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Acellular dermal matrix collagen responds to strain by intermolecular spacing contraction with fibril extension and rearrangement

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ABSTRACT

Acellular dermal matrix (ADM) materials are used as scaffold materials in reconstructive surgery. The internal structural response of these materials in load-bearing clinical applications is not well understood. Bovine ADM is characterized by small-angle X-ray scattering while subjected to strain. Changes in collagen fibril orientation (O), degree of orientation as an orientation index (OI) (measured both edge-on and flat-on to the ADM), extension (from d-spacing changes) and changes to intermolecular spacing are measured as a result of the strain and stress in conjunction with mechanical measurements. As is already well established in similar systems, when strained, collagen fibrils in ADM can accommodate the strain by reorienting by up to 50° (as an average of all the fibrils). This reorientation corresponds to the OI increasing from 0.3 to 0.7. Here it is shown that concurrently, the intermolecular spacing between tropocollagen decreases by 10% from 15.8 to 14.3 Å, with the fibril diameter decreasing from 400 to 375 Å, and the individual fibrils extending by an average of 3.1% (D-spacing from 63.9 to 65.9 nm). ADM materials can withstand large strain and high stress due to the combined mechanisms of collagen reorientation, individual fibril extension, sliding and changes in the molecular packing density.

1. Introduction

In a number of reconstructive surgical procedures, it is necessary to provide reinforcement in the form of scaffold materials. These materials must be immunologically compatible with the body and readily incorporated by the body into living tissue. They must also have sufficient strength to perform the task at hand and have appropriate stiffness and elastic properties. These scaffold materials may be synthesized from a variety of synthetic materials (Lutolf and Hubbell, 2005) or produced by decellularization of native materials. Extracellular matrix materials (ECM) derived from a variety of tissues have been successfully used as scaffolds, including commercially available ADM materials produced from a variety of species including porcine, bovine (Chen et al., 2004; Clemens et al., 2013) and human dermal tissue (Badylak et al., 2009; Bondioli et al., 2014; Cornwell et al., 2009; Shevchenko et al., 2010; Wells et al., 2015b). Clinical applications for these materials include providing support during breast reconstruction (Winters and Colwell, 2014), abdominal hernia repair (Mohsina et al., 2015), tendon repair (Lee, 2008), and rotator cuff repair (Derwin et al., 2010), as well as substituting for skin in serious burns and chronic or difficult-to-close wounds (MacNeil, 2007), vascular grafts (Thomas et al., 2013) and bladder repair (Brown et al., 2002).

Collagen-based materials are suitable as surgical scaffolds because once they have been thoroughly decellularized, they have excellent biocompatibility (Lee et al., 2001) and high mechanical strength or toughness. Natural collagen tissues are sufficiently porous for cells to infiltrate and populate the implanted scaffolds (Loh and Choong, 2013). With time, collagen scaffolds are remodelled and absorbed and are eventually replaced by the patient's own tissue (O'Brien, 2011), however, for load bearing applications the mechanical strength of the implanted scaffold withstands the stresses up until the time that remodelled tissue takes over this function.

The tissues are elastic and are resistant to tearing because of the highly fibrillar nature of type I collagen; the collagen fibrils have an inherent strength and elasticity (Meyers et al., 2013; Wells et al., 2015a) that allows them to rearrange and block a propagating tear (Yang et al., 2015). The ability to block a propagating tear is affected by the arrangement of the collagen fibrils. This fibril arrangement can be described in terms of orientation direction and spread (Basil-Jones et al., 2010). Collagen orientation (reported as the orientation index, OI) has been investigated in heart valve (Sellaro et al., 2007), pericardium (Liao et al., 2005; Sizeland et al., 2014), bladder (Gilbert et al.,

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2008), cornea (Boote et al., 2011), skin (Purslow et al., 1998), aorta (Gasser, 2011), and stomach tissue (Floden et al., 2010). When collagen fibrils are arranged in parallel or almost-parallel sheets (i.e., have a high OI), in general, a stronger material results (Basil-Jones et al., 2010, 2011; Sizeland et al., 2013, 2014). However, while this relationship is true for differences within a species and tissue type, it may not always hold true for a direct comparison between species (Wells et al., 2015b).

Collagen-based materials respond to strain with rearrangement followed by fibril extension (Basil-Jones et al., 2012; Kayed et al., 2015; Sizeland et al., 2017; Yang et al., 2015). The fibrils rearrange in the direction of strain, and then the individual fibrils can extend in length by as much as 6% (Kayed et al., 2015). Fibril sliding likely also occurs. These multiple mechanisms for accommodating strain lead to very high strength and tear-resistant materials.

Constitutive equations have been developed for mechanical models of tissues where there is rearrangement of collagen fibrils during strain (Belkoff and Haut, 1991; Lanir, 1979, 2017; Wren and Carter, 1998). These have typically used a value of 1000 MPa for the elastic modulus of collagen fibrils and measured values for bulk modulus.

The structure of the collagen fibrils may also influence the mechanical properties. The diameter of the collagen fibrils has been correlated with strength in some materials. For example, in human aortic valves, regions of high stress may contain larger-diameter fibrils (Balguid et al., 2008); in mouse tendon, fibril diameter increases with loading (Michna, 1984); and in bovine leather, higher strength materials have larger fibril diameters (Wells et al., 2013). Fibril diameter may also increase with age (Parry et al., 1978).

Small-angle X-ray scattering (SAXS) can provide detailed information on the architecture of collagen materials, including fibril orientation and D-spacing (Basil-Jones et al., 2010; Kronick and Buechler, 1986; Liao et al., 2005; Sasaki and Odajima, 1996; Wells et al., 2015b). Polarized light can also be used for fibril orientation changes (Sacks et al., 1997; Tower et al., 2002) and this may include Raman imaging (Bertinetti et al., 2015).

Here, we investigated the orientation distribution over an area of bovine dermal ADM to get a better understanding of the isotropy or likely directional properties of the materials. We then investigated the response of the ADM materials to strain, specifically the rearrangement and extension of the collagen fibrils, to help us elucidate how these materials adapt to strain and what gives them their remarkable strength.

2. Materials and methods

2.1. Source material

The test material used was the commercially available SurgiMend (TEI Biosciences/Integra, New Jersey) ADM. SurgiMend is derived from the skins of young neonatal bovines that are less than 5 months old. After decellularization, the materials are very soft and pliable; however, they are then freeze dried for preservation and storage, and it is in this state that the material is available commercially. ADM material nominally 3 mm thick was studied here. The material was hydrated prior to testing and maintained hydrated during testing. Measurements on two or three portions taken from each of three sheets of this material (from different animals) are reported here (including in the Supplementary Information file). Two of these portions from each sample were for OI, d-spacing and fibril diameter, and one for intermolecular spacing (for only two of the animals).

2.2. Electron microscopy

Samples of ADM materials were cut into small cubes and fixed for over 8 h at room temperature in modified Karnovsky's fixative. The fixative contained 3% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer (pH 7.2). Following this the samples were washed three times for 10–15 min each in phosphate buffer (0.1 M, pH 7.2) before being dehydrated in a graded series of 10–15 min ethanol washes (25%, 50%, 75%, 95%, and 100%), followed by a final 100% ethanol wash for 1 h. Samples were critical-point (CP) dried using a Polaron E3000 series II critical-point drying apparatus in which liquid CO_2 was used as the CP fluid and 100% ethanol was used as the intermediary fluid. The dry samples were then mounted on to aluminium stubs and sputter coated with gold using a Baltec SCD 050 sputter coater. An FEI Quanta 200 Environmental scanning electron microscope (SEM) with an accelerating voltage of 20 kV was used to view the samples.

2.3. Synchrotron SAXS

Diffraction patterns were recorded on the Australian Synchrotron SAXS/WAXS beamline using a high-intensity undulator source. Energy resolution of 10^{-4} was obtained from a cryo-cooled Si(111) double-crystal monochromator. The beam size (FWHM focused at the sample) was 250 × 80 μm, with a total photon flux of approximately 2×10^{12} ph·s^{-1} using a Pilatus 1 M detector and an active area of 170 × 170 mm. Two experimental setups were used. For low q measurements, a sample-to-detector distance of 3371 mm was used with X-ray energy of 12 keV. For high q measurements, the sample-to-detector distance was 900 mm with an X-ray energy of 15.3 keV. Exposure time for diffraction patterns was 1–5 s, and data were initially processed with Scatterbrain software (developed in house at the Australian Synchrotron).

SAXS analysis was carried out in two directions through the samples. The X-ray beam was passed either through the flat surface of the sample normal to its surface (here, referred to as normal or surface) or edge-on to the sample (here, referred to as edge-on or cross sections) (Fig. 1). Because structure varies across the width of the sample, the edge-on measurements were taken at approximately 0.15 mm intervals through the whole width of each sample. Measurements for the surface samples were taken in a 6 point grid with 0.5 mm spacing between points.

Samples were strained with an apparatus using a linear motor, Linmot PS01 48 × 240/30 × 180-C (NTI AG, Switzerland), mounted on a purpose-built frame. Clamps to hold the pericardium were fitted between the linear motor and an L6D OIML single-point loadcell (Hangzhou Wanto Precision Technology Co., Zhejiang, China). Samples were mounted horizontally without tension and strained in 2 mm increments and maintained for 20 s at each extension to stabilize before SAXS patterns (Fig. 2), the extension and the force information were recorded. This process was repeated until the sample failed; the interval between the strain increments was approximately 100 s.

2.4. Fibril diameter

Fibril diameters were calculated from the SAXS data using the Irena software package (Ilavsky and Jemian, 2009) running within Igor Pro. The data were fitted at the wave vector, Q, in the range of 0.01–0.04 Å^{-1} and at an azimuthal angle of 92.5° (over a 5° segment) to the long axis of most of the collagen fibrils. The average direction of the long axis of the collagen fibrils was determined as the position for the maximum scattering intensity of the D-spacing diffraction peaks with the azimuthal angle. To fit the data, the “cylinderAR” shape model with an aspect ratio of 100 was used for all cases as this was high enough that higher values did not improve the modelling. However, the aspect ratio of unbranched collagen fibrils may be larger than 100.

2.5. D-spacing

The D-spacing was determined from the position of the centre of a Gaussian curve fitted to the 5th order diffraction peak taken from the integrated intensity from the azimuthal range of 45–135°.