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Full-field bulge test for planar anisotropic tissues: Part I – Experimental methods applied to human skin tissue

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The nonlinear anisotropic properties of human skin tissue were investigated using bulge testing. Full-field displacement data were obtained during testing of human skin tissues procured from the lower back of post-mortem human subjects using 3-D digital image correlation. To measure anisotropy, the dominant fiber direction of the tissue was determined from the deformed geometry of the specimen. Local strains and stress resultants were calculated along both the dominant fiber direction and the perpendicular direction. Variation in anisotropy and stiffness was observed between specimens. The use of stress resultants rather than the membrane stress approximation accounted for bending effects, which are significant for a thick nonlinear tissue. Of the six specimens tested, it was observed that specimens from older donors exhibited a stiffer and more isotropic response than those from younger donors. It was seen that the mechanical response of the tissue was negligibly impacted by preconditioning or the ambient humidity. The methods presented in this work for skin tissue are sufficiently general to be applied to other planar tissues, such as pericardium, gastrointestinal tissue, and fetal membranes. The stress resultant–stretch relations will be used in a companion paper to obtain material parameters for a nonlinear anisotropic hyperelastic model.

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1. Introduction

The characterization of human skin biomechanics is essential for a wide variety of applications, from modeling the tissue–device interface of medical devices to tissue engineering. The anisotropic properties of skin arise from the collagen–elastin fiber microstructure and can have important implications for applications such as creating accurate models of an amputee's residual limb, quantifying changes in microstructure during scarring, designing artificial skin, and imaging for cancer detection. The outermost layer of the skin, the epidermis, is 70–120 μm thick and composed primarily of cells. The 1–4 mm thick dermis is the thickest component of skin and is comprised of a collagen–elastin fiber network embedded in a matrix of hydrated proteoglycans [1]. The network consists predominantly of collagen, which contributes 70–80% of the dry weight of skin, while elastin contributes to 2–4%. Scanning electron microscopy has shown that collagen fibers are arranged in the plane of the dermis [2]. The dermis, and specifically the collagen–elastin network, is thought to dominate the finite deformation mechanical properties of skin [3].

Mechanical experiments have repeatedly demonstrated that skin exhibits a nonlinear and anisotropic stress response [4,5]. Much of the current literature concerning the large deformation response of skin tissue has been determined from in vitro uniaxial tension tests [6–9,2]. These tests have the advantage of standard implementation and straightforward analysis. Consequently, they are often used to compare the effect of therapeutic treatments on the tissues, such as the subcutaneous expansion of skin for grafting [6–8] and wound healing [9]. Uniaxial tests have also been applied to investigate the structural origins of the large deformation mechanical behavior of skin tissues. Experiments paired with scanning electron microscopy (SEM) imaging of the initial and deformed states have shown that the nonlinear J-shaped stress response is caused by the recruitment of crimped collagen fibers of the dermis. Stress is carried by the elastin and ground matrix in the toe region [10], the initial compliant portion of the stress response, and by the straightened collagen fibers in the stiffened region [2]. Enzymatic subtraction studies further support the hypothesis that the deformation of collagen is responsible for the large stress response. Rat skin treated with elastase, which removes the elastin component of the tissue, exhibits an extended toe region and incomplete elastic recovery but the same large strain linear region [11].

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Most in vitro studies of skin tissue have used animal models, which can exhibit significant differences in stiffness compared to human skin [12]. For example, Dunn et al. [13] carried out uniaxial tests of human skin tissue from the chest. Evans [12] compared this data to that obtained on pig skin by Shergold et al. [14] and found large differences in the stress response, with the pig skin being approximately 30 times stiffer than human. Similar uniaxial tests carried out using human thigh skin [15], rat skin [16] and bovine dermis [3] also showed wide variation in stress response, with the pig skin exhibiting the stiffest and rat skin the most compliant response. Uniaxial tests have also been used to investigate anisotropy in the stress–strain response of skin tissue from both animal models and human specimens. The anisotropic behavior of skin has been attributed to the preferential alignment of collagen fibers in the dermis [2]. The preferred orientations vary throughout the body and have been historically described by Langer's lines [17]. For example, Dombi et al. [18] tested rat skin samples from the back oriented either parallel or transverse to the spine. Samples were tested to failure and the collagen content of the tissues was measured. It was seen that specimens loaded in the direction transverse to the spine exhibited higher tensile strength than specimens loaded parallel to the spine, and specimens with higher collagen content correlated with higher tensile strength. Similarly, Annaidh et al. [4,5] measured the stress response of human specimens from various orientations on the back and showed a large difference between different orientations.

Biaxial testing applies a stress state that is closer to in vivo loading and allows for the simultaneous comparison of two material directions of the same sample. Lanir and Fung [19,20] first proposed a biaxial test system consisting of sutures on each edge of a square specimen of rabbit skin. The skin was stretched via sutures applied to all edges of a square patch of tissue. Force was applied in the longitudinal and orthogonal direction of the sample. Force–stretch curves were reported for samples where one direction was held at the reference width and the other direction was stretched. A similar biaxial experiment was carried out by Schneider et al. [21] on human abdominal skin. Compared to Lanir's data from rat skin, human skin stiffened earlier and the effect of anisotropy was not as pronounced.

To restore excised skin to its in vivo configuration, Reihsner et al. [22,23] developed an experimental setup to apply simultaneous radial loading at 12 points around a circular sample of human tissue. Like biaxial tension tests, this test produces a biaxial stress state at the center of the sample, but the multiple grips allow the stiffest material direction to be identified. Samples removed from the body were extended from the relaxed state back to the in vivo configuration while force was monitored. The stiffest direction measured was interpreted as the average fiber direction over the sample and incremental elastic constants were reported. Comparing the human data from these tests to uniaxial tests described previously is difficult because of the different stress state, and even within human uniaxial tests, a wide range of stress–strain behavior is observed, probably due to the different body locations tested. A similar radial test has been applied in vivo [24], as well as indentation [25] and suction [26] tests. These tests recruit subcutaneous tissues in addition to skin and require finite-element analysis to extract material properties.

Bulge testing also applies a biaxial stress state when the radial stress components are small compared to in-plane stresses. Radial stresses are negligible for thin membranes and zero at the outer surface of the tissue. The fiber direction can be experimentally determined from the bulge test, but unlike the 12-point test developed by Reihsner and coworkers, bulge testing allows for complete fixation of the edges of the tissue. This may prevent microstructural rearrangements with loading, and mitigate the effects of preconditioning. Preconditioning, or repeated loading prior to testing, is typically used to obtain repeatable test results from uniaxial and biaxial tests. Previous inflation tests on ocular tissue have shown repeatable measurements of the stress response with negligible effects from preconditioning [27,28]. Bulge tests are not commonly used to characterize the mechanical behavior of skin tissues. At least two studies have applied bulge testing to skin but have not determined the stress–strain response or investigated the mechanical anisotropy [29,30].

Bulge testing is typically used to characterize the isotropic, elastic properties of metallic [31–33] and polymer thin films [34–36] that can be modeled as thin membranes. To calculate the membrane stresses, it is assumed that the membrane deforms into a spherical cap. This allows the principal stretches to be calculated from the difference in the arc length of the deformed cap and the initial specimen diameter [33] or the gradient of displacements measured from grid markers on the material surface [37]. Stresses can be calculated from the applied pressure by assuming a deformed spherical shape and neglecting the effects of bending [38]. In cases where the material is too thick to assume membrane behavior and large deformations are present, inverse finite-element methods have been applied to incorporate the effects of bending in calculating the material properties from the pressure–displacement response [39]. Bulge testing has been applied to many biological tissues such as canine pericardium [40], canine jugular vein [41] and murine pulmonary arteries [42]. These studies assumed the tissue could be treated as isotropic and utilized the spherical cap membrane stress approximation to determine stress–strain behavior.

Recent bulge testing methods have advanced to characterize the anisotropic properties of fibrous tissues. The orientation of the stiffest material direction in the plane of the tissue is determined from the deformed shape using 2-D images of the deformed profile in 2–6 planes [43,42] or Moir interferometry [44]. For example, Zioupos et al. [44] used Moir interferometry to determine the stiffest material orientation of bovine pericardium. The authors demonstrated that the minor axis of the ellipsoid formed by inflation of a circular sample corresponded to the preferred fiber orientation using polarized light microscopy. Similarly, Drexler et al. [42] determined the stiffest direction of rat extrapulmonary arteries by imaging the profile of the inflated tissue at 30° increments. The minor axis of the ellipsoid was determined from the angle for which the profile of the tissue was minimized. Rather than determine the stiffest direction of the tissue, Marra et al. [43] imaged the profile of porcine aortic tissue in the axial and circumferential directions to compare these anatomical orientations directly. For all three studies, the normal strain components along the stiffest and least stiff directions [44,42] or axial and circumferential directions [43] were calculated globally from the arc length of the deformed tissue compared to the diameter of the undeformed tissue. The normal components of the membrane stress along these same directions were calculated from the pressure and deformed radii of curvature. However, using the membrane approximation ignores the effect of bending on the stress response of thick samples. Later work by Bischoff et al. [45] accounted for bending in the same experiment developed by Drexler et al. by utilizing inverse finite-element analysis to determine material parameters from bulge test data. The inverse finite-element method is more accurate than the membrane stress approximation but less efficient.

This work presents a bulge test method capable of repeatable measurements of the anisotropic nonlinear properties of human skin. The method utilizes stereoscopic digital image correlation (DIC) to obtain full-field displacement measurements for the surface of the inflating tissue. The full-field measurements allow for the determination of the in-plane material directions of the tissue as well as the calculation of local strains and curvatures in the

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