Replication of annulus fibrosus through fabrication and characterization of polyurethane and cellulose nanocrystal composite scaffolds

Brody A. Frost and E. Johan Foster

Department of Materials Science and Engineering, Macromolecules Innovation Institute, Virginia Tech, Blacksburg, VA, USA

ABSTRACT

This study sought to obtain a simple scaffold for annulus fibrosus (AF) repair or replacement using a combination of polyurethane (PU) reinforced with cellulose nanocrystals (CNCs). Composites containing up to 20 wt% CNCs were solvent casted and fabricated into ribbons and radially layered structures to be mechanically tested in tension, compression, creep, and relaxation. Tension and compression testing on swollen composite films and ringed structures, respectively, revealed that the PU 90/10 and PU 80/20 composites had elastic moduli most closely related to the natural AF tissues. Creep and relaxation revealed that the composite materials show a greater percentage of elastic response and longer relaxation times than natural intervertebral disc (IVD) tissues. It was shown that this approach leads to a scaffold that nearly mimics the mechanical properties of natural IVD tissues, while allowing fine tuning of these mechanical properties by varying CNC content and the ringed structure.
Introduction

Intervertebral disc (IVD) degeneration is the most common cause of neck and lower back pain, affecting anywhere from 12 to 35% of a given population, and chronically disabling roughly 10% of sufferers.\(^1,^2\) The total associated costs for individuals and health care exceed $88 billion annually, making it the third largest medical expense, behind diabetes and cancer.\(^3\) The most common cause of disc degeneration is degenerative disc disease, which can be linked to and exacerbated by many factors, including genetics, aging, obesity, intense exercise, smoking, and other environmental effects within the body.\(^6,^7\) As the disc begins to undergo degeneration, the nucleus pulposus (NP) and annulus fibrosus (AF) begin to degrade through alterations in composition and biomechanical properties, specifically the loss of proteoglycans and subsequent hydration, crosslinking of collagen fibrils within the AF, and inhibited ability to pressurize the NP. These changes eventually lead to the formation of fissures within the AF lamellae, and ultimately, the bulging and herniation of the nucleus pulposus NP onto the spinal cord.\(^2,^8\) This disease occurs most in the lumbar section of the spine since it possesses the greatest load bearing requirements, often times leading to physical therapy and pain management or invasive surgical methods. However, due to the harsh mechanical conditions experienced by the lumbar spine, more efficient treatment options have proven difficult to achieve.\(^2,^11,^12\)

Currently, the most common surgical solutions include spinal fusion surgery and total disc replacement, and although these treatments offer pain relief for the majority of patients, there are multiple limitations that arise with each option.\(^2,^12,^13,^15\) Spinal fusion surgery severely limits the mobility by fusing two vertebrae together, hindering any individual vertebral movement, and total disc replacement can cause hypermobility among the vertebrae, with both options offering little to no shock absorption or cushioning of loads.\(^13,^14,^16,^17\) To help alleviate some of these limitations, a multitude of research is currently going into new techniques such as cellular therapy, gene therapy, and tissue engineered scaffolds, each contributing a vital solution to IVD replacement and AF repair.\(^20\)–\(^25\) Among the most common tissue engineered scaffolds are polymer composites, both natural and synthetic, which have started playing a vital role in the field of biomaterials.\(^21\)–\(^23\),\(^26\)–\(^30\) These scaffolds can be cultured with natural cells and tissues to elicit greater biomimetic properties for a given application, such as IVD replacement.\(^23\)–\(^25,^27\)–\(^30\)

In particular, polyurethane has become a well-known biomedical material due to its resilience to corrosion and abrasion, durability, elastomeric properties, fatigue resistance, and biocompatibility.\(^31\)–\(^33\) As well, it can be combined with different fillers or natural cells/tissues to form versatile composites, in which mechanical and biochemical properties can be finely tuned to adapt to vast potential medical applications.\(^32,^33\)

With the amount of effected population, medical costs associated with disc degeneration, and current treatment options posing multiple limitations to patients, a new solution to AF repair and IVD replacement has been proved essential.\(^34,^35\) Because of this, vast amounts of current research are going into better potential solutions, specifically those of biopolymer scaffolds.\(^34,^35\) However, most only pose a focus on the biological aspects of the scaffold, with little to no attention to the mechanical properties, proving the need for further research in the field, especially with regards to mechanical characterization.\(^22,^24,^25,^27,^29,^30,^34,^35\) This study seeks to progress research in the field of AF repair and replacement through the biomedical applications of polymer composites, by way of solvent casting composites and fabrication methods for radially layered structures consisting of polyurethane (PU) and cellulose nanocrystals (CNCs), Scheme 1. The scope will focus on multiple mechanical characterization techniques, such as tension, compression, creep, and relaxation tests, to establish whether these composites will be suitable for mimicking the mechanical properties of the AF, or supplemental to other current techniques. The novelty of this fabrication technique is its ordered composite structure, mimicking

![Scheme 1. Schematic representing the overall processing method for the fabrication of the AF scaffolds, with steps as followed: solution casting an 80/20 wt/wt PU solution/CNC dispersion and melt pressing into a uniform film to be cut into ribbons; ribbons coiled into a ringed-structured scaffold, fusing the outermost ribbon together with DMF; and capping the scaffold with endplates by DMF fusing, to allow for injection of water into the hollow center. *Note that the capping and injecting of water was solely for the demonstration of possible future work continuing from this research and potential of final application in the spine.]}
the rings of the natural AF, and possessing a hollow center, in which an artificial NP can be introduced to further improve biomimetic properties compared to current solutions, Figure 1. Although the composite will eventually need to go in the body, cell cultures, immune response, toxicity, and other biological testing are out of scope for this study.

Materials and methods

Materials selection

A Texin RxT85A polyurethane (PU 85A) was obtained from Covestro to be used as the polymer matrix for the composite. PU 85A is an FDA approved ether-based polyurethane with a shore hardness of 85A, tensile strength of 36.6 MPa, ultimate elongation of 610%, and exceptional hydrolytic stability, abrasion resistance, and corrosion resistance, with more detailed material properties specified in the given reference. Freeze-dried CNCs were manufactured by the US Forest Service’s Cellulose Nanomaterials Pilot Plant at the Forest Products Laboratory and obtained from University of Maine Process Development Center to be used as the filler for the composites. They demonstrated dimensions of roughly 5–10 nm in diameter by 150–200 nm in length, for an aspect ratio range of 10–40, and contained a cellulose surface sulfation of 0.94 wt% sulfur as sodium form, with more detailed properties specified in the given reference. Freeze-dried CNCs were manufactured by the US Forest Service’s Cellulose Nanomaterials Pilot Plant at the Forest Products Laboratory and obtained from University of Maine Process Development Center to be used as the filler for the composites. They demonstrated dimensions of roughly 5–10 nm in diameter by 150–200 nm in length, for an aspect ratio range of 10–40, and contained a cellulose surface sulfation of 0.94 wt% sulfur as sodium form, with more detailed properties specified in the given reference.36

N,N-Dimethylformamide, anhydrous, 99.8% (DMF) was obtained from Sigma Aldrich to be used as the solvent for both a PU 85A solution and a CNC suspension.

Casting process

Both a 40 mg/mL suspension of CNCs in DMF and an 80 mg/mL solution of PU 85A in DMF were created prior to casting of the films. The CNC suspension was created by weighing out 16 g of dry CNCs in 400 mL of DMF, and vigorously shaking by hand, mixing for 10 min with a Fisher Scientific digital vortex mixer, and sonicating for 30 min at 110 W and frequency of 40 kHz with a Branson M2800 ultrasonic bath or until well dispersed. Quality of dispersion was determined by stability of the CNC dispersion in DMF as a function of storage time, Figure S1 in supplementary materials, following an established procedure.38 The PU 85A solution was created by weighing out 32 g of PU 85A pellets in 400 mL of DMF, and using a hot plate at 140°C while stirring at 1000 rpm for 1 h or until all the PU 85A pellets had dissolved. Once the CNC suspension and PU 85A solution were completely homogenous, they were mixed together, using a stir bar, in composition ratios shown in Table 1. These concentrations were chosen to determine the aggregation threshold of the CNCs within the PU 85A matrix, as well as variations in mechanical properties as CNC content is increased. The PU 70/30 composition was shown to surpass the threshold, causing aggregation of CNCs within the matrix upon drying, Figure S2 in supplementary materials, and ultimately resulting in brittle fracture, Figures S3 and S4 in supplementary materials.

The combined mixture was placed in an oil bath at 140°C while stirring at 750 rpm until enough DMF was evaporated to increase viscosity (to the ‘viscosity of molasses’). The viscous mixture was then transferred into a Teflon dish, set on a hot plate at 80°C, and left overnight to slowly evaporate the DMF. After drying overnight, the sample was removed from the hot plate, and put into a vacuum oven at 80°C and –27 in*Hg for 4 h or until all DMF was removed. Thermogravimetric analysis (TGA) was employed utilizing a TA Instruments Q50 TGA with a sample weight of 20 mg, heating rate of 10°C/min from 25 to 150°C, and a dwell time of 10 min at 150°C, to confirm the removal of all
solvent. This ensures that the solvent will not act as a plasticizer during mechanical characterization, to obtain accurate material properties measurements.

Sample fabrication method

After complete removal of the solvent, each sample composition was hot pressed using a 3851-0 Carver Press at 140°C and 3 MPa of pressure for 5 min, using 0.9–1.0 mm aluminum spacers to ensure uniformity of thickness throughout the film. The films were cut with a razor blade into 1.0 cm wide ribbons for tension testing and 2.0 cm wide ribbons for creep testing. Three ribbons of each composition were placed into a beaker filled with deionized water to swell the samples, while three of each remained dry.

In an analogous procedure, ribbons were used to create the ringed samples, with dimensions of 0.3 mm thick and 9.0 mm wide. Each composite composition had 500 and 650 mm of ribbons measured out in total length to be coiled together, using inner diameter of roughly 15 mm and 12 mm, respectively, specific dimensions in Table 2. The 500 mm (thin) samples used the same inner to outer diameter dimension ratio as a natural AF to NP, while the 650 mm (thick) samples used a greater ratio of AF to NP diameter to determine the effects that adding additional rings had on the mechanical properties, and discussed in detail in the subsequent section. After each sample was measured, the respective inner diameter was measured on one ribbon and held together by fusing the contact points of the ribbon with DMF. All the remaining ribbons were wrapped around the inner ring, with the ends of the outermost ring being dipped for 3 s in DMF, to fuse the ring and hold the middle rings together. This ensured that the middle rings would not unravel, however were still able to delaminate to act as individual rings when tested, instead of a chunk of composite material. All ringed samples were dried in the vacuum oven, using the same procedure previously mentioned. Every ringed sample was then swollen in DI water for at least 48 h prior to testing to ensure swelling throughout the entire sample.

It should be noted that during the casting and melt pressing processes, the CNCs were subjected to oxidation at the increased temperatures, leading to a yellowish coloring. As the CNC content increased, the yellow color became deeper within the composite, as expected with higher oxidation. Figure S2 in supplementary materials represents the coloring effect of the thermal processes on the CNCs, showing the discoloration as CNC content increases. This discoloration can also be noted in Scheme 1 and Figure 5, in which the PU 80/20 composition is shown.

Swelling procedure

Swelling tests were performed on six square samples of each composition with dimensions of 1 cm² in area and 0.3 mm in thickness. Each sample was subjected to a 48-h dipping time in 100 mL of DI water at 25°C. After 48 h, there was no additional water absorption, therefore the samples were removed and measured for weight and volume differentials.

Mechanical testing procedures

Tension testing

Tension testing was performed utilizing a TA Q800 Dynamic Mechanical Analyzer (DMA) on six of each composite composition (three dry and three wet for 48 h) to determine the initial mechanical properties of the composite material. Each sample was tested using an isostatic force test with a force ramp rate of 3 N/min at 25°C until a maximum of 18 N was reached.

Compression testing

Compression testing was performed utilizing an Instron 5900 Series Universal Testing System on three ringed samples of each composite composition for each total coil length, all of which had been previously swollen for at least 48 h, to determine the compressive mechanical properties of the ringed structures. Each sample was tested on parallel plates using an isostatic crosshead displacement rate of...
5 mm/min at 25°C until the 1 kN load cell was maxed out around 950 N. The force and crosshead displacement were recorded, and converted into stress and strain using cross-sectional area and initial sample height, respectively.

**Creep testing**

Creep testing was performed utilizing an Instron 5900 Series Universal Testing System on three ribbons of each composite composition at 25°C, all of which had been previously swollen for at least 48 h, to determine the viscoelastic properties of the composite material. The tops of the ribbons were fixed into the tension clamp attachment on the upper crosshead, while a weight was attached to the bottoms of the ribbons, to induce creep. An extensometer was then attached to the middle of the ribbons, to receive more accurate strain data, since crosshead displacement could not be recorded. As the crosshead was raised, the initial elastic response and delayed viscous response were recorded over a 12-min time period. Due to the stiffness of the PU 80/20 composite, a greater weight of 30 N was needed to observe creep trends similar to that of the PU 100/0 and PU 90/10 with a 17 N weight. To ensure accurate data comparison between compositions, all elastic and viscous strain data was normalized to the lowest induced stress, which was calculated using the applied force and cross-sectional area of each ribbon.

**Relaxation testing**

Relaxation testing was performed utilizing an Instron 5900 Series Universal Testing System on three ringed structures of each composition for the intermediate crosshead displacement (50 mm/min) to reach a kN load cell was maxed out around 950 N. The force and crosshead displacement were recorded, and converted into stress and strain using cross-sectional area and initial sample height, respectively.

**Results**

**Swell testing**

While swelling the samples in preparation for mechanical testing, it was observed that the composite materials absorbed water, increasing by greater amounts of mass and volume as CNC content increased, Table 3. After 48 h of swelling, the samples absorbed negligible amounts of additional water, therefore all data for longer swelling times were omitted. It should also be noted that the samples maintained the same amount of water uptake (mass and volume) after reaching an equilibrium point for over two years. This leads to the assumption that when placed in the body for long periods of time, the samples will not dry out or increase in swelling, which could cause potential problems for the long-term mechanical properties or the finite space in which they are inserted. Also, because the fluid in the spine contains some ionic strength, there is a possibility that fluid absorption within the body could differ from the DI water used in this study. However, based on a study by Tham et al., simulated body fluid and water have similar absorption properties. Although the composite system being compared is not identical to the composite system within this study, it has been shown that water and simulated body fluid (SBF) have relatively equal maximum fluid absorption. However, SBF was shown to have a larger diffusion coefficient, which could be beneficial to the relaxation times of the AF scaffold. Therefore, it can be assumed that the swelling property of the scaffold will act similarly in the spine as it does in DI water.

**Tension testing**

Data acquired from DMA tension testing for each composite composition, both dry and swollen, agreed with predicted correlations between the effects of CNC content and moisture uptake on the resulting mechanical properties, Figures 2 and 3, and Table 4. As the CNC concentration increased, the composites showed an increase in moduli and yield strength, with a decrease in yield strain. This results from the CNCs within the composite contributing reinforcement from a filler-filler network, instead of solely following the rule of mixtures, leading to higher mechanical reinforcement, specifically stiffness. As the CNC concentration increase up to 30 wt%, like that of PU 70/30, aggregate defects can form within the composite, Figures S2 and S3 in supplementary materials, leading to stress-concentrators and brittle-like qualities, Figure S4 in supplementary materials. However, as moisture content increased, the composite softened due to the cleaving of the hydrogen bonds between the hydrogen bonds.
CNCs. This leads to a decrease in the stronger CNC–CNC interactions, and therefore, overall mechanical reinforcement, as shown by the decrease in modulus and yield strength, and increase in yield strain and overall toughness from the dry state to the swollen state, Table 4. This is to be expected as moisture increases, the hydrogen bonds between adjacent CNCs are broken, leading to a composite with individual CNCs acting as the filler instead of potential aggregates or networks of CNCs.43 Therefore, water uptake increases load bearing within the matrix, leading to higher elongations and weaker moduli. Also, CNCs acting individually increases interfacial interactions between the CNCs and the PU 85A matrix, allowing the composite to act more similar to an isotropic material, which can be compared to the exceedingly large error bars for the dry composites, Figure 3. Ultimate tensile stresses and strains for the composites could not be determined since each sample reached either maximum displacement or force limitation of the DMA.

Note that although the DMA was run at 25 °C, both materials within the composite are thermally stable well past the temperature of the body. As well, neither material demonstrates any morphological transition within the temperature range of the body, therefore was assumed to act the same mechanically at both room and body temperatures.

Ultimately, tension testing revealed that the composite material exhibits mechanical properties most similar to the AF using wet PU 100/0, PU 90/10, and PU 80/20 composites, while the wet PU 70/30 composite showed high aggregation of CNCs, leading to defects and brittle fracture failure, as shown in Figure S1 in supplementary materials. Therefore, PU 70/30 was determined to surpass the percolation threshold of CNCs, and was omitted from the rest of the study. As well, all samples further characterized were swollen for at least 48 h prior to testing.

Table 4. Tensile properties of dry and wet composites as determined from DMA.

| Composition | Dry Modulus (MPa) | Wet Modulus (MPa) | Dry Yield Strength (MPa) | Wet Yield Strength (MPa) | Dry Elongation at Yield (%) | Wet Elongation at Yield (%) |
|-------------|------------------|------------------|--------------------------|--------------------------|-----------------------------|-----------------------------|
| PU 100/0    | 16.0 ± 0.3       | 17.1 ± 0.2       | 2.2 ± 0.0                | 2.4 ± 0.0                | 18.2 ± 0.9                  | 19.31 ± 0.9                 |
| PU 90/10    | 38.7 ± 12.4      | 24.4 ± 0.2       | 2.5 ± 0.2                | 2.3 ± 0.1                | 8.4 ± 1.5                   | 13.6 ± 1.6                  |
| PU 80/20    | 98.1 ± 29.4      | 32.3 ± 0.3       | 3.2 ± 0.5                | 2.2 ± 0.1                | 5.4 ± 2.5                   | 9.7 ± 0.6                   |

Standard deviations were taken from three samples of each composition, both dry and wet.
Compression testing of the wet PU 100/0, PU 90/10, and PU 80/20 ringed structures utilizing the Instron, resulted in expected trends for mechanical reinforcement of CNCs, similar to those observed in tension testing acquired from the DMA. As well, the increase in overall ribbon length proved to further strengthen the mechanical properties of the ringed structures, leading to higher compressive moduli, yield strength, and overall toughness, while retaining similar yield strain, Figure 4 and Table 5. Each sample showed an initial toe region of roughly 7–10% strain before reaching the linear elastic region, which can be explained by either a lack of uniformity between the widths of the rings or initial relaxation of polymer chains. Also, the thin samples showed a longer plateau region after yielding, resulting in an increased strain at 950 N by roughly 20%, which can be attributed to lower mechanical support from a lesser number of concentric rings and a larger middle void. This shows the improvement of mechanical integrity for the thick samples, due to the addition of concentric rings and overall cross-sectional area. Each curve shows a yield close to 15% strain and an exponential increase in stress after a larger amount of compressive strain, in which two phenomena occurred. The first agrees with compression of a polymeric material, in which the polyurethane chains collapse on themselves and reduce the amount of free volume within the system. Eventually, the reduction of free volume reached a maximum densification, resulting in an exponential increase of force needed to further strain the sample. If the Intron was able to increase force past the 950 N limit, the slope of the stress–strain curve would appear to asymptotically approach infinity. The second agrees with compression of a hollow cylinder, in which the samples yield due to buckling. Once the applied stress reached a certain threshold, the ringed structure alleviated this stress by allowing the rings to buckle into the vacant middle, dissipating stress into strain. It should be noted that to prevent overload of the 1 kN load cell, all samples were only tested until a maximum load 950 N was achieved, therefore the observation of a lower final stress for the thick samples compared to the thin samples is a result of the same force over a greater cross-sectional area, Figure 4. The composites were shown to be incompressible, therefore ultimate compressive stresses and strains could not be determined, even with a larger load cell. Although the processing and fabrication methods resulted in almost negligible error for repeatability of the sample dimensions, Table 2, the slight differences in heights of the coiled ribbons were just enough to cause some variations in the first 5–20 N of the compression tests. This was due to the greater width ribbons coming into contact with the compression plates before others, but since the effects caused could only be seen over a small force range, it was assumed to cause negligible effects on the resulting mechanical properties. Also, it was observed that once a given compressive stress was reached, failure due to buckling occurred in each of the samples tested during yield, Figure 5. This can be attributed to a lack of internal pressure within the system, such as that caused by the NP of the natural disc, which would hinder the ability to buckle, and instead cause barreling during deformation. In most of the samples, the buckling caused a decrease in the stress needed to further induce strain for a given period of time, which can be observed by the drop in the stress–strain curves at yield, followed by a slight plateau region.

**Figure 4.** Compressive stress–strain curves of thin samples (black) and thick samples (red) ringed samples obtained from an Instron ramping displacement at 5 mm/min until a maximum force of 950 N was achieved.

**Table 5.** Compressive properties of wet ringed composites as determined by the Instron.

|          | Thin samples | PU 100/0 | PU 90/10 | PU 80/20 |
|----------|--------------|-----------|-----------|-----------|
| Bulk modulus (MPa) | 5.9 ± 1.4 | 9.1 ± 1.3 | 14.1 ± 2.2 |
| Yield strength (MPa) | 0.4 ± 0.0 | 0.6 ± 0.0 | 0.9 ± 0.1 |
| Yield strain (%) | 14.7 ± 0.9 | 15.6 ± 1.5 | 16.8 ± 1.5 |
| Strain at 950 N (%) | 83.3 ± 0.9 | 78.9 ± 2.6 | 77.9 ± 1.0 |
| Thick samples | PU 100/0 | PU 90/10 | PU 80/20 |
| Bulk modulus (MPa) | 10.5 ± 1.0 | 13.8 ± 1.5 | 18.8 ± 1.7 |
| Yield strength (MPa) | 0.7 ± 0.0 | 0.9 ± 0.1 | 1.3 ± 0.1 |
| Yield strain (%) | 13.9 ± 0.4 | 16.2 ± 1.4 | 14.8 ± 0.5 |
| Strain at 950 N (%) | 63.7 ± 0.5 | 58.4 ± 1.1 | 52.5 ± 3.1 |

Standard deviations were taken from three samples for each total coil length of each composition.

**Compression testing**

Compression testing of the wet PU 100/0, PU 90/10, and PU 80/20 ringed structures utilizing the Instron, resulted in expected trends for mechanical reinforcement of CNCs, similar to those observed in tension testing acquired from the DMA. As well, the increase in overall ribbon length proved to further strengthen the mechanical properties of the ringed structures, leading to higher compressive moduli, yield strength, and overall toughness, while retaining similar yield strain, Figure 4 and Table 5. Each sample showed an initial toe region of roughly 7–10% strain before reaching the linear elastic region, which can be explained by either a lack of uniformity between the widths of the rings or initial relaxation of polymer chains. Also, the thin samples showed a longer plateau region after yielding, resulting in an increased strain at 950 N by roughly 20%, which can be attributed to lower mechanical support from a lesser number of concentric rings and a larger middle void. This shows the improvement of mechanical integrity for the thick samples, due to the addition of concentric rings and overall cross-sectional area. Each curve shows a yield close to 15% strain and an exponential increase in stress after a larger amount of compressive strain, in which two phenomena occurred. The first agrees with compression of a polymeric material, in which the polyurethane chains collapse on themselves and reduce the amount of free volume within the system. Eventually, the reduction of free volume reached a maximum densification, resulting in an exponential increase of force needed to further strain the sample. If the Intron was able to increase force past the 950 N limit, the slope of the stress–strain curve would appear to asymptotically approach infinity. The second agrees with compression of a hollow cylinder, in which the samples yield due to buckling. Once the applied stress reached a certain threshold, the ringed structure alleviated this stress by allowing the rings to buckle into the vacant middle, dissipating stress into strain.

It should be noted that to prevent overload of the 1 kN load cell, all samples were only tested until a maximum load 950 N was achieved, therefore the observation of a lower final stress for the thick samples compared to the thin samples is a result of the same force over a greater cross-sectional area, Figure 4. The composites were shown to be incompressible, therefore ultimate compressive stresses and strains could not be determined, even with a larger load cell. Although the processing and fabrication methods resulted in almost negligible error for repeatability of the sample dimensions, Table 2, the slight differences in heights of the coiled ribbons were just enough to cause some variations in the first 5–20 N of the compression tests. This was due to the greater width ribbons coming into contact with the compression plates before others, but since the effects caused could only be seen over a small force range, it was assumed to cause negligible effects on the resulting mechanical properties. Also, it was observed that once a given compressive stress was reached, failure due to buckling occurred in each of the samples tested during yield, Figure 5. This can be attributed to a lack of internal pressure within the system, such as that caused by the NP of the natural disc, which would hinder the ability to buckle, and instead cause barreling during deformation. In most of the samples, the buckling caused a decrease in the stress needed to further induce strain for a given period of time, which can be observed by the drop in the stress–strain curves at yield, followed by a slight plateau region.
Figure 4. Although all the samples underwent buckling deformation, when the applied force was removed and the samples allowed to swell and relax overnight, each returned to its original shape, showing minute, if any, remaining plastic deformation.

Creep and relaxation testing
Creep and relaxation testing of the wet PU 100/0, PU 90/10, and PU 80/20 ribbons and ringed structures, respectively, resulted in similar mechanical reinforcement trends as tension and compression from the addition of CNCs. However, the differences in viscoelastic properties between the PU 90/10 and PU 80/20 composites were not as significant, while comparatively, PU 100/0 exhibited a drastic difference in properties. When compared to PU 90/10 and PU 80/20, PU 100/0 showed an increase in elastic response and viscous flow by a factor of roughly 3 and 7, respectively, and a decrease in the relaxation time of 8–10 s with a higher overall relaxation by roughly 8% of its initial stress, Figure 6 and Table 6. As CNCs were added to reinforce the composite, interfacial interactions between the matrix and filler increased, resulting in a hindrance of polymer chain movement by way of bonds and potential impedance. This caused the composite to exhibit a much lower viscous response during creep and relaxation, when compared to PU 100/0. It was observed that the initial elastic response for both creep and relaxation were also diminished due to the reinforcement of the CNCs within the matrix, which causes the material to become stiffer from interfacial interactions as well as allowing greater load transfer onto the particles. The stiffer a material becomes, the less likely it is to have an initial response to an immediate load, therefore causing less elastic strain during creep testing.

Discussion
To create a polymer composite scaffold that could be easily fabricated and finely tuned, the two materials chosen were a simple, FDA approved polyurethane and CNCs. The polyurethane exhibited a modulus that was close to the range of the natural AF, Figure 3, however it was too flexible for application in the spine. Therefore, the addition of CNCs

![Figure 5. (a) Fabricated ringed structure from the PU 80/20 ribbons shown (b) before and (c) after the sample is loaded past its yield point, resulting in buckling deformation.]

![Figure 6. Creep (black) and relaxation (red) curves of PU 100/0, PU 90/10, and PU 80/20 ribbons and ringed structures, respectively, showing the effect of CNC content on the viscoelastic properties.]

was crucial due to their ability to increase the mechanical properties of the polymer composite, while adding a negligible amount of weight, given their high strength to weight ratio.\textsuperscript{51} It should be noted that there is a potential for CNC aggregations to occur after casting the composites, however, a similar study by Rueda et al. show good dispersion quality in samples up to 30 wt\% CNCs using a similar solvent casting method.\textsuperscript{62} Based on the dispersion quality of the CNCs in DMF at 40 mg/mL and similar casting techniques, it was assumed that the CNCs were well dispersed throughout the entire composite at concentrations up to 20 wt\% CNC. This can also be seen by the aggregations in Figures S2 and S3 in supplementary materials. As well, the minute variability within the mechanical properties obtained from tension testing led to the assumption of a high level of dispersion, as seen in the similar study by Rueda et al.\textsuperscript{52} Furthermore, research has shown the benefit of adding CNCs to a polymeric matrix for mechanical tunability.\textsuperscript{40,53,54} Consequently, when tested dry, the samples demonstrated a drastic increase in modulus as shown in previous work,\textsuperscript{40,55–57} but significantly out of the accepted range. To mitigate this increase, the samples were swollen to reach the needed range of moduli, since the presence of CNCs in the composite increased the hydrophilicity, allowing for the absorption of water. Due to the minimal uptake of water within the PU 100/0 composition, as well as the increasing moisture absorption as CNC concentration increased, the composite does not seem to be porous, but instead the mechanism behind which the composite absorbs water is assumed to be from the water “wicking” along the CNC network. As the hydrogen bonds are cleaved between CNCs, a pathway is opened in which the water can be transported from the surface further into the center of the composite, allowing for swelling of the entire sample over a given period of time. Although no microscopy data was obtained, the assumption is based off of the swelling measurements and small deviation in mechanical properties for each composition in its swollen state, Tables 3 and 4. If water was not able to “wick” along a dispersed network to penetrate the entire composite, the variability in the mechanical data would be much higher. This also shows the compatibility of the composite when introduced to the fluid within the spine. Utilizing a composite system with these two materials, increased the tunability of the samples, allowing for better biomimeticity of the mechanical properties to match those of the natural AF.

The ability of the composites to retain the same equilibrium point of water absorption is crucial for maintaining ideal mechanical properties and sample dimensions throughout the entirety of its use.\textsuperscript{40,58,59} Although the CNCs in the wet state act individually, there may still be small swollen aggregates of CNCs within the composite. Therefore, if the composites begin to dry out from loss of moisture, the swollen CNC aggregates within the matrix will start to bond together through hydrogen bonding, causing the scaffold to become stiffer and contain defects, as well as decrease in height and cross-sectional area. Eventually, when introduced to enough loading cycles, the scaffold will have the propensity to propagate fissures throughout the individual layers due to the rigidity and stress concentrations of the aggregated CNCs.\textsuperscript{60,61} This is similar to the cause of fissure formation within the natural AF, in which there is a decrease in proteoglycan content and increase in collagen crosslinking during IVD degeneration, leading to a loss of hydration and rigidity of the lamella.\textsuperscript{26} On the other hand, if the composites begin to absorb more water, the scaffolds will continue to swell and increase in dimensions, inducing excess pressure on the adjacent vertebrae and potentially on the spinal cord. The composites used for the scaffolds in this study showed the ability to retain the same equilibrium of water for over 2 years, resulting in no physical or mechanical changes, therefore exhibiting ideal swelling characteristics for in vivo applications.

Neither spinal fusion surgery nor total disc replacement show any water absorption within the system since all materials used are either metals, such as titanium and stainless steel, or hydrophobic polymers, such as UHMWPE, PEEK, and PU/PC copolymer.\textsuperscript{13,16} Recently researched polymer composite scaffolds are being embedded with natural cells and tissues, such as proteoglycans, glycosaminoglycans, and type I collagen, to maintain hydration of the scaffold by the same mechanisms of the natural AF, however this also opens up the possibility for degeneration by the same mechanisms. This shows the potential advantages of using a nonbiodegradable AF scaffold, in which there is an absence of natural cells and tissues that could cause the material to undergo the same degeneration as the natural AF.

The mechanical properties of the composite materials and axially layered scaffolds showed promising similarities to those exhibited by the natural

Table 6. Viscoelastic properties of the composite materials and ringed structure as determined by creep and relaxation testing.

|                  | PU 100/0 | PU 90/10 | PU 80/20 |
|------------------|----------|----------|----------|
| Elastic strain (%) (creep) | 12.3 ± 1.3 | 4.1 ± 0.1 | 3.4 ± 0.3 |
| Viscous flow (%) (creep) | 7.0 ± 1.6 | 1.4 ± 0.0 | 1.3 ± 0.4 |
| $\tau_{50}$ (s) (relaxation) | 18.8 ± 0.9 | 26.5 ± 4.9 | 28.2 ± 6.3 |
| $\sigma_{50}$ (%) (relaxation) | 615.6 ± 9.6 | 693.2 ± 5.3 | 704.5 ± 10.0 |

Standard deviations were taken from three samples for each composition.
disc. The elastic moduli for both the wet PU 90/10 and PU 80/20 of 24.4 and 32.3 MPa, respectively, reached the 18–45 MPa range of the natural AF when subjected to similar tensile forces associated with flexion and extension motions of the spine. This indicates the effectiveness of submerging the composites in fluid, similar to that of the spine. The fairly large modulus range of the AF is due to the alignment in collagen fibers throughout the lamellae, radially increasing from roughly 30° to 45°. However, when subjected to tensile forces parallel and perpendicular to the fibers alignment, the modulus changes significantly from roughly 100 MPa to 0.22 MPa, respectively. The elongation at yield during tension for both the PU 90/10 and PU 80/20 of 13.6% and 9.7% strain, respectively, fell below the 20–30% experienced by the natural AF. This is most likely explained by the natural AF going through two separate regions before yielding, the toe region and linear elastic region, in which the collagen fibers experience an un-crimping behavior followed by the actual elongation of the fibers. The composites in this study only experienced a linear elastic region before yielding, therefore resulted in lesser elongations when compared to natural tissue. The compressive moduli for both the thin and thick samples exhibited properties more closely resembling to that of the natural IVD rather than specifically the AF, however both are much higher than either natural tissue. The natural IVD demonstrates a compressive modulus of 3–10 MPa and the AF demonstrates a modulus of 0.27–0.44 MPa, while the thin and thick samples demonstrated moduli above 10 MPa for all compositions, Table 5. This should be attributed to the strength of the CNC reinforcement in the PU 85A being greater than the collagen fibers reinforcing the AF lamella, however with the addition of the pressurized NP, the IVD as a whole is able to withstand greater loads. Although the moduli for the fabricated ringed structures were above the expected range, the trends of the stress–strain curves show much more similarities to that of the natural AF and IVD. Each stress–strain curve for the ringed samples, as well as the natural AF and IVD, exhibited a toe region, linear elastic region, yield, plateau region, and incompressible region, where stress exponentially increases. This shows the capability of the ringed structures to mimic the same compressive trends as the natural AF and IVD, however the actual mechanical properties need to be further tailored to more closely agree with literature values. It should also be noted that compression tests performed on natural IVDs within the stress range of this study showed no permanent compromise to the mechanical properties nor did it lead to any changes in the structure, suggesting that biological changes occur before structural damage occurs. Again, this shows the potential advantages of a nonbiodegradable scaffold without any biological components to degrade and change over time, leading to failure of the disc. Although the experimental parameters used for creep and relaxation in this study are different compared to those found in literature for natural IVD and AF, similarities between general observations and trends can be deduced. As well, very little creep and relaxation analysis has been performed on the AF individually, with the majority of studies investigating the IVD as a whole, therefore most comparisons will be to that of the IVD. The elastic response for PU 100/0, PU 90/10, and PU 80/20 accounted for roughly 64%, 74%, and 72% of the total creep deformation, respectively, compared to the natural IVD, which shows an elastic response of only 30–45% of the total creep deformation. Of the other 55–70% of deformation corresponding to viscous flow experienced by the IVD, the AF contributes about 50%, leaving the NP to contribute the remaining 10–15%. Therefore, the AF is shown to play a vital role in the creep response of the IVD. In comparison, significantly less viscous flow was observed for each of the compositions tested, however, a much greater creep time was allotted for the IVD ranging in hours as opposed to minutes. During a creep test in which the viscous response was recorded over a span of only 32 min, the IVD was shown to experience a deformation of roughly 30% due to viscous flow, which is much closer to the values obtained for the composites in this study. Also, depending on the amount of time allowed for the IVD to undergo creep, the immediate recovery can change significantly from 70% for shorter times to 20% for much longer times. However, when given anywhere between 14 and 36 h, the IVD reaches 99% recovery. Although specific values are not reported, immediate recovery of the composite samples was shown to increase as CNC content decreased, with full recovery after swelling the samples overnight. Relaxation times have been shown to directly correlate to degeneration of a disc, with healthier discs having longer relaxation times and decreasing as degeneration gets worse. This can be attributed to the loss of proteoglycan content within the disc as it begins to degrade, therefore reducing the absorbance of water into the disc. A similar trend can be seen with the PU 90/10 and PU 80/20 composites, where an increase in water absorption directly correlates to the increase in relaxation time from 18.8 s to 26.5 s and 28.2 s, respectively, although relaxation times for IVDs were recorded in the range of milliseconds. Another comparison can be observed in the stress relaxation that occurs for the
same amount of time for the composites compared to a natural IVD. When given 720 s to relax, PU 100/0, PU 90/10, and PU 80/20 exhibited stress relaxations of around 39%, 31%, and 30% of the original stress, respectively, which can be compared to the natural IVD exhibiting a stress relaxation of roughly 34% of the original stress.

Along with compression, creep, and relaxation properties, cyclability of the scaffold should be assessed as well. Although cyclability of the scaffold was not directly tested, the scaffold relaxation times and recovery to normal position after compression testing, shows promise for cyclic loading. Post-compression test swelling showed that when swollen in water for 7 h based on average sleep time of 7–9 h for individuals of all ages, the composite scaffolds returned to normal position with no plastic deformation. Due to the lack of spinal compression during an individual’s sleep cycle, the natural IVD returns to its normal swollen state by the mornings. This leads to the assumption that after every night of sleep when swollen in the fluid of the spine, the composites should be able to return to their original swollen state. As well, the material properties of the polyurethane, with respect to an ultimate strain of roughly 610% nor a yield strain in compression, will not be reached within the normal spinal motion, therefore should behave exclusively elastically and return to its normal state.

A few other studies have been shown to incorporate CNCs into elastomeric materials, including polyurethane, latex, and bagasse, with equal success in reinforcement, however, most of these studies tended to utilize a much lower CNC concentration. Nevertheless, the mechanical reinforcement trends remained the same. As CNC concentration within the polymer matrix increased, so did the mechanical reinforcement, leading to a higher modulus and tensile strength, and a lower ultimate strain. The composite system in this study agrees with the reinforcement trends of the other comparative studies, but could most similarly be compared to a study by Rueda et al., in which up to 30 wt% CNC composition was incorporated into a segmented thermoplastic elastomeric polyurethane matrix. Utilizing a similar solvent casting procedure, Rueda et al. shows relatively uniform CNC dispersion in the polyurethane matrix, leading to almost equal reinforcement properties as the results in Table 4.

When comparing the micromechanical models of these studies, most appear to follow the rule of mixtures for well dispersed fibers/particles in a polymer composite. However, when the CNC concentration rises past a certain value, an additional contribution from a filler–filler network can be observed due to the hydrogen bonding between CNCs. This characteristic can lead to a change in the linearity of the mechanical properties, as shown by the increasing variation of the modulus, tensile strength, and ultimate strain in this study as well as that of Rueda et al. The composite system in this study most likely resembles a micromechanical model following the rule of mixtures, similar to the other studies, however, receives an additional mechanical reinforcement contribution from the CNC–CNC network formed within the matrix at higher CNC concentrations. However, when in its wet state, the hydrogen bonds between the CNCs begin to break leading to a lower mechanical reinforcement than even that of the rule of mixtures, causing some very interesting properties, as shown in Table 4.

Furthermore, of the CNC/elastomeric composite systems that have been researched, none show the fabrication of a complex scaffold, as well as the change in mechanical properties due to water absorption, to the best of the author’s knowledge. Therefore, only the dry tensile data can be directly compared, while the wet state mechanical properties and compression testing on the rings cannot. This shows the ability of this composite system to perform differently in a wet environment than it does in dry leading to novel mechanical properties and applications.

The composite material, as well as the ringed structures, were mainly compared to the mechanical properties of other CNC/elastomer composite systems, as well as the natural AF and IVD, to determine the suitability of this system as a replacement for disc degeneration. However, a comparison must also be made to current treatment options, such as spinal fusion surgery and total disc replacement, as well as current research in the field, such as cellular therapy and other tissue engineered scaffolds for IVD replacement, to better understand the novelty and viability of this study. Spinal fusion surgery and total disc replacement offer little to no shock absorption or cushioning of loads applied to the spine, therefore, forces are redistributed to the adjacent IVDs, most often times leading to further degeneration. Along with these drawbacks, are the complete hindrance of individual movement between vertebrae for spinal fusion, and a lack of resistance during movement causing hypermobility in the joints for total disc replacement. The PU 90/10 and PU 80/20 composites will not only allow for individual movement of vertebrae, but have shown to offer resistance within the property range of the natural AF when stress is applied, to eliminate hypermobility. Also, the PU 90/10 and PU 80/20 composites contain a soft, durable, and highly wear resistant
polyurethane, which will further eliminate currently used metals and other hard polymers associated with large wear volume, wear debris, corrosion, inflammation, and potential rejection.\textsuperscript{16,17}

Cellular therapies and tissue engineered scaffolds are becoming widely researched for IVD repair and replacement, with a specific focus on mimicking the biological components of the natural tissues. IVDs begin going through biological changes before structural and mechanical degeneration occur, therefore cellular therapies focus on repairing and healing the natural cells and tissues before the IVD fails, especially those of collagen, hyaluronan, proteoglycans, and glycosaminoglycans.\textsuperscript{22,77} Multiple approaches have been taken to aid in the healing and generation of these natural tissues, such as embedding mesenchymal stem cells, TGF-\(\beta\) growth factors, and other AF cells into different types of collagen rich, hyaluronan rich, and fibrinogen rich extracellular matrices.\textsuperscript{22,23,25} However, cellular therapies are only feasible for IVDs that have yet to reach the stage of degeneration requiring surgical intervention, leading to further studies in tissue engineered scaffolds embedded with these biological components. The majority of these scaffolds are comprised of aligned polymer fibers with a type I collagen rich extracellular matrix for the AF and an alginate or type II collagen hydrogel for the NP, with varying polymers and embedded biological components throughout different studies.\textsuperscript{26,27,78,79} These scaffolds, however, focused more on the biological aspect of the system rather than the manipulation of the mechanical properties. Therefore, none of these tissue engineering options demonstrate the tunability shown in this study through the variability of CNC concentration and amount of rings to manipulate the mechanical properties of the scaffolds. The tunability of the CNC concentration also leads to the possibility of gradient scaffolds, such as making the outer rings stiffer and inner rings softer.\textsuperscript{27,78,79}

Although the composite materials in this study do not contain any biological components, an argument can be made for the advantages of the nonbiological scaffold. Most of the current research approaches rely on the biological components to yield the properties necessary to replace the natural AF and IVD, however, these biological components are what lead to IVD degeneration.\textsuperscript{22–27} Therefore, being able to create a biocompatible composite material and structure it to mimic the natural AF, yet comprise no biological components for potential degeneration later in a patient’s life, shows benefits for future IVD replacement. As well, one of the major problems with current tissue engineering approaches is the scalability of the scaffolds for clinical application.\textsuperscript{29,80} The average tissue engineered scaffold is a fraction of the natural human IVD, which can cause problems and unreliable mechanical characterization when scaled. In particular, it has been shown that as size increases, the scaffolds under-perform in mechanical properties, as well as lead to reduced cell viability, glycosaminoglycans content, and heterogeneous matrix distribution.\textsuperscript{80} The scaffold in this study was constructed to real IVD height, but slightly smaller cross-sectional area. However, the ease of fabrication allows for a much simpler manipulation of sample dimensions than those of other tissue engineered scaffolds.

**Conclusions**

To determine the suitability for AF repair or replacement, multiple composites comprised of different PU 85A and CNCs compositions were solvent casted and fabricated into ribbons and radially layered structures, and characterized utilizing multiple mechanical characterization techniques. The effects of CNC content, moisture uptake, and total length of coiled ribbons for ringed structures on the mechanical properties of the composites were investigated in depth. The tensile properties of the PU 90/10 and PU 80/20, when wet, showed the greatest comparison to those found for the natural AF tissues. However, when formed into radially layered structures, they lacked the same characteristics as the AF in compression, therefore fluctuations of the amount of rings on mechanical properties should be further analyzed. Creep and relaxation tests achieved preliminary viscoelastic properties for the composites and ringed structures, showing the elastic and viscous response, as well as the relaxation time and total stress relaxation. Although not all of the results obtained from this study correlate to those found in literature for the natural AF, it was proven that the mechanical properties of the composites can be finely tuned through the addition of different CNC contents, as well as fluctuating the amount of rings in the radially layered structure. As well, the scaffolds created in this study show superior tunability of the system’s mechanical properties and ease of processing, that other tissue engineering scaffolds studies do not obtain. Thus, this system has greater potential to mimic the needs of individual patients based on their specific injured discs. This work provides adequate progress towards the field of AF repair or replacement, as well as supplementing additional research, utilizing polymer composites for biomedical applications with successful mechanical properties characterization of a biocompatible composite.
Acknowledgments

This research was supported through the James F. Powell Fellowship. The authors would like to thank Dr. Mark Van Dyke (VT BEAM) and Dr. Aaron Goldstein (VT CHE) for their input on this document and Mr. Mac McCord for his assistance with compression, creep, and relaxation testing.

Disclosure statement

The authors declare that the contents have no conflict of interest towards any individual or organization.

Notes on contributors

Brody Frost has graduated with both a B.S. and M.S. in Materials Science and Engineering, and is currently pursuing a Ph.D. in Macromolecular Science and Engineering at Virginia Tech. Brody’s research focuses are in the fields of biomaterials and nanocomposites under his advisor E. Johan Foster. His research projects have included extraction of CNCs from agricultural waste products, utilizing CNCs as a filler in a polyurethane matrix for annulus fibrosus replacement, and 3D printing scaffolds for tissue engineering.

E. Johan Foster is an Associate Professor in Materials Science and Engineering at Virginia Tech. Johan’s expertise are in the design, synthesis, processing, and investigation of functional nanocomposites, biomaterials, supramolecular materials and polymers. His research group focuses on the chemistry and engineering of functional bio(nano)materials, imbibing materials with ‘smart’ functionality, often utilizing CNCs as a filler. Johan has many national and international collaborators, from countries as far reaching as Australia, Ghana, Kenya, France, and Switzerland. His research program is supported through federal, industrial and state research grants.

References

1. Freemont AJ, Peacock TE, Goupille P. Nerve ingrowth into diseased intervertebral disc in chronic back pain. Lancet. 1997;350:178–181.
2. Urban JGP, Roberts S. Degeneration of the intervertebral disc. Arthritis Res Ther. 2003;5:120–130.
3. Buttacavoli FA, Delamarter RB, Kanim LEA. Cost comparison of patients with 3-level artificial total lumbar disc replacements versus 360° fusion at 3 contiguous lumbar vertebral levels: an analysis of compassionate use at 1 site of the US investigational device exemption clinical trial. SAS J. 2010;4:107–114.
4. Gaskin DJ, Richard P. The economic costs of pain in the United States. In: Institute of Medicine (US) Committee on Advancing Pain Research, Care, and Education. Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research, Washington (DC): National Academies Press (US); 2011. Appendix C.
5. Crow WT, Willis DR. Estimating cost of care for patients with acute low back pain: a retrospective review of patient records. J Am Osteopath Assoc. 2009;109:229–233.
6. Roberts S, Evans H, Trivedi J, et al. Histology and Pathology of the human intervertebral disc. J Bone Joint Surg Am. 2006;88:10–14.
7. Battie M, Videman T, Levalahti E, et al. Genetic and environmental effects on disc degeneration by phenotype and spinal level: a multivariate twin study. Spine. 2008;33:2801–2808.
8. Buckwater JA. Aging and degeneration of the human intervertebral disc. Spine. 1999;20:1307–1314.
9. Inoue N, Espinoza Oria AA. Biomechanics of Intervertebral disk degeneration. Orthop Clin North Am. 2011;42:487–499.
10. Adams MA, Hutton WC. Gradual disc prolapse. Spine. 1985;10:524–531.
11. Kortelainen P, Puranen J, Koivistö E, et al. Symptoms and signs of sciatica and their relation to the localization of the lumbar disc herniation. Spine. 1985;10:88–92.
12. Taher F, Essig D, Lebl DR, et al. Lumbar degenerative disc disease: current and future concepts of diagnosis and management. Adv Orthop. 2012;2012:970752.
13. Nouh MR. Spinal fusion-hardware construct: basic concepts and imaging review. WJR. 2012;4:193–207.
14. Multilevel Spinal Fusion for Low Back Pain [Internet]. Spine Health; 2007 [cited 2018 May 28] https://www.spine-health.com/treatment/spinal/fusion/multilevel-spinal-fusion-low-back-pain.
15. Quirno M, Goldstein JA, Bendo JA, et al. The incidence of potential candidates for total disc replacement among lumbar and cervical fusion patient populations. Asian Spine J. 2011;5:213–219.
16. Reeks J, Liang H. Materials and their failure mechanisms in total disc replacements. Lubricants. 2015;3:346–364.
17. Serhan H, Mhatre D, Defossez H, et al. Motion-preserving technologies for degenerative lumbar spine: the past, present, and future horizons. SAS J. 2011;5:75–89.
18. Lee AJ, Lee M. Comparative analysis on the nanoindentation of polymers using atomic force microscopy. Polym Test. 2010;29:95–99.
19. Panayotov IV, Orti V, Cuisinier F, et al. Polyetheretherketone (PEEK) for medical applications. J Mater Sci Mater Med. 2016;27:118.
20. Guterl CC, See EY, Blanquer SBG, et al. Challenges and strategies in the repair of ruptured annulus fibrosus. Eur Cell Mater. 2013;25:1–21.
21. Vadalà G, Mozetic P, Rainer A, et al. Bioactive electrospun scaffold for annulus fibrosus repair and regeneration. Eur Cell Mater. 2012;21:20–26.
22. Alini M, Roughley PJ, Antoniou J, et al. A bio-analogous approach to treating disc degeneration: not for today, but maybe for tomorrow, In: Gunzburg R, Mayer HM, Szpalski M, Aebi M, editors. Arthroplasty of the spine. Berlin, Heidelberg: Springer; 2004; p. 159–164.
23. Sakai D, Mochida J, Iwashina T, et al. Regenerative effects of transplanting mesenchymal stem cells embedded in atelocollagen to the degenerated intervertebral disc. Biomaterials. 2006;27:335–345.
24. Hudson KD, Bonassar LJ. Hypoxic expansion of human mesenchymal stem cells enhances three-dimensional maturation of tissue-engineered intervertebral discs. Tissue Eng Part A. 2017;23:293–300.
fibrous defects in intervertebral discs. Tissue Eng Part A. 2018;24:187–198.

26. Hudson KD, Mozia RI, Bonassar LJ. Dose-dependent response of tissue-engineered intervertebral discs to dynamic unconfined compressive loading. Tissue Eng Part A. 2015;21:564–572.

27. Iu J, Massicotte E, Li SQ, et al. In vitro generated intervertebral discs: toward engineering tissue integration. Tissue Eng A. 2017;23:1001–1010.

28. D’Angelo F, Armentano I, Cacciotti I, et al. Tuning multi/pluri-potent stem cell fate by electrospun poly(lactic acid)-calcium-deficient hydroxyapatite nanocomposite mats. Biomacromolecules. 2012;13:1350–1360.

29. Burke A, Hasirci N. Advances in experimental medicine and biology: polyurethanes in biomedical applications. Acta Biomater. 2018;78:13–22.

30. Buckley CT, Hoyland JA, Fujii K, et al. Critical aspects and challenges for intervertebral disc repair and regeneration: harnessing advances in tissue engineering. JOR Spine. 2018;1:e1029.

31. Joseph J, Patel RM, Wenham A, et al. Biomedical applications of polyurethane materials and coatings. Trans IMF. 2018;96:121–129.

32. Burke A, Hasirci N. Advances in experimental medicine and biology: polyurethanes in biomedical applications. Boston (MA): Springer; 2004.

33. Zdrahala RJ, Zdrahala IJ. Biomedical applications of polyurethanes: a review of past promises, present realities, and a vibrant future. J Biomater Appl. 1999;14:67–90.

34. Wheeler AJ. Procedural rates, economic costs, and geographic variation in the surgical treatment of orthopedic surgery (ABOS) quality improvement initiative; part II candidates. Spine (Phila Pa 1976). 2013;38:57–75.

35. McGuire KJ, Harrast J, Herkowitz H, et al. Geographic variation of primary and revision lumbar total disc replacement [dissertation]. Logan (UT): Utah State University; 2010.

36. Product Center Thermoplastic Polyurethanes [Internet]. Covestro; 2015 [cited 2018 May 28]. https://www.tpu.covestro.com/en/Products/Texin/ProductList/201403080434/Texin-RxT85A.aspx.

37. The Process Development Center – Nanocellulose Data Sheets [Internet]. The University of Maine; 2019 [cited 2019 February 4]. https://umeaine.edu/pdc/nanocellulose/nanocellulose-spec-sheets-and-safety-data-sheets/

38. Camarero Espinosa S, Kuhn T, Foster EJ, et al. Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis. Biomacromolecules. 2013;14:1223–1230.

39. Tham WL, Chow WS, Mohd Ishak ZA. Simulated body fluid and water absorption effects on poly (methyl methacrylate)/hydroxyapatite denture base composites. Express Polym Lett. 2010;4:517–528.

40. Fallon JJ, Kolb BQ, Herwig CJ, et al. Mechanically adaptive thermoplastic urethane/cellulose nanocrystal composites: understanding process driven structure property relationships. J Appl Polym Sci. 2019;136:46992.

41. Sullivan EM, Moon RJ, Kalaizidou K. Processing and characterization of cellulose nanocrystals/poly-lactic acid nanocomposite films. Materials. 2015;8:8106–8116.

42. Mathew AP, Gong G, Bjorngrim N, et al. Moisture absorption behavior and its impact on the mechanical properties of cellulose whiskers-based polyvinylacetate nanocomposites. Polym Eng Sci. 2011;51:2136–2142.

43. Khazraji AC, Robert S. Interaction effects between cellulose and water in nanocrystalline and amorphous regions: a novel approach using molecular modeling. J Nanomater. 2013;2013:1–10.

44. Nerurkar NL, Elliot DM, Mauck RL. Mechanical design criteria for intervertebral disc tissue engineering. J Biomech. 2010;43:1017–1030.

45. Yi J, Boyce MC, Lee GF, et al. Large deformation rate-dependent stress-strain behavior of polyeurea and polyeurethanes. Polymer. 2006;47:319–329.

46. Wu CP, Chen YC, Peng ST. Buckling analysis of functionally graded material circular hollow cylinders under combined axial compression and external pressure. Thin Wall Struct. 2013;69:54–66.

47. Koeller W, Meier W, Hartmann F. Biomechanical properties of human intervertebral discs subjected to axial dynamic compression. A comparison of lumbar and thoracic discs. Spine. 1984;9:725–733.

48. Meakin JR, Hukins DWL. Effect of removing the nucleus pulposus on the deformation of the annulus fibrosus during compression of the intervertebral disc. J Biomech. 2000;33:575–580.

49. Lu H, Wang B, Ma J, et al. Measurement of creep compliance of solid polymers by nanoindentation. Mech Time-Depend Mat. 2013;7:189–207.

50. Findley WN, Lai JS, Onaran K. Creep and relaxation of nonlinear viscoelastic materials. New York (NY): Elsevier; 2012.

51. Dufresne A. Nanocellulose: a new ageless bionano-material. Mater Today. 2013;16:220–227.

52. Rueda L, Sarageui A, Fernandez d’Arlas B, et al. Cellulose nanocrystals/polyurethane nanocomposites. Study from the viewpoint of microphase separated structure. Carbohyd Polym. 2013;92:751–757.

53. Oksman K, Aitomaki Y, Mathew AP, et al. Review of the recent developments in cellulose nanocomposite processing. Compos Part A: Appl Sci. 2016;83:2–18.

54. Cacciotti I, Fortunati E, Puglia D, et al. Effect of silver nanoparticles and cellulose nanocrystals on electrospun poly (lactic acid) cat mats: morphology, thermal properties and mechanical behavior. Carbohyd Polym. 2014;103:22–31.

55. Smyth M, M’Bengue M-S, Terrien M, et al. The effect of hydration on the material and mechanical properties of cellulose nanocrystal-alginato composites. Carbohyd Polym. 2018;179:186–195.

56. Nicharat A, Shirole A, Foster EJ, et al. Thermally-activated shape memory behavior of melt-mixed polyurethane and upscalable phosphorylated-cellulose nanocrystal nanocomposites. J Appl Polym Sci. 2017;134:45033.

57. Sapkota J, Natterodt J, Shirole A, et al. Fabrication and properties of polyethylene/cellulose nanocrystal composite. Macromol Mater Eng. 2017;302:1600300.

58. McMillan S, Rader C, Jorfi M, et al. Mechanically switchable polymer fibers for sensing in biological conditions. J Biomed Opt. 2017;22:27001

59. Smyth M, Fournier C, Driemeier C, et al. Tunable structural and mechanical properties in liquid of...
cellulose nanofiber substrates for stem cell culture. Biomacromolecules. 2017;18:2034–2044.

60. Kargarzadeh H, Sheltami RM, Ahmad I, et al. Cellulose nanocrystal: a promising toughening agent for unsaturated polyester nanocomposites. Polymer. 2015;56:346–357.

61. Xu S, Girouard N, Schueneman G, et al. Mechanical and thermal properties of waterborne epoxy composites containing cellulose nanocrystals. Polymer. 2013;54:6589–6598.

62. Best BA, Guilak F, Setton LA, et al. Compressive mechanical properties of the human annulus fibrosus and their relationship to biochemical composition. Spine. 1994;19:212–221.

63. Schechtman H, Robertson PA, Broom ND. Quasi-static and cyclic compressive loading studies of the intervertebral disc with combined flexion and torsion. RBEB. 2012;28:311–318.

64. Korecki CL, MacLean JJ, Iatridis JC. Dynamic compression effects on intervertebral disc mechanics and biology. Spine. 2008;33:1403–1409.

65. O’Connell GD, Jacobs NT, Sen S, et al. Axial creep loading and unloaded recovery of the human intervertebral disc and effect of degeneration. J Mech Behav Biomed Mater. 2012;4:933–942.

66. Keller TS, Spengler DM, Hansson TH. Mechanical behavior of the human lumbar spine. I. Creep analysis during static compressive loading. J Orthop Res. 1987;5:467–478.

67. O’Connel GD, Vresilovic EI, Elliott DM. Human intervertebral disc internal strain in compression: the effect of disc region, loading position, and degeneration. J Orthop Res. 2011;29:547–555.

68. O’Connell GD, Malhotra NR, Vresilovic EI, et al. The Effect of nucleotomy and the dependence of degeneration of human intervertebral disc strain in axial compression. Spine. 2011;36:1765–1771.

69. Johannessen W, Vresilovic EI, Wright AC, et al. Intervertebral disc mechanics are restored following cyclic loading and unloaded recovery. Ann Biomed Eng. 2004;32:70–76.

70. Roenneberg T, Kuehnle T, Juda M, et al. Epidemiology of the human circadian clock. Sleep Med Rev. 2007;11:429–438.

71. Saralegi A, Rueda I, Martin L, et al. From elastomeric to rigid polyurethane/cellulose nanocrystal bionanocomposites. Compos Sci Technol. 2013;88:39–47.

72. Pei A, Malho JM, Ruokolainen J, et al. Strong nanocomposite reinforcement effects in polyurethane elastomer with low volume fraction of cellulose nanocrystals. Macromolecules. 2011;44:4422–4427.

73. Haji P, Cavaillé JY, Favier V, et al. Tensile behavior of nanocomposites from latex and cellulose whiskers. Polym Compos. 1996;17:612–619.

74. Bras J, Hassan ML, Bruzesse C, et al. Mechanical, barrier, and biodegradability properties of bagasse cellulose whiskers reinforced natural rubber nanocomposites. Ind Crop Prod. 2010;32:627–633.

75. Ciocci M, Cacciotti I, Seliktar D, et al. Injectable silk fibroin-hydrogels functionalized with microspheres as adult stem cells-carrier systems. Int J Biol Macromol. 2018;108:960–971.

76. Cacciotti I, Ciocci M, Di Giovanni E, et al. Hydrogen sulfide-releasing fibrous membranes: potential patches for stimulating the human stem cells proliferation and viability under oxidative stress. Int J Mol Sci. 2018;19:e2368.

77. Bron JL, Helder MN, Meisel HJ, et al. Repair, regenerative and supportive therapies of the annulus fibrosus: achievements and challenges. Eur Spine J. 2009;18:301–313.

78. Bhunia BK, Kaplan DL, Mandal BB. Silk-based multilayered angle-ply annulus fibrosus construct to recapulate form and function of the intervertebral disc. Proc Natl Acad Sci USA. 2018;115:477–482.

79. Yang J, Yang X, Wang L, et al. Biomimetic nanofibers can construct effective tissue-engineered intervertebral disc for therapeutic implantation. Nanoscale. 2017;9:13095–13103.

80. Gullbrand SE, Kim DH, Bonnevie E, et al. Towards the scale up of tissue engineered intervertebral discs for clinical application. Acta Biomater. 2018;70:154–164.