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Effect of sample geometry on the apparent biaxial mechanical behaviour of planar connective tissues

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Abstract

Mechanical testing methodologies developed for engineering materials may result in artifactual material properties if applied to soft planar connective tissues. The use of uniaxial tissue samples with high aspect ratios or biaxial samples with slender cruciform arms could lead to preferential loading of only the discrete subset of extracellular fibres that fully extend between the grips. To test this hypothesis, cruciform biaxial connective tissue samples that display distinctly different material properties (bovine pericardium, fish skin), as well as model textile laminates with predefined fibrous orientations, were repeatedly tested with decreasing sample arm lengths. With mechanical properties determined at the sample centre, results demonstrated that the materials *appeared* to become stiffer and less extensible with less slender sample geometries, suggesting that fibre recruitment increases with decreasing sample arm length. Alterations in the observed shear behaviour and rigid body rotation were also noted. The only truly reliable method to determine material properties is through in vivo testing, but this is not always convenient and is typically experimentally demanding. For the in vitro determination of the biaxial material properties, appropriate sample geometry should be employed in which all of the fibres contribute to the mechanical response.

Keywords: Biaxial; Mechanical testing; Sample geometry; Pericardium; Skin; Artifacts

1. Introduction

The mechanical characterization of planar connective tissues (e.g. pericardium) is of particular interest for physiology and for the design of bioprosthetic replacements. The bulk of this information has been obtained through in vitro testing since the determination of mechanical properties in vivo can be extremely difficult. These in vitro observations are then routinely used as predictors of material performance in situ with the inherent assumption that the testing methodology has not influenced the observed behaviour. Nevertheless, the geometry of the sample and the method of gripping the sample edges may have profound effects on any measured mechanical properties since they directly influence how the load is transferred to the underlying fibrous network. Although these issues are believed to be important, they have been essentially ignored for the biaxial mechanical testing of connective tissue materials.

Historically, two methods have been used to determine biaxial mechanical properties of planar connective tissues: inflation studies [1–3] and the deformation of square specimens with sutured edges [4–9]. Hildebrant et al. [1] first reported the inflation method in which a circular portion of tissue was clamped and one side was subjected to a positive pressure in order to deform the membrane. The other, and arguably more popular method of biaxial testing has been the use of square samples with sutured edges based on the work of Lanir

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and Fung [4]. Interestingly, none of these works have made mention of the vast literature regarding biaxial testing of engineering materials. The most popular sample geometry for the biaxial testing of engineering materials has been the cruciform (cross-shaped) sample developed by Mönch and Galster [10]. This geometry has been extensively used in the testing of metallic materials [11–13] and composite materials [14–17]. Recent studies on fabric [18] and planar connective tissues [19,20] have also used similar sample geometries.

Although not explicitly mentioned by Mönch and Galster [10], the adaptation of long sample arms to biaxial testing is loosely based upon the work of the French mathematician and engineer Adhémar Barré de Saint-Venant (1797–1886). Saint-Venant's principle essentially states that stress (and presumably the strain) distribution may be assumed to be independent of the actual mode of load application—except in the immediate vicinity of where the load is applied. The method of gripping sample edges imposes local stresses and strain constraints at the grip-sample interface and these local influences are considered to become negligible at some distance away from the grip. The Saint-Venant principle has thus been the primary motivation for the use of long slender samples in uniaxial material testing, with extensometer-based strain measurement at the sample mid-region.

Previous studies have demonstrated the Saint-Venant effect in uniaxial connective tissue samples [21,22], stating that strain measurements based on the grip-to-grip displacement may grossly overestimate the specimen strain and suggested measurement of local strains at the sample centre. Jimenez et al. [23] also demonstrated negligible influence of the grips on central specimen strains when the tendon sample length was greater than eight diameter multiples: a ratio similar to the aspect ratio (ratio of sample length to width) used when testing engineering materials.

In connective tissues subjected to tensile loads (e.g. tendon, pericardium, etc.), the extracellular reinforcing fibres are presumably orientated along lines of principle stresses or strains to effectively distribute the load throughout the structure. Observations of these fibres in the pericardium [24–28] and fish skin [29,30] at a light microscopic level (100–200X magnification) reveal continuous bands or sheets of collagen fibrils with interspersed elastic fibres that also appear to be continuous in nature. However, there is some evidence that collagen fibrils in fish skin [31] as well as in tendons and ligaments are not continuous but display a spectrum of characteristic lengths [32–34]. If collagen fibrils are in fact discontinuous, then there must be some level of continuity within the structure in order to distribute the applied load since the amorphous matrix likely does not mediate stress transfer between adjacent fibrils [35]. Although certain proteoglycans bind specifically to collagen [36] it is unlikely that their relatively long filaments are completely responsible for holding the entire structure together. The more plausible explanation is that physical interactions between the adjacent fibrils, such as interweaving [35] is the predominant mechanism by which load is transmitted throughout the tissue. Therefore, either these tissues exhibit continuous fibre reinforcement, or it can be assumed that an "effective" continuous fibre reinforcement exists at some level within the tissue.

This continuous nature of the reinforcing fibres in planar connective tissues may lead to unanticipated difficulties when attempting to ascertain the mechanical properties of these tissues by conventional testing methods. The use of uniaxial samples with high aspect ratios or biaxial samples with slender cruciform arms could lead to the situation where a discrete subset of fibres that fully extend from grip-to-grip are preferentially loaded—especially if the fibres are not completely aligned with the testing axes. This subset of fibres is dependent on the aspect ratio of the specimen and the orientations of the fibre populations. By altering sample geometry to reduce the influence of the grips, the problem of loading a smaller proportion of the reinforcing fibres may be exacerbated. Therefore, any measured mechanical properties are to some extent artifactual, with properties reflecting sample geometry. There have been a few studies that reported alterations in mechanical behaviour with varying sample length or aspect ratio [37,38]; unfortunately these studies based their strain measurements on the grip-to-grip displacement and their conclusions must be viewed with caution.

The purpose of this study was to test the hypothesis that, as the length of the sample arms are reduced, an *apparent* increase in stiffness (tangent modulus) and decrease in extensibility (maximum strain) of the material sample will occur presumably due to an increasing proportion of collagen fibres that fully extend between the grips. Equibiaxial tension experiments were undertaken on both pericardial heart valve materials and fish skin: two planar connective tissues that have different structures and display distinctly different material properties. After testing of connective tissue samples, similar experiments were performed on model textile laminates with simultaneous measurement of fibre orientation during deformation to help determine the underlying mechanism responsible.

2. Methods

2.1. Materials

Three different materials were used in this study: (i) fish skin which exhibits a simple orthogonal array of collagen fibres [31,39], (ii) bovine pericardium which displays a more complex