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Compressive properties of fibrous repair tissue compared to nucleus and annulus

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ABSTRACT

The wound healing process includes filling the void between implant and tissue edges by collagenous connective repair tissue. This fibrous repair tissue may load share or stabilize implants such as spinal disc replacements. The objective of this study was the biomechanical characterization of human fibrous tissue compared to annulus fibrosus and nucleus pulposus. Human lumbar discs (10 nucleus and annulus) and 10 lumbar deep wound fibrous tissue specimens were sectioned into 12 mm diameter × 6 mm high cylindrical samples. Confined compression testing, after 2 h swelling at 0.11 MPa, was performed at 5%, 10% and 15% strain over 3.5 h. Unconfined dynamic testing (2–0.001 Hz) was performed at 5–15% strain. Semi-quantitative histology estimated the proportion of proteoglycan to collagen. Fibrous tissue exhibited a decrease in height during the swelling period whereas annulus and nucleus tissues did not. The aggregate modulus was significantly less for fibrous tissue ($p < 0.002$). Percent stress relaxation was greatest for the fibrous tissue and similar for annulus and nucleus. Dynamic testing found the storage modulus (E') was greater than the loss modulus (E'') for all tissues. Annulus were found to have greater E' and E'' than nucleus, whereas E' and E'' were similar between annulus and fibrous tissue. Fibrous tissue had the greatest increase in both moduli at greater frequencies, but had the lowest hydration and proteoglycan content. Fibrous tissue would not be a substitute for native tissue within the disc space but if adjacent to a disc prosthesis may impart some degree of intersegmental stability during acute loading activities.

1. Introduction

Wound healing is a process of filling a void or gap between tissue edges by collagenous connective repair tissue (Broughton et al., 2006). The process also occurs at the interface between host tissues and implants (Tomida et al., 2011; Sevastjanova et al., 1987; Therin et al., 1994; Hacking et al., 2000). This fibrous tissue must have the physical properties to withstand the stresses imposed by the adjacent tissues.

Prior biomechanical evaluations of fibrous repair tissue have typically been performed using tensile tests (Viidik and Gottrup, 1986; Quaglini et al., 2005; Hacking et al., 2000; Kinneberg et al., 2011; Tohyama et al., 2003, Thornton et al., 2000). Typically, the fibrous repair tissue has been found to have substantially lower yield strength and tensile Young's modulus vs. the native tissue. For example, the static tensile modulus has been reported as 286 MPa vs. over 700 MPa for fibrous scar tissue within the central 1/3 defect of a rabbit patellar tendon vs. native intact central 1/3 tendon, respectively (Tohyama et al., 2003; Kinneberg et al., 2011).

In weight-bearing regions such as the spine, wound repair tissue is often under compression. Quantifying deep wound properties under compression may provide insight into the performance of spinal disc replacements where voids in and around the device will be filled with fibrous tissue (Fig. 1). Fibrous tissue may affect disc device mobility, load sharing, or even prevent device dislodgement, migration, or disassembly of multi-component devices. Spine surgeons may also find the properties of this tissue of interest when considering the long-term prognosis of non-painful pseudarthrosis developing after a fusion procedure. This may guide the surgeon as to when to operatively intervene.

The purpose of this study was to evaluate the viscoelastic compressive properties of deep wound fibrous tissue in comparison to annulus fibrosis and nucleus pulposus tissue. We hypothesized that fibrous tissue would have intermediate viscoelastic properties compared to native annulus and nucleus. As a gross approximation, confined compression testing simulated the condition of fibrous tissue surrounding a nucleus implant with a competent annulus,

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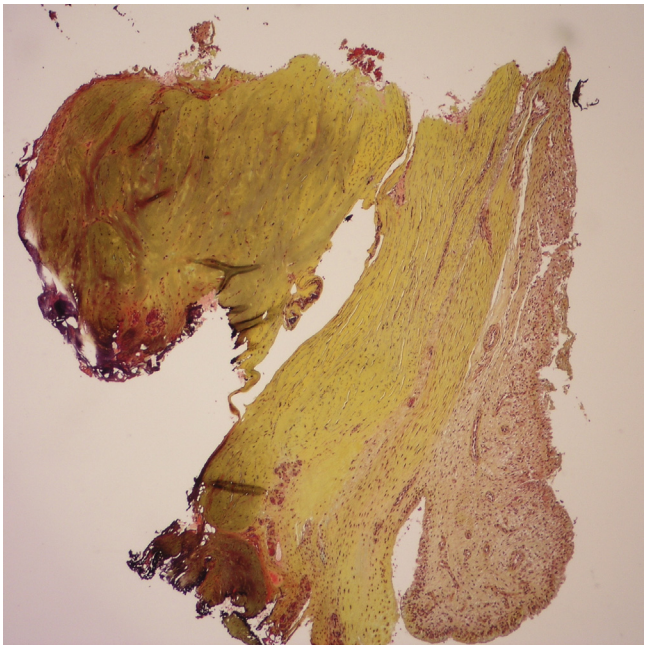

Fig. 1. Histology of tissue excised that was adjacent to a cervical total disc replacement, Movat's pentachrome stain for proteoglycan (green), collagen (yellow) and muscle or fibrin (red). This clinical image has no green and thus no substantial proteoglycan. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

and unconfined compression testing approximated the environment of a total disc replacement in which the majority of the annulus fibrosus has been resected. Specifically, confined compression testing was used to determine the aggregate modulus and stress relaxation behavior. Unconfined cyclic loading was used to determine the elastic (E') and viscous (E'') moduli at various frequencies. Semiquantitative histology approximated the relative proportion of proteoglycan to collagen for the various tissues.

2. Methods

2.1. Tissue procurement

After IRB approval, 10 patients having revision posterior lumbar spinal surgery had 1–5 cm^3 of deep subfascial wound scar tissue excised during the exposure (Table 1). The time from tissue harvest to freezing was less than 30 min so that the tissue was in its normal state of hydration. At the time of preparation, tissue was double-wrapped in air-tight plastic bags and frozen at −20 °C and stored until use.

Comparative annulus fibrosus and nucleus pulposus tissue was obtained from 10 fresh human lumbar spines, and discs were harvested intact and graded for degree of degeneration (Table 2) (Thompson et al., 1990).

2.2. Tissue preparation

Each frozen specimen had a 12.0 mm diameter sample removed using a sharp punch (Acuderm, Fort Lauderdale, FL). Fibrous tissue samples were clinically harvested at various orientations; comparative annulus and nucleus tissues were obtained from the anterior annulus and the central nucleus and were axially oriented. Punched samples were inserted into a 6 mm thick aluminum plate with a 12 mm hole (Fig. 2). After visual confirmation that tissue was exposed on each side of the plate, the plate was frozen for 20 min at −20 °C. A sharp, flat blade was used on the frozen sample to face the tissue off to a 6 mm height. A second sample from each tissue type was prepared for histological analysis.

2.3. Confined compression

Incremental stress relaxation testing used confined compression methods similar to those used in previous studies (Périé et al., 2005; Johannessen and Elliott, 2005). A fixture having a 12.1 mm diameter cylindrical chamber was affixed to a servohydraulic test frame with a 670 N load cell (Interface, Scottsdale, AZ). Each

Table 1
Fibrous tissue characteristics.

Origin of fibrous tissue samples	Donor age at time of tissue harvest (range, years)	In vivo maturation period (range, months)
Instrumentation removal ($n=5$)	21–63	25–66
Fusion extension ($n=3$)	51–59	62–119
Pseudoarthrosis repair ($n=2$)	66–72	20–32

Table 2
Nucleus and annulus characteristics.

Origin of nucleus and annulus tissue samples	Patient age and gender at time of tissue harvest	Disc grade[a]
L1-2	52 y/o male	4
L2-3	34 y/o male	2
L2-3	47 y/o male	2
L3-4	33 y/o female	2
L3-4	49 y/o female	2
L3-4	68 y/o male	3
L4-5	22 y/o female	1.5
L4-5	51 y/o male	3.5
L4-5	53 y/o male	3.5
L4-5	59 y/o male	4.5

[a] Grade 1 is normal while grade 5 is advanced degeneration.

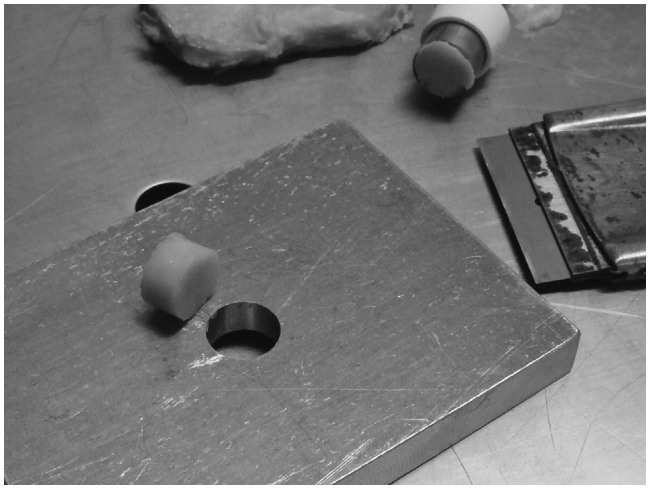

Fig. 2. The 12 mm punch, 6 mm aluminum plate and blade used for sample preparation.

frozen specimen was placed at the bottom of the compression chamber and allowed to thaw to room temperature for 30 min. Thawed specimens were then bathed in normal saline at room temperature. A 12 mm diameter porous impounder made of sintered 316L stainless steel (40 μm pore size, 50% porosity, Applied Porous Technologies, Tariffville, Conneticut, USA) was used to apply a 0.11 MPa (12 N) compressive stress and allowed to equilibrate for 7200 s. This preload was based on the intrinsic pressure of the human disc as measured *in vivo* and *in vitro*, and was similar to the equilibrium swelling pressure observed in previous spinal soft tissue studies (Johannessen and Elliott, 2005; Best et al., 1994; Ekström et al., 2004; Nachemson et al., 1979; Brinckmann and Grootenboer, 1991; Buttermann et al., 2009; Iatridis et al., 1996; Proctor et al., 1989; Urban and McMullin, 1985). Swelling and testing times were based upon specimens achieving a minimum 90% of maximal creep and relaxation, yet also allowing all the testing to be completed in 12 h to minimize tissue degradation (Johannessen and Elliott, 2005; Heneghan and Riches, 2008). Prior to each test free impounder travel was confirmed. The impounder was cleaned after each test to prevent clogging. The test order was randomized for nucleus, annulus and fibrous tissue samples.

The initial tissue height, L_0, of each specimen was determined under a 0.11 MPa (12 N) load at the end of the swelling period. Then an incremental relaxation test was performed by advancing the porous impounder, at a rate of 0.05 mm/min, to