the elastic region and the plastic region in which the deformations in the tissue are no longer reversible.

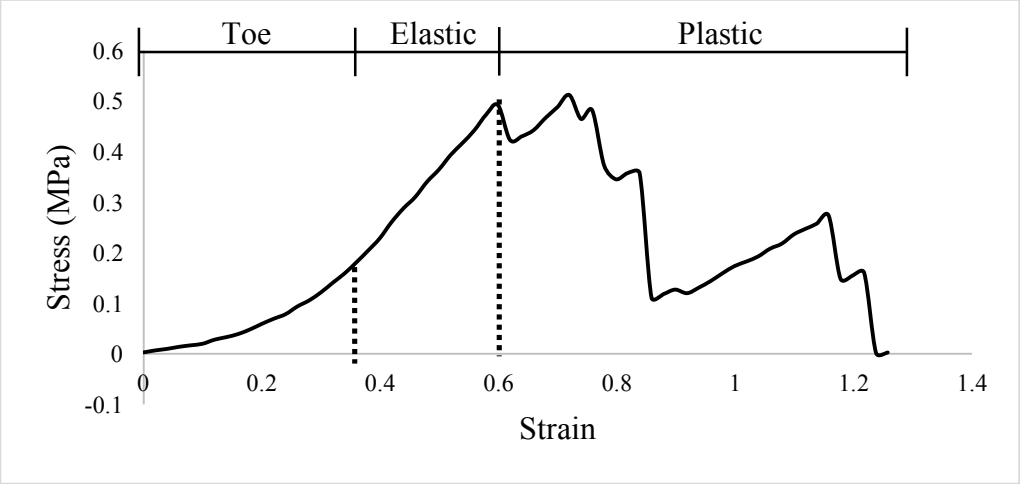

Figure 4. A typical stress-strain curve demonstrating the toe region, linear region, and plastic region.

1.2.2 Tensile Properties

As mentioned, the IVD contains high levels of proteoglycans which attract water into the NP. This hydration allows the NP to generate hydrostatic pressure which is an important characteristic of the IVD that enables it to resist compression. Under compression, this hydrostatic pressure within the NP causes it to bulge onto the AF, resulting in tensile and shear stresses to the AF. Therefore, when studying the mechanical properties of the IVD, specifically of the AF, the type of stress that is often examined is tension. There are regional variations of the tensile properties of the AF with the fibres of the OAF being stiffer than the IAF and the fibres from the anterior region being stiffer than those from the posterior region (Holzapfel et al., 2005; Skaggs, Weidenbaum, Iatridis, Ratclife, & Mow, 1994).

Early research studying tensile properties of the AF concluded that, although the tensile strength was highest when applied in the direction of the collagen fibres, the intralamellar matrix also contributed to the tensile strength of the AF and therefore warranted further research (Adams & Green, 1993; Green, Adams & Dolan, 1993). Research studying single lamellae

subjected to tension has consistently demonstrated that they exhibit a typical non-linear stress-strain response as described above (Ebara et al., 1996; Holzapfel et al., 2005; Skaggs et al., 1994). However, this research focused mainly on the tensile properties of the collagen fibres themselves and not of the intralamellar matrix. Holzapfel and colleagues (2005) stretched single layers of the AF both parallel and perpendicular to the collagen fibre orientation of cadaver lumbar spines in order to determine the tensile strength of both the collagen fibres and the intralamellar matrix. The authors found the Young's modulus to be much lower (0.22 MPa) when pulled perpendicular to the collagen fibres compared to when pulled parallel to the collagen fibres (28-78 MPa). They concluded that the intralamellar matrix had an insignificant load carrying capacity under tension, with the collagen fibres themselves being the main contributor. Similar results were seen by Pezowicz and colleagues (2005) who found an average initial peak stress of 0.15 MPa when they pulled samples of single lamellae from bovine tail IVDs perpendicular to the collagen fibre orientation. Additionally, the authors observed cleft formation within the intralamellar matrix when pulled perpendicularly and therefore concluded that the intralamellar matrix plays a smaller role in resisting tension than the collagen fibres themselves. They also hypothesized that, due to the ease with which they separated adjacent collagen fibres and the observation of clefts, herniation likely progressed through the separation of these fibres (Pezowicz et al., 2005). Similar clefts were observed by Tampier, Drake, Callaghan, & McGill (2007) who initiated herniation in porcine cervical IVDs. They thus concluded that these clefts are an important mechanism of herniation progression and that, despite the low strength of the intralamellar matrix, it is an important contributor to resisting herniation progression.

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In addition to the tensile properties of single lamellae and the intralamellar matrix, the tensile properties of multiple lamellae of the AF and the interlamellar matrix have been examined. Similar to studies looking at the tensile properties of the AF in single lamellae, those looking at the tensile properties of multiple layers of the AF have demonstrated a typical stress-strain response (Adams & Green, 1993; Fujita, Duncan, & Lotz, 1997; Iatridis, MaClean, & Ryan, 2005). Fujita and colleagues (1997) demonstrated that during radial stretching of multiple layers of the AF from cadaver lumbar spines, the observed deformation was primarily within the interlamellar matrix and not the actual collagen fibres. They concluded that a gradual disruption of the interlamellar matrix at a localized area resulted in failure of the samples and not deformation of the collagen fibres themselves. A similar delamination response within the interlamellar matrix when subjected to tensile loading has been demonstrated by Gregory, Bae, Sah, and Masuda (2012); Iatridis and colleagues (2005); Iatridis & Gwynn (2004); and Mengoni and colleagues (2015). Furthermore, Iatridis & Gwynn (2004) demonstrated that, in addition to tensile stress, shear stress within the interlamellar matrix ranged from 0.4 to 1.0 MPa during a circumferential tensile test. They hypothesized that this shear stress between adjacent lamellae contributed to delamination.

When looking at the mechanical properties of the IVD during twist, there are several unique characteristics that are important to consider. Strain within the AF has been shown to be quite substantial in twist (Stokes & Greenapple, 1985; Stokes, 1988); up to five times larger than in compression (Stokes & Greenapple, 1985). The high strain in twist is likely contributing to delamination. In addition, Schmidt and colleagues (2007) found that axial rotation resulted in the largest tensile strains on the collagen fibres (11.9%), despite being limited by the facet joints, when compared to lateral bending (8.9%), flexion (7.2%), and extension (5.9%). Finally, static

twist has been shown to decrease the compressive strength of the spine, resulting in increased endplate fractures (Aultman, Drake, Callaghan, & McGill, 2004).

In conclusion, twist results in both shear and tensile stresses on the AF. As mentioned previously, tension has been demonstrated to disrupt both the interlamellar matrix and the intralamellar matrix of the AF. This disruption of the inter- and intralamellar matrices due to the tensile and shear forces the AF is subjected to during twist may result in damage to these structures.

1.3 Bovine Model for Human IVD Biomechanics

A number of studies have used bovine tail IVDs to study the mechanical properties of human IVDs and the development of IVD disorders (Iatridis et al., 2005; Pezowicz et al., 2005; Pezowicz et al., 2006; Schechtman, Robertson & Broom, 2006; Simunic, Roberston & Broom, 2004; Stokes & Greenapple, 1985). *In vitro*, the most suitable model to study human IVD biomechanics is a human cadaver IVD. However, they are often more expensive and less readily available than other models, such as bovine tail IVDs. In addition, a bovine model allows for control over certain variables such as physical activity level, age, and diet, making a much more homogeneous sample compared to humans. Bovine tail IVDs have been demonstrated to be a suitable model for human IVDs due to a number of anatomical, biological, and biomechanical similarities described below.

1.3.1 Anatomy

The geometry of bovine tail IVDs, specifically IVD height and area, are comparable to human lumbar IVDs (Beckstein et al., 2008; Monaco, DeWitte-Orr, & Gregory, 2016; O'Connell, Vresilovic, & Elliot, 2007). O'Connell and colleagues (2007) determined that the

geometry of bovine tail IVDs deviated from human IVDs by 22% when considering IVD height, width, and NP area. One major difference between bovine tail and human lumbar IVDs is their shape, with bovine IVDs having a more circular shape compared to the limacon-shaped human IVDs (Monaco et al., 2016; O'Connell et al., 2007). It has been hypothesized that the shape of the IVD contributes to the herniation process, with circular IVDs herniating in the posterior direction and in a more diffuse manner and limacon IVDs herniating in the posterolateral direction and in a more concentrated manner (Yates, Giangregorio, & McGill, 2010). Another difference between bovine tail and human lumbar specimens is the absence of facet joints in bovine tail vertebrae (Alini et al., 2008). Therefore, in a bovine tail, twist is not limited by the facet joints as it is in human lumbar spines.

1.3.2 Biology

Bovine tails and human IVDs have a similar distribution of extracellular matrix proteins, with a higher water and proteoglycan content in the NP than in the AF and a higher collagen content in the AF than in the NP (Beckstein, Sen, Schaer, Vresilovic, & Elliot, 2008; Demers, Antoniou, & Mwale, 2004). Bovine tail IVDs have also been found to have a similar amount of proteoglycans in the AF and a similar amount of type II collagen in the NP as human lumbar IVDs (Beckstein et al., 2008; Demers et al., 2004). Conversely, Demers and colleagues (2004) found that bovine tail IVDs had less type II collagen in the AF than human lumbar IVDs and therefore concluded that bovine tail IVDs can serve as a good model to study healthy human IVDs but may not be suitable for older, more degenerated IVDs. An important component of both the inter- and intralamellar matrices is elastin. Elastin has been found to have a similar organization in bovine tail IVDs and human IVDs in which it is concentrated between adjacent

lamellae and between adjacent collagen fibres within lamellae (Yu, Fairbank, Roberts, & Urban, 2005; Yu et al., 2007).

1.3.3 Biomechanical Properties

Biomechanically, bovine IVDs have also been shown to be comparable to human lumbar IVDs. Bovine tail IVDs have similar torsional stiffness ranges to human lumbar IVDs (Showalter et al., 2012). Showalter and colleagues (2012) found that bovine tail IVDs had torsional stiffness values within 25% of those values found in human IVDs whereas the values from porcine and ovine IVDs were significantly higher than those from human IVDs. The compressive ROM of bovine tail IVDs (1.24 mm) was also the most similar to humans (1.21mm) compared to porcine, ovine, mice, and rat IVDs (Beckstein et al., 2008). Finally, bovine tail IVDs support similar ranges of compressive forces as humans due to the surrounding musculature (Alini et al., 2008). In conclusion, bovine tail IVDs have been deemed a suitable alternative to healthy young human IVDs when studying their mechanical properties.

1.4 IVD Herniation

IVD herniation is a common injury to the lower back, with 95% of herniated IVDs occurring in the lumbar spine in those aged 25-55 (Jordon, Konstantinou, & O'Dowd, 2009). It is characterized by the NP migrating radially through the layers of the AF and either completely migrating through all the layers of the AF, entering the spinal canal (prolapse) or partially migrating through the layers, resulting in bulging of the AF onto the spinal canal (protrusion) (Yasuma, Makino, Saito, & Inui, 1986). IVD herniation generally occurs in the posterior direction. This may be due, in part, to a smaller number of lamellae and more incomplete layers of lamellae on the posterior side of the IVD (Cassidy et al., 1989; Tsuji et al., 1993). This may

also be due to repetitive or prolonged flexion, a common cause of IVD herniation in which the anterior AF/NP is under compression while the posterior AF is under tension. This results in the posterior AF supporting most of the tension being applied by the NP, making it more likely to weaken and fail.

The mechanisms of IVD herniation progression are two-fold: through breaching the intralamellar matrix and through delamination of the interlamellar matrix. Tampier and colleagues (2007) initiated herniation in porcine cervical IVDs through repetitive flexion/extension combined with compression and discovered clefts formed by the NP in the intralamellar matrix of each lamella and no rupturing of the actual collagen fibres. Similarly, Gooyers and colleagues (2015) demonstrated clefts within the intralamellar matrix as well as delamination within the interlamellar matrix. They suggested that herniation occurred through migration of the NP between adjacent lamellae until it found the weakest spot of the next lamella and protruded, through clefts, between adjacent collagen fibres (Gooyers et al., 2015). Figure 5 illustrates this migration pattern of herniation through a combination of delamination and cleft formation.

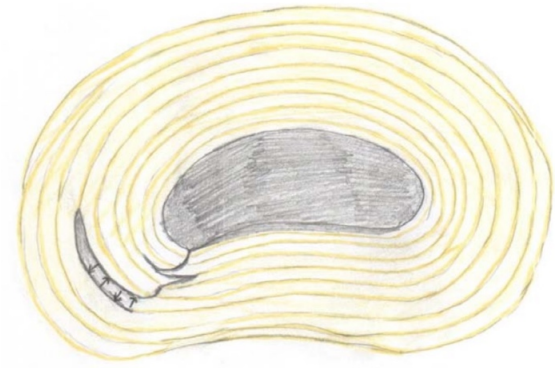

Figure 5. Illustration of the migration pattern of the NP through the AF during herniation. Image reproduced from Gregory (2009), with permission.

1.4.1 Risk Factors

Several risk factors have been linked to IVD herniation such as repetitive flexion/extension (Callaghan & McGill, 2001; Tampier et al., 2007), vibration (Gregory & Callaghan, 2011), and twisting (Drake et al., 2005; Farfan, 1969; Marshall & McGill, 2010). Contrarily, compression generally results in endplate fractures and is unlikely to damage the IVD on its own (Adams & Hutton, 1981). Several *in vivo* studies have concluded that, when combined with repetitive flexion, twisting is associated with LBP (Kelsey et al., 1984; Shan et al., 2013) and IVD herniation (Greenough & Fraser, 1994). Although twist has been demonstrated to cause LBP and contribute to IVD herniation, the results of several *in vitro* studies looking at the effect of twist on initiating IVD damage have not been as conclusive.

1.4.1.1 Twist

Some of the first studies looking at twist and its effect on the IVD were conducted by Farfan and colleagues (1969, 1970). In 1969, Farfan applied an axial twist moment alone and in combination with compression to the FSUs of lumbar cadaver spines with and without facet joints. The results indicated circumferential annular tears, similar to delamination, to the superficial layers of the IVD in the posterior and posterolateral regions, and no damage to any bony structures such as the facet joints. This damage was less severe when combined with compression, likely due to the closer approximation of the facet joints (Farfan, 1969). Therefore, the author concluded that, although the facet joints act as a protective mechanism to the IVD, the IVD can become damaged from twisting alone without any damage to the bony structures. A follow-up study concluded that the facet joints and the IVD contribute equally to resisting applied twist (Farfan et al., 1970). They found that the whole intervertebral joint failed at 22°, the isolated IVD failed at 16°, and the facet joints failed at 12°. These results were supported by

Krismer et al. (1996) who demonstrated, at 8° of twist, weakening of the AF fibres that were oriented in the direction of twist. Additionally, torsional strength decreased more in the FSUs in which the AF fibres were intentionally cut than in the FSUs in which the facet joints were removed, indicating that the AF provided more resistance to twist than the facet joints. They concluded that, although the facet joints contribute to resisting twist, they are a secondary barrier with the IVD being the primary barrier to resisting twist.

However, Adams & Hutton (1981) did a similar study to Farfan (1969) looking at the effect of twist on the IVD and found that it was the facet joints that provided the majority of the resistance to twist and not the IVD. They subjected human cadaver FSUs to compression and axial twist with several conditions: an intact FSU, an FSU without ligaments, an FSU without ligaments or facet joints, and the IVD in isolation. They found that the IVD played an important role in resisting twist within the first 2° of rotation but past that it was the facet joints that resisted twist with the IVD only playing a minor role (Adams & Hutton, 1981). Therefore, they concluded that it is the facet joints that will ultimately become damaged as a result of twist and not the IVD (Adams & Hutton, 1981). These results were supported by Shirazi-Adl and colleagues (1986).

These studies have several important limitations. Other than the study by Shirazi-Adl and colleagues (1986), these studies all used human cadaveric tissue with a large age range (18-86 years). Age can influence the mechanical properties of the IVD and therefore may have influenced the results of these studies as it was not always controlled for. Additionally, Adams & Hutton (1981) were unable to apply a constant level of compression and twist moments across the conditions, making it difficult to compare the results from the different groups. A final

limitation of these studies is that the type and degree of damage done to the AF was assessed qualitatively.

Since these controversial studies, research has primarily focused on twist in combined loading scenarios such as in combination with flexion. This research has concluded that twist does increase the vulnerability of the IVD and accelerates the progression of herniation when combined with flexion (Berger-Roscher, Casaroli, Rasche, Villa, Galbusera, & Wilke, 2016; Drake et al., 2005; Lu, Hutton, & Gharpuray, 1996; Marshall & McGill, 2010; Schmidt et al., 2007; Veres et al., 2010). Berger-Roscher and colleagues (2016) exposed ovine IVDs to 4° of twist combined with flexion and lateral bending and found that twist resulted in increased annular damage and increased endplate joint fractures. Additionally, Drake and colleagues (2005) subjected porcine cervical FSUs to either static compression and static axial torque combined with repetitive flexion, or compression and repetitive flexion alone. They found that IVD herniation occurred quicker in the group exposed to repetitive flexion combined with axial torque than in the group exposed to flexion alone. They also found a higher number of facet fractures in the flexion group exposed to torque and therefore concluded that failure of the IVD and the facets were not independent of one another but rather, damage to one structure accelerated damage to the other. These results were supported by a similar study by Veres and colleagues (2010) who concluded that although repetitive twist did not increase the number of herniated IVDs, it did facilitate herniation progression in those IVDs that were already at risk. Additionally, Marshall & McGill (2010), through X-ray images and computed tomography scans, demonstrated increased delamination in the interlamellar matrix when repetitive twist was combined with flexion/extension, concluding that twist does contribute to IVD herniation.

In summary, recent *in vivo* and *in vitro* studies looking at twist in combined loading scenarios have confirmed that twist does increase the risk of IVD herniation, however the mechanisms behind how twist increases this risk are not as well understood. It is possible that twist causes microscopic damage to the structures of the AF which indirectly predispose the IVD to herniation and further damage when combined with flexion, allowing herniation to progress more easily. In order to determine the mechanisms and isolate the damage twist in combined loading scenarios is causing to the AF, twist should be looked at in isolation. The current study focused on research on the effect of twist on the mechanical properties of the IVD in order to address this gap.

2. Purpose & Hypotheses

The research studying twist in combined loading scenarios suggests that an element of LBP and IVD injury is due to twist but there is limited research on the mechanisms behind this relationship. Therefore, the primary purpose of the current study was to determine the effect of twist on the mechanical properties of the AF of the IVD, specifically of the inter- and intralamellar matrices, as these matrices are involved in preventing IVD herniation (Gooyers et al., 2015; Tampier et al., 2007). The secondary purpose was to examine the extent of damage to the IVD caused by twist using histological analyses.

It was hypothesized that twist would decrease the interlamellar matrix strength and intralamellar matrix strength and stiffness as a result of the tensile and shear forces they are subjected to during twist. It was further hypothesized that damage (i.e. delamination and lamellae disruption) to these matrices would be greatest in the IVDs that were exposed to twist compared to non-twisted, non-compressed IVDs.

3. Methods

3.1 Specimens

Thirty-six bovine tail FSUs were obtained fresh from the tails of 18 skeletally mature animals. This ensured that the IVDs, vertebrae, and vertebral endplates were completely developed. Previous research has demonstrated that bovine tail IVDs are a suitable alternative to human lumbar IVDs due to anatomical (O'Connell et al., 2007), biological (Beckstein et al., 2008) and biomechanical similarities (Showalter et al., 2012). One major difference between bovine and human IVDs is the shape, with bovine IVDs having a more circular shape. However, for the current study this difference was beneficial as it allowed for twist to be more evenly distributed around the IVD. The spines were obtained from a common source in the Kitchener-Waterloo region allowing for control over certain variables such as physical activity levels, diet, and age. Two FSUs were removed from each spine (caudal (Ca) 1/2 and Ca 3/4), dissecting through every other IVD. The FSUs were stored in the freezer at -20°C until testing. Before testing, they were removed from the freezer and thawed at room temperature overnight. Much of the surrounding fat and muscle tissue was removed from the FSU with some muscle tissue being left around the IVD to minimize dehydration.

3.2 FSU Loading Protocol

Each FSU was secured into two custom aluminum cups through a custom twisting jig. The custom twisting jig had six pointed self-tapping screws (three for the superior vertebrae and three for the inferior vertebrae), which were used to secure to the upper and lower vertebrae (Figure 6). The twisting jig functioned to both secure the FSU and allow for the application of

static twist of the superior vertebrae with respect to the inferior vertebrae as the top ring could rotate (Figure 6b). The FSU was then wrapped in gauze soaked with phosphate buffered saline (PBS) and plastic wrap to ensure it stayed hydrated during the loading protocol. Finally, the FSU was mounted into a uniaxial electromechanical materials testing system (MTS, Eden Prairie, MN) (Figure 7).

Once mounted, each FSU was preconditioned with 125N of compression and 0° of twist for 15 minutes using the MTS. This reversed any post-mortem swelling that occurred in the specimens (Marshall & McGill, 2010). The FSUs were preconditioned with 125N of compression because previous *in vitro* studies using porcine models preconditioned FSUs with 300N of compression (Drake et al, 2005; Marshall & McGill, 2010), which when normalized to the area of a bovine IVD is approximately 125N. Following preconditioning, each FSU was subjected to one of four loading protocols for two hours: (1) 0° of twist and 0N of compression, these FSUs were considered the control specimens; (2) 0° of twist and 1000N of compression; (3) 12° of twist and 0N of compression; (4) 12° of twist and 1000N of compression.

There were nine FSUs per group for a total of 36 FSUs. The FSUs subjected to twist were removed from the MTS and then manually twisted at a constant rate of 2.7°/sec using a metronome. During pilot testing, several FSUs were twisted to end range in order to determine twist range of motion. The average of these end ranges was determined to be 12° therefore, in the current study, the FSUs in either twist group were twisted 12°.

The applied compression was 1000N because, when normalized to the area of a human IVD (~4000N), it is considered a moderate level of compression, falling between NIOSH's lifting guidelines action limit and maximum permissible limit (NIOSH, 1981). Following the

loading protocol, each FSU was removed from the custom twisting jig and the muscles and ligaments surrounding the IVD were removed in order to access the IVD.

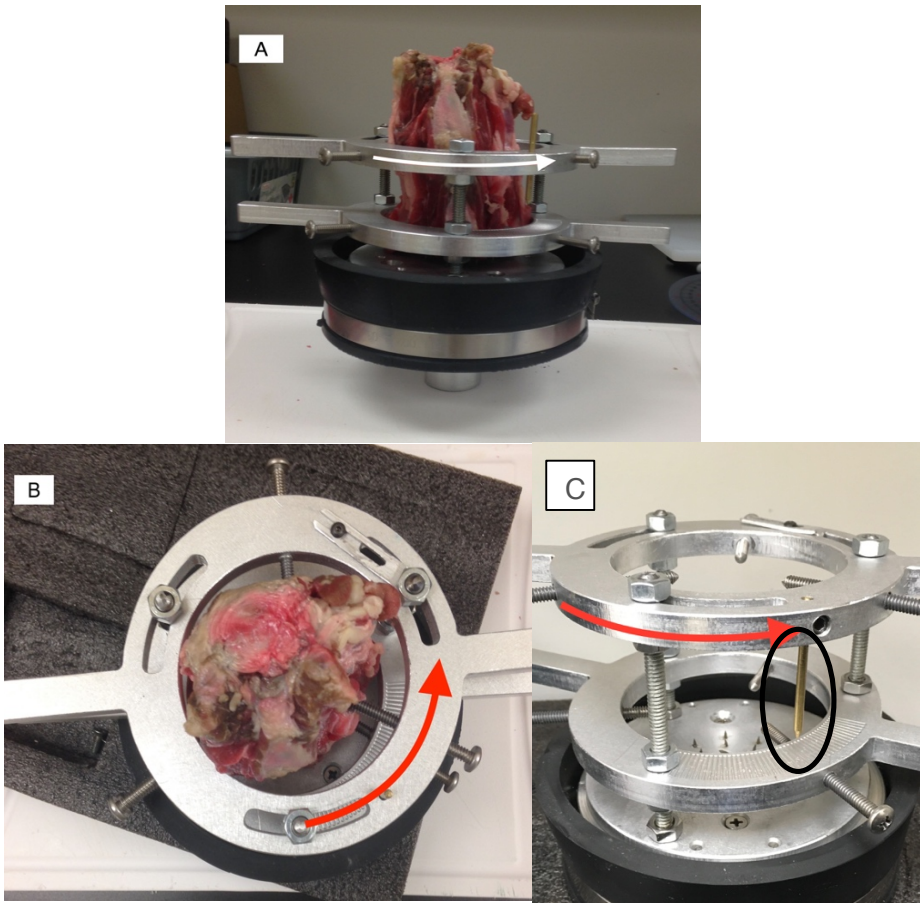

Figure 6. A) Sagittal view of an FSU in twist using the custom twisting jig. Arrow indicates direction of twist. B) Transverse view of an FSU in twist using the custom twisting jig. C) Custom twisting jig indicating the pin (circled in black) used to indicate degrees.

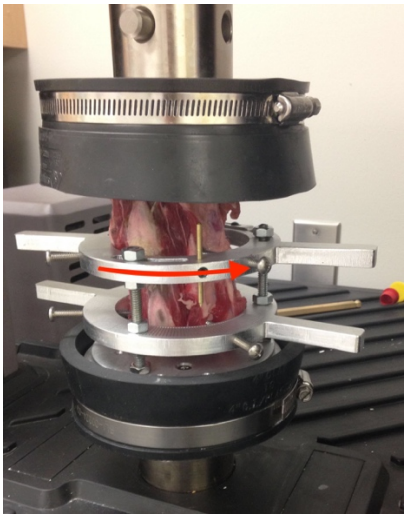

Figure 7. An FSU in twist using the custom twisting jig and mounted in the MTS. Arrow indicates direction of twist.

3.3 Annulus Fibrosus Mechanical Testing Protocol

3.3.1 Tensile Testing of the Intralamellar Matrix

Thirty-two of the 36 mechanically loaded IVDs were used for further mechanical testing (8 IVDs per condition), while the remaining four IVDs were reserved for histological testing. Two single layer samples were dissected from the anterior OAF of each of the 32 IVDs for a total of 60 viable samples (four samples were not large enough for mechanical testing). The collagen fibres within each dissected sample from the IVDs subjected to twist were oriented in the direction of twist (Figure 8). This was because there was likely more tensile and shear forces within the intralamellar matrix in layers in which the collagen fibres were being stretched due to twist. A dissection microscope was used to dissect the samples to ensure dissection of only one layer and to minimize damage to the sample during dissection (SD-SCD Trinocular Stereoscope, Leica, Wetzlar, Germany).

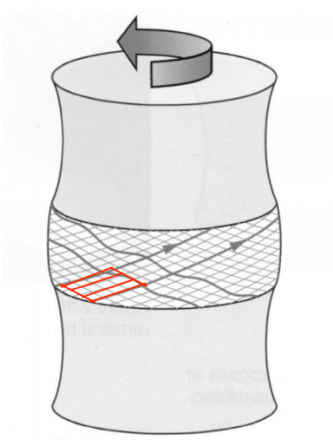

Figure 8. The orientation of the collagen fibres of the AF during twist where the red rectangle represents a dissected single layer oriented in the direction of twist. Image adapted from Adams et al. (2013), reproduced with permission.

Following dissection, the tissue thickness was measured using a laser displacement sensor (Keyence, Mississauga, ON). The sensor calculated the difference between the distance between the laser and the base of the plate used to hold the tissue and the distance between the laser and the tissue sample. The obtained tissue thickness was then multiplied by the width of the tissue to calculate the cross-sectional area of the sample which was used to normalize force (Figure 9). The average tissue thickness and cross-sectional area for each condition is summarized in table 1. No significant differences in tissue thickness or cross-sectional area were observed between any of the exposure conditions. Additionally, the average thickness of the untwisted samples was 0.30mm (±0.08) compared to 0.31mm (±0.11) for the twisted samples ($p=0.79$) and the average cross sectional area of the untwisted samples was 0.84mm^2 (±0.30) compared to 0.81mm^2 (±0.33) for the twisted samples ($p=0.65$).

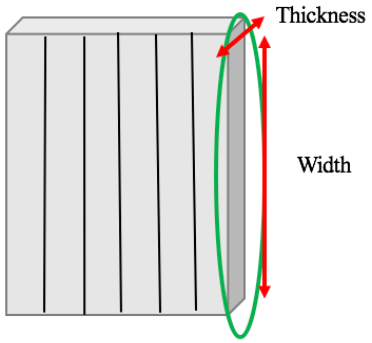

Figure 9. Measurement of the cross-sectional area of a sample (green circle) using tissue thickness and tissue width.

Table 1. Average single layer sample thickness and cross-sectional area for each exposure condition. No significant differences were observed between the conditions ($p>0.05$).

	Tissue Thickness (mm)	**Cross-sectional Area (mm^2)**
Control Samples	0.33 (±0.07)	0.91 (±0.27)
Compression-only Samples	0.27 (±0.09)	0.75 (±0.32)
Twist-only Samples	0.33 (±0.11)	0.82 (±0.31)
Combination Samples	0.29 (±0.11)	0.79 (±0.36)

The samples were then mounted into the BioTester 5000 (CellScale, Waterloo, ON) via tungsten rakes. The rakes used small hooks to puncture the tissue to allow a strong grip while stretching it. The samples were mounted such that tension was applied perpendicular to the orientation of the collagen fibres in order to isolate the intralamellar matrix (Figure 10). Prior to tensile testing, the samples were soaked with PBS to ensure they stayed hydrated during testing. The mounted tissues were then stretched to approximately 5mN to pull them taut. Each tissue was preconditioned with 3 repeats of 10% strain at a rate of 1% strain/sec. Following preconditioning, the tensile test began with the samples being pulled at a rate of 2% strain/sec until failure.

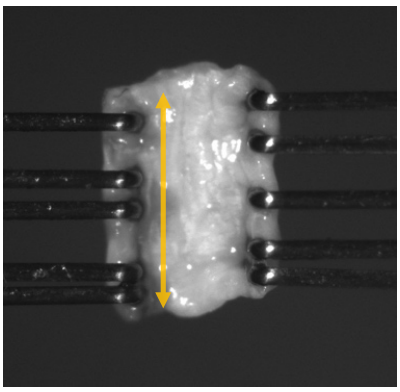

Figure 10. Single layer sample mounted via rakes with tension being applied perpendicular to the collagen fibres. Note the orientation of collagen fibres represented by the orange arrow.

3.3.2 Tensile Testing of the Interlamellar Matrix

From the 32 IVDs dissected for the single layer tensile test, a sample of the AF was dissected from the posterior OAF of each of the IVDs for a total of 32 samples. Each sample contained approximately 6 adjacent layers of the AF. Delamination between the two most centroid adjacent layers of a sample was initiated manually by cutting between these central layers, isolating the interlamellar matrix. This created a T shaped tissue that was suitable for the T-peel test. The T-peel test was the most appropriate test for examining the tensile properties of the interlamellar matrix because it is best when the two materials adhered to one another are both flexible, such as two adjacent AF layers (ASTM Standard D1876-01, 2001). Additionally, the T-peel test mimicked how the interlamellar matrix is loaded *in vivo*.

Prior to mechanical testing, the T shaped samples were soaked with PBS to ensure the samples remained hydrated during testing. The bond width of each sample was also measured using digital callipers (Figure 11). The tissue samples were then clamped in the Ustretch (Cellscale, Waterloo, ON) at the manually created tabs (Figure 12). Once secured, the tissue samples were stretched until the applied tension was approximately 10mN without creating

delamination. At this point, the peel test began with the tissue being peeled at a rate of 0.5 mm/second until failure.

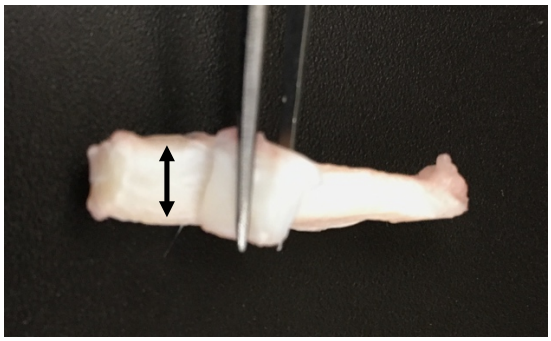

Figure 11. Tissue width was measured for each sample. Note the black arrow indicating the tissue bond width.

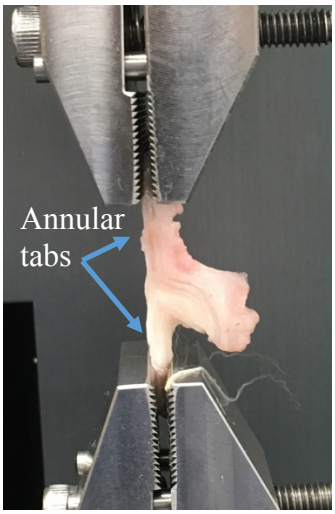

Figure 12. AF sample clamped at the manually created tabs during the T-peel test.

3.4 Endplate Geometry Measurements

IVD endplate measurements, including AP and lateral widths, were taken using digital callipers in order to calculate IVD area. The mean area was 539.15mm^2 (±28.70) for the control IVDs, 517.23mm^2 (±16.74) for the compressed-only IVDs, 575.19mm^2 (±66.70) for the twist-only IVDs and 528.33mm^2 (±70.99) for the combination IVDs. No significant differences in

endplate area were observed between conditions. Additionally, the mean area of the untwisted IVDs was 529.41mm^2 (±25.50) compared to 548.30mm^2 (±69.47) for the twisted IVDs (p=0.38).

3.5 Histological Analysis

Following the two-hour loading protocol, one IVD per group (4 IVDs total) was carefully dissected from each FSU. Each IVD was cut in half and placed in 10% formalin (Fisher Scientific) for 48 hours until histological testing. Tissue embedding and Hematoxylin and Eosin (H&E) staining was done at the Ontario Veterinary College (Guelph, Ontario) in order to visualize type I and type II collagen and any damage that may have occurred as a result of the loading protocol. An Axiolab microscope (Zeiss, Jena, Germany) was then used to view the stained slides in order to assess any visible damage to the AF.

3.6 Data Analysis

3.6.1 Single Layer (intralamellar matrix) Tensile Test

Each tensile test recorded force and displacement data, obtained using the LabJoy software (Cellscale, Waterloo, ON), and was sampled at 10Hz. Force and displacement data were then normalized to obtain stress and strain. Force was normalized by dividing the recorded force by the calculated cross-sectional area. Displacement was normalized by dividing the recorded displacement of the tissue by the original length of the tissue. From the calculated stress-strain curves, the variables of interest were the stress and strain at the end of the toe region, Young's modulus, and yield point (Figure 13). The toe region was defined as the non-linear region preceding the linear elastic region. Young's modulus was determined by calculating the slope of the linear region, determined by fitting the data in this region with a linear line. The

trendline was first applied to the linear region, including a small portion of the non-linear toe region, and then was gradually brought in towards the linear region until the R^2 value of the trendline was at least 0.98. The point where this established linear region began was also considered the stress and strain at the end of the toe region. Finally, the yield point, located at the end of the linear region, was considered the point at which stress either suddenly dropped or if the slope of the linear region decreased.

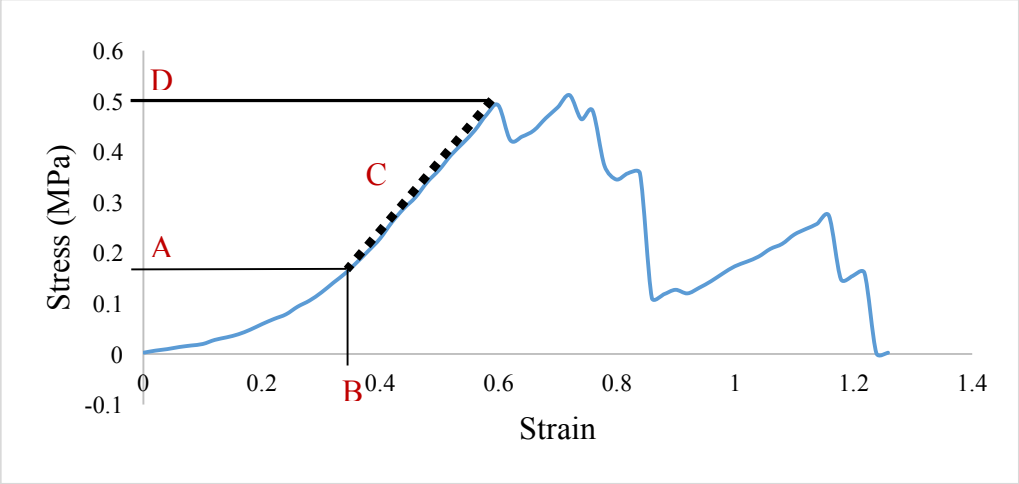

Figure 13. Stress-strain curve of a single layer tensile test indicating variables of interest: A) stress at the end of the toe region, B) strain at the end of the toe region, C) Young's modulus (slope of linear region), and D) yield point.

3.6.2 Peel test

Force and displacement data were recorded using the LabJoy software (Cellscale, Waterloo, Ontario) and sampled at 10Hz during each peel test. Force was normalized to the tissue bond width to obtain peel strength; displacement was not normalized to original length, as the original length is irrelevant in a mechanical peel test. From the peel strength-displacement curve, peel strength and peel strength variability were calculated. Peel strength was the average

stress of the plateau region (N/mm) and peel strength variability was the standard deviation of the plateau region (N/mm) (Figure 14).

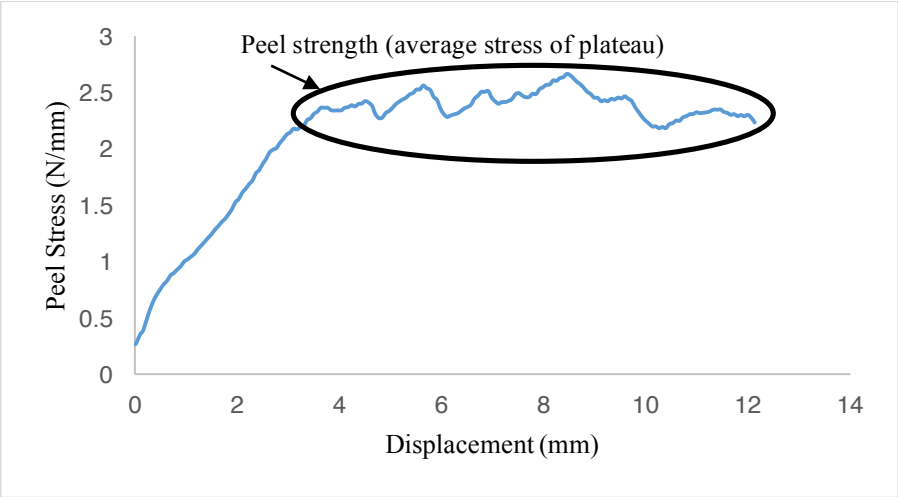

Figure 14. A typical peel stress-displacement curve obtained from a T-peel test.

3.7 Statistical Analysis

Two-way analyses of variance (ANOVA) were conducted to determine the effect of 1) twist and 2) compression on the variables of interest from the peel test and the single layer tensile test. The first factor (twist) had two levels: 0° and 12° and the second factor (compression) had two levels: 0N and 1000N. An alpha level of 0.05 was used.

4. Results

4.1 Single Layer (intralamellar matrix) Tensile Properties

4.1.1 Effect of Twist

Thirty-two single layer samples were obtained from twisted FSUs and 28 samples from untwisted FSUs. Overall, twist, regardless of compression exposure, had a significant effect on a number of the variables of interest from the single layer tensile test. There was a significant main effect of twist observed for all of the variables of interest with the exception of strain at the end of the toe region, which demonstrated a significant interaction between twist and compression. First, stress at the end of the toe region was lower in twisted samples compared to untwisted samples ($p=0.006$) (Figure 15). Twisted samples had a mean stress of 0.10 MPa (±0.06) while untwisted samples had a mean stress of 0.18 MPa (±0.14) at the end of the toe region. Second, twisted samples also had lower Young's moduli, indicating decreased stiffness, than untwisted single layer samples ($p=0.010$) (Figure 16). Specifically, twisted samples had a mean Young's modulus of 0.011 MPa (±0.008) while untwisted samples had a mean Young's modulus of 0.019 MPa (±0.015). Finally, twisted samples had lower yield points compared to untwisted samples ($p<0.001$) (Figure 17). Twisted samples had a mean yield point of 0.42 MPa (±0.23) while untwisted samples had a mean yield point of 0.81 MPa (±0.46). These findings are summarized in Table 2.

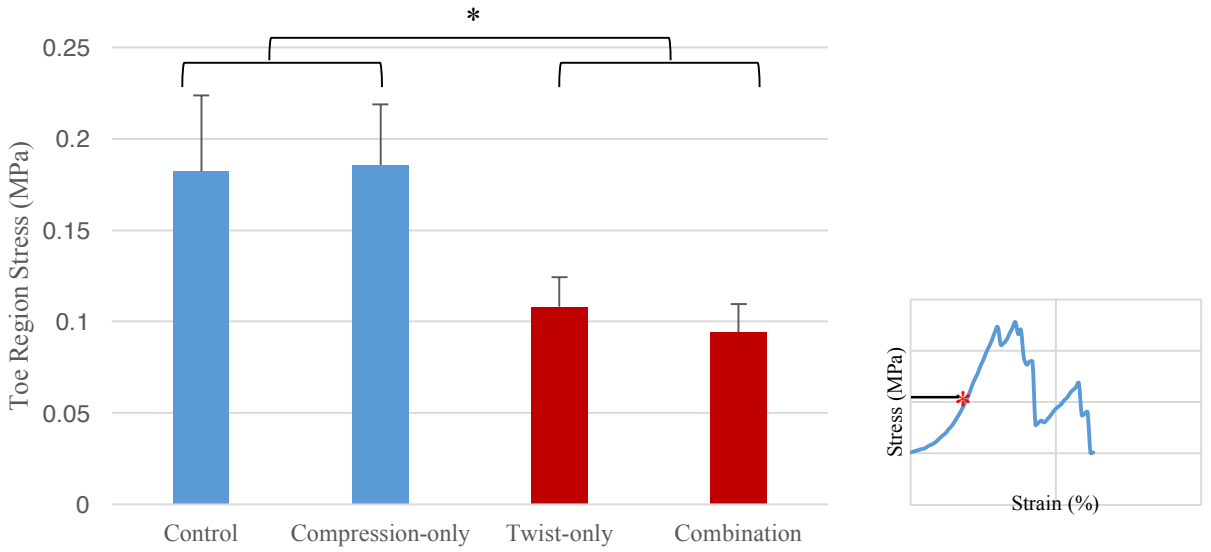

Figure 15. Average stress at the end of the toe region for each condition. Blue represents untwisted samples and red represents twisted samples. Significant differences identified by an asterisks. No significant interaction between compression and twist was observed. Standard error bars shown. Note the stress-strain curve indicating the variable of interest with a red asterisk (stress at the end of the toe region).

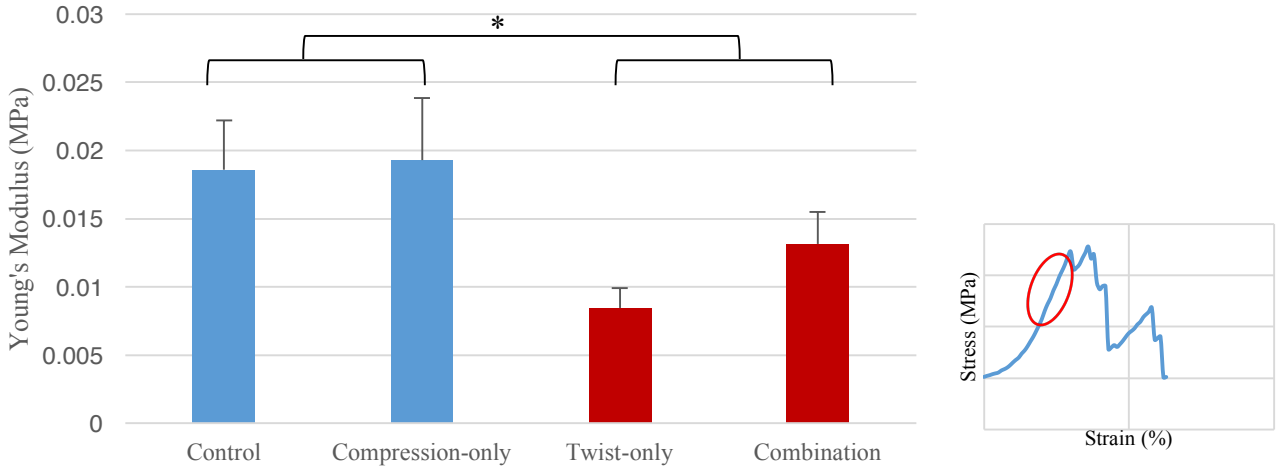

Figure 16. Average Young's modulus for each condition. Blue represents untwisted samples and red represents twisted samples. Significant differences identified by an asterisk. No significant interaction between compression and twist was observed. Standard error bars shown. Note the stress-strain curve indicating the variable of interest with a red circle (Young's modulus).

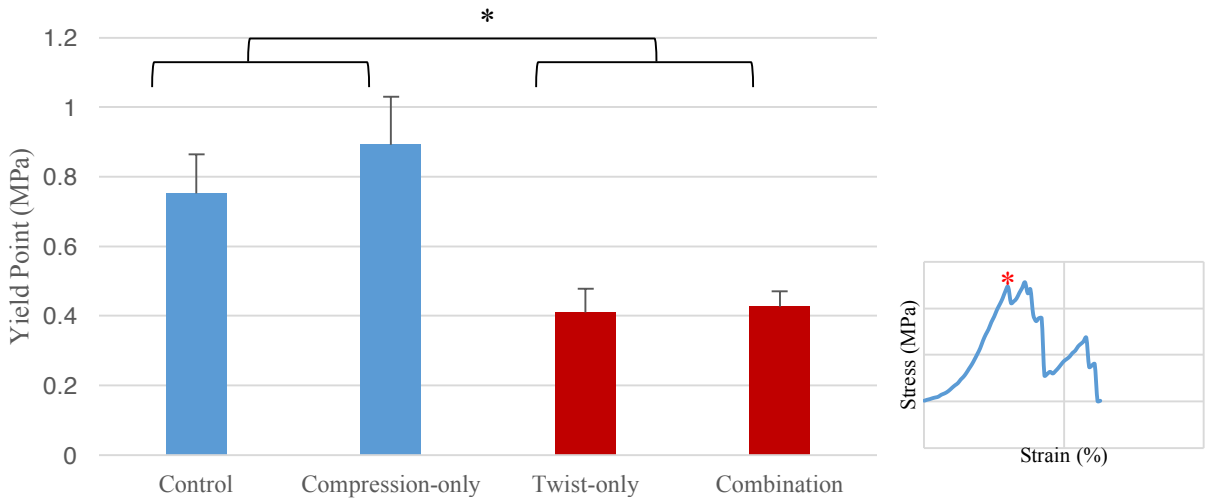

Figure 17. Average yield point for each condition. Blue represents untwisted samples and red represents twisted samples. Significant differences identified by an asterisk. No significant interaction between compression and twist was observed. Standard error bar shown. Note the stress-strain curve indicating the variable of interest with a red asterisk.

Table 2. Average values for the variables of interest from the single layer tensile tests collapsed across compression exposure for untwisted and twisted samples. Significant differences identified with an asterisk ($p<0.05$).

	End of Toe Region Strain (%)	**End of Toe Region Stress (MPa)**	**Elastic Modulus (MPa)**	**Yield Point (MPa)**
Untwisted (n=28)	38.17 (±25.29)	0.18 (±0.14) *	0.02 (±0.01) *	0.81 (±0.46) *
Twisted (n=32)	36.09 (±32.35)	0.10 (±0.06)	0.01 (±0.01)	0.42 (±0.23)

4.1.2 Effect of Compression

There was no significant main effect of compression observed for any of the variables of interest from the single layer tensile test. These findings are summarized in Table 3.

Table 3. Average values for the variables of interest from the single layer tensile tests collapsed across twist exposure for uncompressed and compressed samples. No significant main effect of compression was observed ($p>0.05$).

	End of Toe Region Strain (%)	**End of Toe Region Stress (MPa)**	**Elastic Modulus (MPa)**	**Yield Point (MPa)**
Uncompressed (n=32)	40.92 (±32.28)	0.15 (±0.13)	0.01 (±0.01)	0.58 (±0.41)
Compressed (n=28)	32.65 (±24.68)	0.13 (±0.10)	0.02 (±0.01)	0.63 (±0.41)

4.1.3 Effect of Twist Combined with Compression

The single layer tensile test yielded a significant interaction between twist and compression for the strain at the end of the toe region ($p=0.022$). Specifically, in specimens that were not exposed to twist, the magnitude of compression (either 0N or 1000N) did not alter the strain at the end of the toe region. However, for samples that were twisted, if they were also compressed with 1000N, the strain at the end of the toe region was significantly lower than specimens that were twisted but not compressed. (Figure 18).

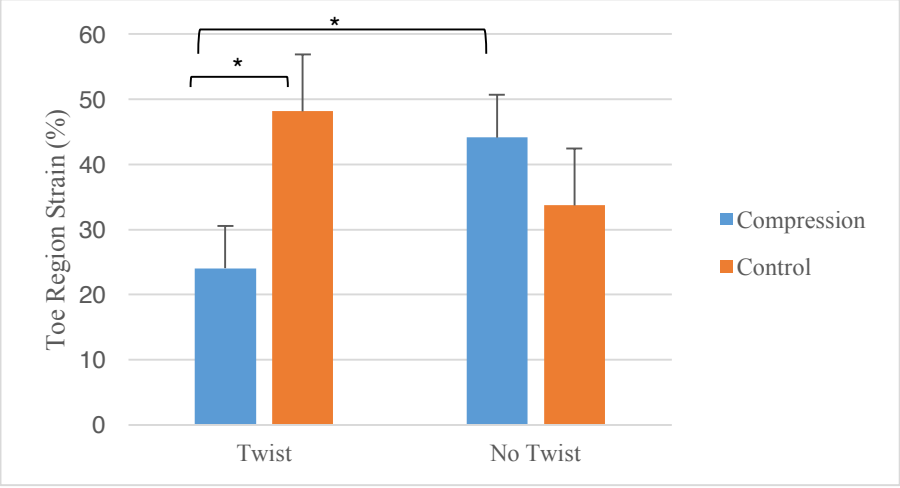

Figure 18. Interaction between twist exposure and compression exposure for strain at the end of the toe region ($p=0.022$). Significant differences between conditions identified with an asterisk ($p<0.05$). Standard error bars shown.

4.2 Peel Test (interlamellar matrix) Properties

Sixteen multi-layered annular samples were obtained from twisted FSUs and 16 samples were obtained from untwisted FSUs. There was no significant main effect of twist or compression for any of the variables of interest from the peel test. There were also no significant interactions observed for any of the variables. These findings are summarized in Tables 4 and 5.

Table 4. Average values for the variables of interest of the peel test collapsed across compression exposure (control vs compressed) for untwisted and twisted samples. No significant main effect of twist was observed for either variable ($p>0.05$).

	Peel Strength (N/mm)	**Peel Strength Variability (N/mm)**
Untwisted (n=16)	3.47 (±1.67)	0.74 (±0.58)
Twisted (n=16)	2.86 (±0.90)	0.58 (±0.29)
p value	0.223	0.352

Table 5. Average values for the variables of interest of the peel test collapsed across twist exposure for uncompressed and compressed samples. No significant main effect of compression was observed for either variable ($p>0.05$).

	Peel Strength (N/mm)	**Peel Strength Variability (N/mm)**
Uncompressed (n=16)	3.15 (±1.43)	0.60 (±0.31)
Compressed (n=16)	3.17 (±1.33)	0.72 (±0.56)
p value	0.954	0.482

4.3 Histological Analysis

Two samples per condition were obtained for histological analysis for a total of eight samples. There were several differences observed between the four conditions in terms of level of damage to the AF. In general, there appeared to be a loss of integrity of the AF in the twisted samples, as seen primarily by increased disruption of individual lamellae (Figure 19, 20). There also appeared to be an increased prevalence of delamination between lamellae of twisted samples

compared to untwisted samples. The control samples were largely intact with some disruption of individual lamellae (20a) and minimal delamination between lamellae observed. Similarly, the compression-only samples were largely intact with minimal delamination between lamellae and negligible disruption of individual lamellae (20b). The twist-only samples displayed more severe disruption of individual lamellae compared to the control samples (20c). Interestingly, the disruption of individual lamellae is visible approximately every other layer; presumably in the layers in the direction of twist, and therefore under strain. Additionally, there was some delamination occurring between lamellae within the AF. Finally, the combination samples, exposed to compression and twist, displayed the most severe disruption of individual lamellae yet minimal delamination between lamellae (20d). Similar to the twist-only sample, this disruption was evident in approximately every other single layer. The delamination observed in the twisted samples (both twist-only and combination samples), although minimal, was more prevalent than the observed delamination in the compression-only samples. The severity of the observed disruption of individual lamellae was similar in the combination sample to that observed in the twist-only sample (Figure 19).

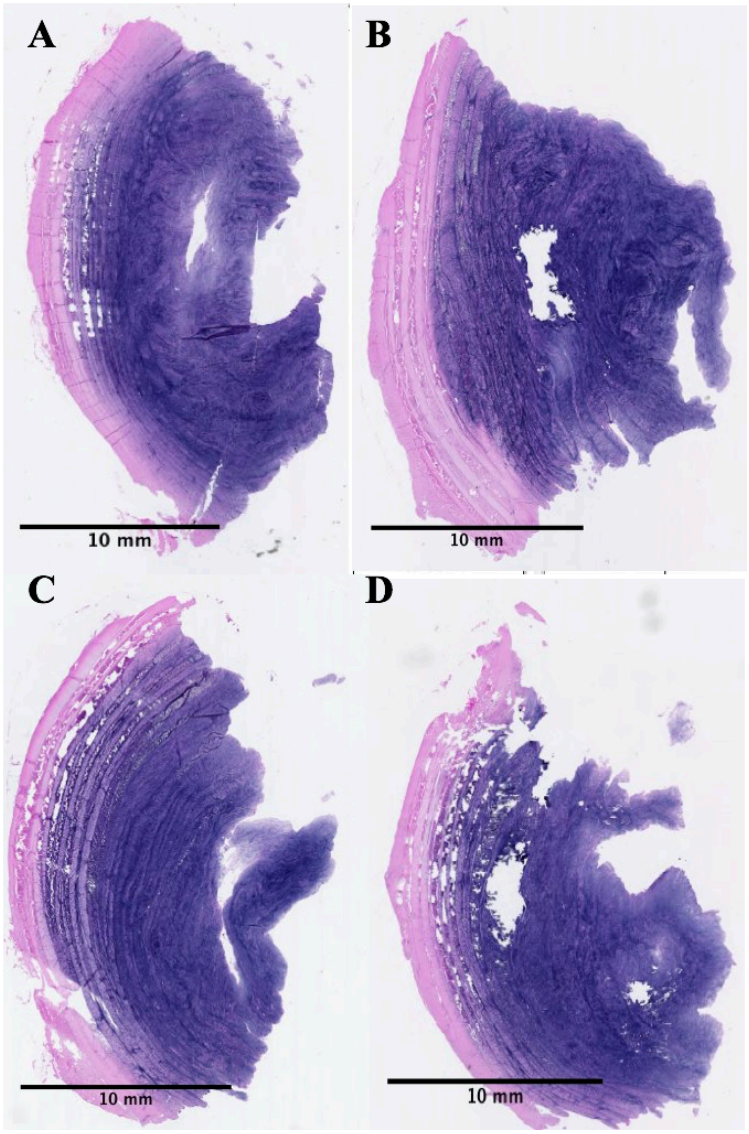

Figure 19. IVDs exposed to (A) control, (B) compression-only, (C) twist-only, and (D) combination loading stained with H&E to show type I (pink) and type II (purple) collagen fibres and damage to the AF. Scale bar: 10mm.

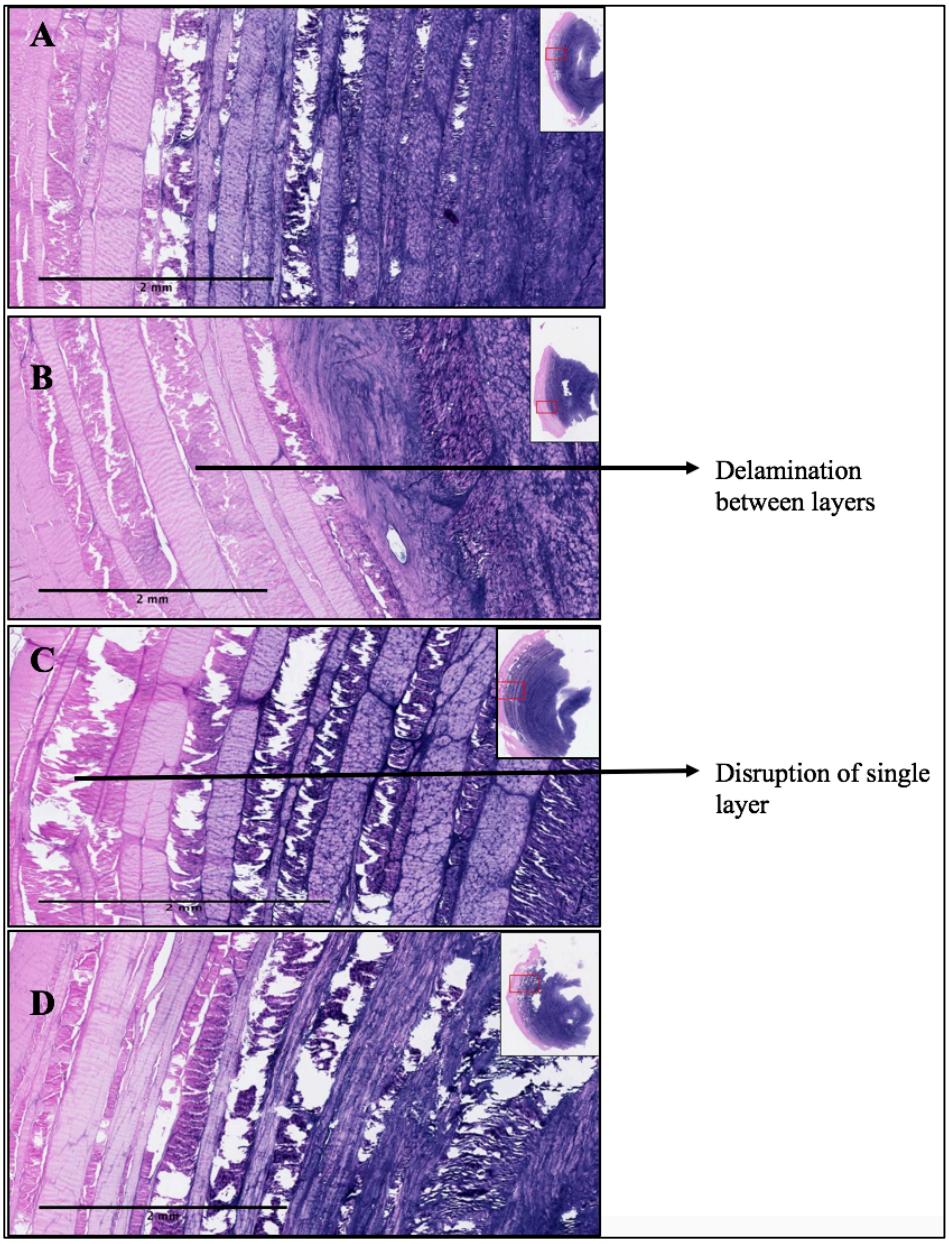

Figure 20. A section of IVDs exposed to (A) control, (B) compression-only, (C) twist-only, and (D) combination loading stained with H&E showing delamination between layers and disruption within a single layer. Scale bar: 2mm.

5. Discussion

5.1 Revisiting the Purpose and Hypotheses

Twist has been shown to increase the risk of IVD herniation in combination with repetitive flexion (Drake et al., 2005; Veres et al., 2010) however, the mechanisms behind this are not well understood given that IVD herniation progresses through both inter- and intralamellar matrices (Tampier et al., 2007; Gregory & Callaghan, 2011; Gooyers et al., 2015). Therefore, the primary purpose of the current study was to determine the effects of static, prolonged twist on the mechanical properties of both aforementioned matrices. It was hypothesized that twist would decrease the interlamellar matrix strength and the intralamellar matrix strength and stiffness due to the tensile and shear forces they are subjected to during twist. Twist did result in decreased intralamellar matrix strength and stiffness, as seen by significantly lower yield points, Young's moduli, and stress at the end of the toe region in the twisted single layer samples compared to the untwisted samples. However, twist did not result in reduced interlamellar matrix strength, as seen by no significant difference in peel strength or peel strength variability between the twisted and untwisted samples.

The secondary purpose of the current study was to examine the extent of damage to the IVD as a result of twist using histological analyses. It was hypothesized that damage (i.e. delamination and lamellae disruption) would be more severe in the IVDs exposed to twist compared to the IVDs that were not exposed to twist. Twist resulted in increased disruption within individual lamellae, indicating that twist damaged the intralamellar matrix. Twist also appeared to result in minimal delamination within the interlamellar matrix, suggesting that twist may have caused a small degree of damage to the interlamellar matrix.

5.2 Effect of Twist on the Interlamellar Matrix

Contrary to the proposed hypothesis, the interlamellar matrix appeared to be unaffected by twist as there were no significant differences in the peel test results between the twisted and untwisted samples. This finding suggests that static isolated twist, even to end range, does not affect the mechanical properties of the interlamellar matrix over a two-hour period. It is likely that, although twist applies tensile and shear forces to the interlamellar matrix (Schmidt et al., 2007; Stokes, 1988), these forces are not sufficient to disrupt the interlamellar matrix. Michalek, Buckley, Bonassar, Cohen, & Iatridis (2009) subjected AF samples from bovine IVDs to shear loading and determined, using confocal microscopy, that under shear there is minimal sliding between AF layers. This suggests that, in a bovine model, although twist subjects the entire AF to shear, the amount of sliding may be minimal within the interlamellar matrix, minimizing the risk of delamination. This was further corroborated by the minimal visible disruption within the interlamellar matrix histologically. Additionally, Adam, Rouch, & Scalli (2015) used a finite element model to analyze the interlamellar matrix of bovine tail IVDs and concluded that bovine IVDs had a high degree of interlamellar shear resistance. This ability to resist shear may have minimized damage to the interlamellar matrix in the current study; however, it is unknown if this same resistance to shear exists in the human IVD.

Despite the results from the peel test, the histological analysis demonstrated a small degree of delamination in the samples exposed to twist. It is possible that, because the delamination was mostly isolated to small regions and was not present across the entire IVD, the samples used for the peel test were not from a region in which delamination was present. It is also possible that the observed delamination was minimal enough that, if it was present within

the sample used for the peel test, it did not disrupt the mechanical properties of the interlamellar matrix and therefore is not a major cause for concern.

5.3 Effect of Twist on the Intralamellar Matrix

Although twist did not have an effect on the mechanical properties of the interlamellar matrix, it did have a substantial effect on the mechanical properties of the intralamellar matrix. In particular, twist, regardless of the magnitude of compression, resulted in a significant decrease in the intralamellar matrix stiffness (Young's modulus), strength (yield point), and the stress at the transmission between the toe region and elastic region (Figure 21). In each case, the findings suggest a weakening effect of twist on the intralamellar matrix.

One key component of the intralamellar matrix that may have been affected by twist is elastin. Elastin is an important component within the intralamellar matrix because it keeps the parallel aligned type I collagen fibres within a single layer strongly bound together and assists in AF recovery following tensile loading (Smith & Fazzalari, 2009). Smith, Byers, Costi, & Fazzalari (2008) demonstrated that, when tension was applied perpendicular to the orientation of the collagen fibres, AF stiffness decreased following elastin degradation. Likewise, the current study found the samples exposed to twist were also significantly less stiff indicating that elastin may have been affected by twist. Additionally, Michalek and colleagues (2009) found that elastin was responsible for stiffening AF fibre bundles when exposed to shear. They hypothesized that damage to elastin, such as in IVD injury, can change the AF's ability to resist shear thus making it more susceptible to microfailures when exposed to motions that cause high shear stresses such as bending and twisting. Similar results were seen in the current study in

which twist resulted in microfailures to the AF, seen within the intralamellar matrix, possibly as a result of damage to elastin.

Samples exposed to twist also demonstrated significantly lower stress values at the end of the toe region as well as at failure (yield point) than untwisted samples (Figure 21). Similar to the effect on stiffness, this is likely attributable to damage to elastin as well as other components of the intralamellar matrix, namely type VI collagen and proteoglycans (Melrose et al., 2008), making the twisted samples less resistant to loading. In the case of the toe region stress, twist likely affected the type VI collagen fibres such that they required less stress to reach an uncrimped state prior to deformation in the elastic region. This may be due to damage or may be a result of a residual load, possibly due to fibre reorientation as a result of the twist exposure, on the collagen fibres even after the two-hour loading protocol. However, further research using high-resolution imaging of the fibres is needed to confirm this hypothesis. This information would be of particular importance, as it is often within the toe region that single layers of the AF and the intralamellar matrix are strained *in vivo* (Skaggs et al., 1994).

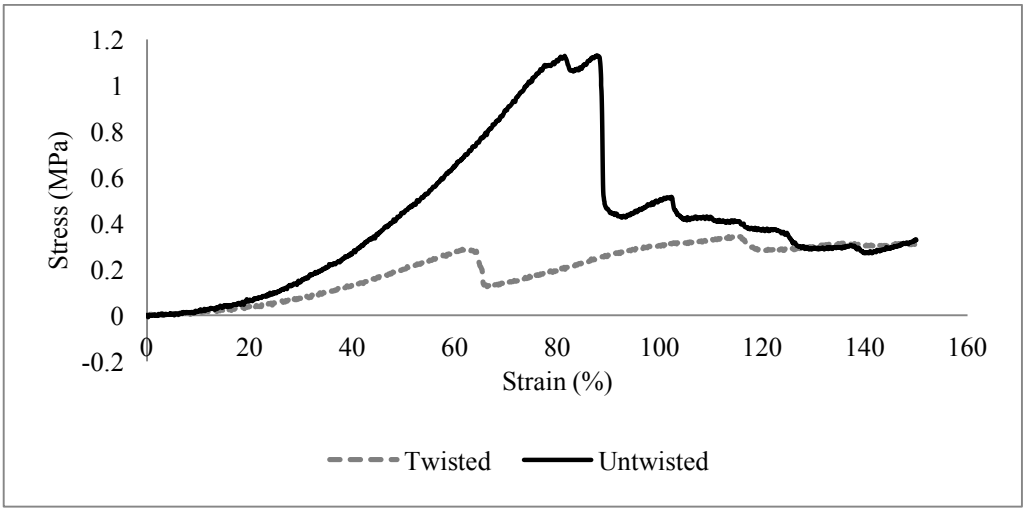

Figure 21. Representative stress-strain curves of a single layer tensile test from a twisted and an untwisted IVD. Note the lower toe region stress, Young's modulus, and yield point in the twisted sample.

In the case of reduced failure strength, it is more likely a result of damage to the intralamellar matrix components, rather than residual loading due to fibre orientation. This is because type VI collagen fibres within the intralamellar matrix are involved in bearing the majority of the tensile load applied to the matrix (Adams & Green, 1993) and therefore would provide the matrix with its tensile strength.

The last variable affect by twist was the strain at the end of the toe region. However, in this case, a significant interaction between magnitude of twist and magnitude of compression was observed such that samples exposed to both twist and compression had significantly lower strain values at the end of the toe region than those exposed to compression-only and twist-only. Similar to toe region stress, this may be due to some sort of load on the intralamellar matrix resulting in decreased required strain before entering into the elastic region of the stress-strain profile. Interestingly, unlike toe region stress, this was further affected by prolonged compression, indicating a cumulative effect of twist and compression on the uncrimping of the type VI collagen fibres.

Twist had a significant effect on all of the variables of interest from the single layer tensile test. Additionally, the histological analysis indicated that twist appeared to disrupt the intralamellar matrix, supporting the results from the single layer tensile test. The intralamellar matrix bears applied tensile loads and assists in recovery following tensile strain in order to provide structural support to the AF (Adams & Green, 1993; Smith & Fazzalari, 2009). Therefore, when the components of the intralamellar matrix became damaged due to the twist, the matrix lost its ability to provide structural support to the collagen fibres within each lamella and keep them tightly bound together. As a result, the collagen fibres likely became disrupted and disorganized, as seen in the histological analysis. Gunzburg and colleagues (1992), using histology, discovered annular lesions in the AF that were exposed to axial torsion, supporting the results of the current study.

5.4 Effect of Compression on the Inter- and Intralamellar Matrices

Overall, compression did not have an effect on the mechanical properties of the inter- or intralamellar matrices. This was demonstrated by no differences in the variables of interest from the peel test or the single layer tensile test between compressed and uncompressed samples. The only variable affected by compression was the strain at the end of the toe region, in which there was an interaction between twist and compression described above. The histological analysis also demonstrated no effect of compression on the matrices, as seen by no major disruptions of individual lamellae or delamination between lamellae in samples exposed to compression-only loading. These results support previous research examining the effect of compression on the interlamellar matrix of bovine IVDs in which there was no difference in peel strength between compressed and uncompressed samples (Clayton & Gregory, 2017). Similarly, Korecki,

MacLean, & Iatridis (2008) used bovine IVDs to determine the effect of compression on IVD mechanics. They found that the observed changes in stiffness and IVD height loss recovered after each exposure to compression, proposing that compression is a healthy loading condition for bovine IVDs. Additionally, Stokes & Greenapple (1985) found that compression resulted in less strain to the AF fibres of human IVDs than twist did, providing additional support to the current study. The results of these studies are encouraging because, *in vivo*, human IVDs are subjected to compression for a large part of the day due to the force of gravity and the surrounding musculature. Therefore, if compression was a significant risk factor for AF damage, the prevalence of IVD injury would likely be much higher.

In summary, the results of the current study suggest that static isolated twist damages the intralamellar matrix. Given that part of herniation progression is migration through the layers of the AF through clefts in the intralamellar matrix (Tampier et al., 2007), these results help explain why the risk of herniation is higher when the IVD is twisted. With weakening of the intralamellar matrix as a result of twist, it is likely easier for the NP to migrate through clefts between the intralamellar matrix. Additionally, these findings help explain results found in previous research looking at twist in combined loading scenarios (Drake et al., 2007; Marshall & McGill, 2010; Veres et al., 2010). Veres and colleagues (2010) subjected ovine lumbar IVDs to 7° of flexion and 2° of axial rotation and determined that, when flexion was combined with twist, the number of herniations did not increase compared to flexion alone, however herniation occurred more easily in the IVDs exposed to twist and flexion. Similarly, Drake and colleagues (2007) subjected porcine cervical IVDs to repetitive flexion and static compression combined with axial torque. They concluded that when repetitive flexion was combined with axial torque, the occurrence of IVD herniation was accelerated compared to repetitive flexion alone. These

studies suggest that twist, when combined with repetitive flexion, facilitates herniation progression within the IVD. This inspired the current study, focusing on potential mechanisms to explain what was occurring in the groups exposed to both flexion and axial twist/torque to cause an increased risk of IVD herniation. The current study provides a proposed mechanism behind this accelerated herniation progression which is that twist weakens the intralamellar matrix. With a weaker intralamellar matrix, clefts will form more easily, allowing IVD herniation to progress more easily, and subsequently more quickly, when twist is combined with repetitive flexion.

5.5 Limitations

The main limitation in the current study was the use of bovine IVDs as a representative of human IVDs. As a result, it is unknown if the findings of the current study are comparable to human IVDs. However, bovine IVDs have been shown to be a suitable model to study human IVDs (Beckstein et al., 2008; O'Connell et al., 2007; Showalter et al., 2012) therefore they are a good first step in order to determine the effect of twist on the IVD. Another limitation was that samples for the peel test and the single layer tensile test were only taken from the OAF because of the difficulty of dissecting from the IAF. It is unknown whether the IAF, which is where herniation progression would start, responds in the same way to twist as the OAF. However, the OAF should have been more severely damaged as a result of twist because it is further away from the point of rotation (the centre of the IVD in the case of the circular bovine IVD) and likely experienced more tensile and shear forces compared to the IAF. When considering the histological analysis, a limitation of the current study was that it was difficult to determine whether changes to the AF were a result of twist exposure or were due to processing (cutting and/or staining of the samples). Another limitation of the histological analysis was the use of

only one IVD per condition. This made it difficult to compare the degree of damage between the four conditions. A final limitation was that twisting torque was not measured in the current study. This may have confirmed that 12° of twist in the bovine IVD is comparable to end range twist in humans in terms of torque magnitude.

5.6 Future Directions

The current study provided important information regarding the mechanisms of injury of twist on bovine IVDs and demonstrated that twist can induce damage to the microstructures of the AF, supporting the need for further research into twist. Future research should look at twisting within physiological ranges and not just end range in order to determine if damage is possible within ranges experienced *in vivo*. Additionally, research should examine dynamic twist and its effect on the mechanical properties of the microstructures of the AF because dynamic twist is more commonly experienced *in vivo* compared to static twist. Future research should also look at the effects of twist on the AF using high-resolution imaging in order to test the proposed hypothesis that damage to the intralamellar matrix results in reorientation and disorganization of the fibres within the intralamellar matrix and individual lamellae of the AF. Finally, future research should look at twist combined with flexion, similar to previous studies (Drake et al., 2007; Marshall & McGill, 2010; Veres et al., 2010) but with a focus on the microstructures of the IVD and the mechanisms of injury. That is, examining the mechanical properties of the inter- and intralamellar matrix, as in the current study, but in combination with flexion in order to further understand what is occurring within these matrices when combined with twist to increase the risk of IVD herniation. Together, this research will allow for a better understanding of the

effects of twist on the IVD and may point towards a need to minimize individuals' exposure to twist in order to minimize the risk of IVD injury.

6. Conclusion

The current study examined the effect of twist on the mechanical properties of the inter- and intralamellar matrices. The interlamellar matrix was not affected, however twist did appear to damage the intralamellar matrix, which is responsible for adhering adjacent collagen fibres within a single layer. Elastin and type VI collagen fibres were likely the components of the intralamellar matrix affected by twist resulting in an overall weakening of the matrix. The intralamellar matrix has been studied previously with respect to IVD herniation and appears to play an important role in herniation through cleft formation (Tampier et al., 2007). The current study suggests that the tensile and shear forces twist applies to the AF result in damage to the intralamellar matrix and consequently, increase the risk of cleft formation, which would subsequently accelerate the occurrence of IVD herniation when exposed to additional loading. This suggests that axial twist may need to be minimized when treating IVD herniations in order to prevent further injury. The results of the current study shed light on the involvement of the microstructures of the AF when the IVD is exposed to twist, suggesting that damage to the intralamellar matrix is likely the source of the increased risk of IVD herniation seen in previous research when combined with repetitive flexion. Therefore, the current study improves our understanding of the involvement of the microstructures of the AF in herniation development when twist is combined with additional loading.

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