Mechanical characterization of the rat and mice skin tissues using histostructural and uniaxial data

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The skin tissue has been shown to behave like a nonlinear anisotropic material. This study was aimed to employ a constitutive fiber family equation to characterize the nonlinear anisotropic mechanical behavior of the rat and mice skin tissues in different anatomical locations, including the abdomen and back, using histostructural and uniaxial data. The rat and mice skin tissues were excised from the animals' body and then the histological analyses were performed on each skin type to determine the mean fiber orientation angle. Afterward, the preconditioned skin tissues were subjected to a series of quasi-static axial and circumferential loads until the incidence of failure. The crucial role of fiber orientation was explicitly added into a proposed strain energy density function. The material coefficients were determined using the constrained nonlinear optimization method based on the axial and circumferential extension data of the rat and mice samples at different anatomical locations. The material coefficients of the skins were given with $R^2 \geq 0.998$. The results revealed a significant load-bearing capacity and stiffness of the rat abdomen compared to the rat back tissues. In addition, the mice abdomen showed a higher stiffness in the axial direction in comparison with circumferential one, while the mice back displayed its highest stiffness in the circumferential direction. The material coefficients of the rat and mice skin tissues were determined and well compared to the experimental data. The optimized fiber angles were also compared to the experimental histological data, and in all cases less than 11.85% differences were observed in both the skin tissues.

Introduction

Skin is a 3-layered structure which protects the human body from the external biochemical, biological, immunological, physical, mechanical, and thermal reactions as a physical barrier. It is biomechanically defined as a nonlinear, viscoelastic material.1,2 It is also known to tolerate almost large deformation loads when removed from a subject's body.3,4 The mechanical behavior of many biological tissues, such as skin, has a close relationship with their microstructure.3 The elastin, as one of the main microstructural components, has been shown to have a key asset in strain recovery of the skin and its contribution to the elasticity modulus is small.3 However, the type I collagen fiber has a vital role not only in large deformation but in the anisotropy and tensile strength of the skin.4,6 Collagen is a primary element of soft tissues, especially the skin tissue. Although skin tissue consists of collagen fibers with almost same structures, its mechanical behavior may vary according to the loading direction which applied to the skin.

Both uniaxial and biaxial tensile tests can be employed to capture the mechanical properties of soft biological tissues. The uniaxial tensile test is a highly recommended mechanical test on the grounds of simplicity, small sample size, and availability of commercial uniaxial devices. Hence, many works have been carried out to obtain the 2-dimensional (2D) or 3-dimensional (3D) constitutive equations from uniaxial data but not for skin tissues.7,8 The small sample size is another reason which might be related to the samples and multi-layered structure of the skin tissues.10,11 These limitations trigger bounding the application of biaxial tensile tests in determining the mechanical properties of soft biological tissues. The recently reported constitutive equations showed that the uniaxial tests on 2 orthogonally cut samples enable us to determine the anisotropic mechanical properties of soft biological tissues same as that achieved by biaxial tests.7,12

The mechanical properties of the skin tissue so far have been measured by applying deformation forces, including traction, tension, suction, torsion or indentation in various ways to the skin samples.13,14 Uniaxial extension setups are beneficial as
the can be used to evaluate in-plane directional differences in material properties and can be non-invasive, applicable and easy to use in vivo. Characterization of the mechanical properties of murine (rat or mice) skin would be important to allow its use as an animal model for human skin diseases. Investigation of the biomechanical properties in such murine could be beneficial particularly with regard to directional mechanical properties of the skin and forensic as well as cosmetic surgeries. Examination of the biomechanical properties of the murine skin would also be of interest in veterinary medicine as regards the healing of canine skin particularly during application of bioactive wound dressings.

The skin tissue can simply be defined to behave like a hyperelastic material. Many works either chose a purely phenomenological approach like the Fung-type model, or consider histostructural information, such as the Holzapfel-Gasser-Ogden constitutive model or fiber family model to capture the nonlinear hyperelastic mechanical behavior of the soft biological tissues, especially the arterial wall. Since the fiber family constitutive based material model has the potential ability to address the mechanical properties of the skin tissue better than that of phenomenological model, it would be useful and practically valuable to determine the material coefficients of the rat and mice skins in different anatomical locations, including the abdomen and back, from uniaxial data with the previously proposed model by Holzapfel. Therefore, the objective of this study is to experimentally and numerically determine the anisotropic mechanical properties of the rat and mice skin tissues using histostructural and uniaxial test data.

Results

The axial and circumferential direction of (a) a skin tissue and (b) its condition under the uniaxial load in the testing machine are presented in Figure 1. The removed skin tissues

Figure 1. A (a) skin tissue sample for the mechanical measurements. The (b) skin tissue samples were subjected to the axial and circumferential loads.

Figure 2. A 1 mm thick histological section of (a) rat abdomen, (b) rat back, (c) mice abdomen, and (d) mice back with circumferential orientation. Due to a planar sectioning, the in-plane fiber orientations are observed. Black lines indicate mean fiber orientation characterized by $\alpha$. 
from the rat and mice were divided into 4 groups, including the rat abdomen, rat back, mice abdomen, and mice back. The skin tissue samples were imaged no longer than 1-hour postmortem to minimize tissue degradation and any subsequent effects which may be related to the postmortem time issue. The skin tissue samples were then cut and stained for histological analysis. The obtained histological images, including (a) rat abdomen, (b) rat back, (c) mice abdomen, and (d) mice back, are indicated in Figure 2. The samples were placed in the circumferential direction and the mean fiber angle orientation of collagens in the skin tissue (α) was determined based on the dominant fiber orientations. The samples were then subjected to a series of axial and circumferential tensile tests and the mean stress-strain diagrams of samples before the incidence of failure was recorded. The stress-strain diagrams of the rat skin tissue under (a) axial and (b) circumferential loadings are depicted in Figure 3. Besides, the stress-strain diagrams of the mice skin tissue under (a) axial and (b) circumferential loadings are demonstrated in Figure 4. The rat abdomen, mice abdomen and back skins showed anisotropic responses while rat back displayed almost isotropic response (compare the stress-strain behaviors for the axial and circumferential), with nonlinear stiffening which is typical for most soft biological tissues (Figs. 3 and 4). Regarding the rat skin, the highest stress in the axial direction was observed in the abdomen skin, while in the circumferential direction the highest one was seen in the back skin. This is in complete agreement with our experimental results which showed that the mean fiber orientation for the abdomen and back skin is aligned in the axial and circumferential directions. On the other hand, the results of the mice skin revealed that the abdomen skin has the highest stress in the axial direction, while the back skin has its highest stress in the circumferential direction. This is also in good agreement with our experimental results which showed that the mean fiber orientation for the abdomen and back skin is aligned in the axial and circumferential directions.

The polynomial coefficients (α1, ..., α5; β1, ..., β5) of the rat and mice skin tissues at different anatomical locations, which were determined using nonlinear optimization method, are listed in Table 1. The values of parameters C, c1, c2, c3, c4, c5, and θ in the fiber family constitutive model were estimated and provided in Table 2 where the computations were based on the average of 6 sets of data for both type of skin tissues.

Discussion

From an analysis of the relationship between strain components in uniaxial tests, this study performed an analysis to identify the constitutive parameters of the rat and mice skin tissues by utilizing the nonlinear optimization method on data from the histostructural analyses and uniaxial extension tests, under the assumption that the constitutive model is governed by the fiber family model.

There have been some reports on the measurement of the mechanical properties of the rat and mice skin tissues using uniaxial or biaxial tensile tests. However, so far no study has investigated the anisotropic mechanical properties of the rat and mice skin tissues at different anatomical locations of their body, i.e., abdomen and back, using histostructural and uniaxial extension data. There have been some studies which used Ogden, Neo-Hookean, Mooney-Rivlin, and Fung-type material model to address the nonlinear mechanical behavior of the skin tissue. However, none of those studies implemented the role of fiber orientations into a constitutive equation to capture the anisotropic nonlinear mechanical properties of the skin tissue. In the
In this study, the angles between the fiber direction and the circumferential direction of the rat and abdomen skins were experimentally and numerically determined using histostructural and optimization approach, respectively. The histostructural analyses revealed the angle of 47.32° ± 3.52°, 14.10° ± 1.20°, 52.25° ± 2.85°, and 29.10° ± 1.98° for the rat abdomen, rat back, mice abdomen, and mice back, respectively. Interestingly, the results obtained from the optimization method were in good agreement with those of experimental data with 41.71°, 13.12°, 48.35°, and 28.59°, for the rat abdomen, rat back, mice abdomen, and mice back, respectively.

Although the methods of determination of constitutive model based on uniaxial tests have been reported, this study benefited from both the experimental and numerical method which enable us to compare our results to that of numeric ones. Although uniaxial tensile test has its obvious limitation, this study could have benefited from the biaxial extension test which have to be taken into account in future studies. Generally, it is difficult to fully assess the anisotropy of planar tissues since the data obtained in uniaxial tests are insufficient for complete constitutive formula. The strategy to replace 2 uniaxial measurements with one biaxial test is important, as 2 perpendicular uniaxial tests represent only the 2 extreme cases of 2D stretch. Besides, the tissue response under uniaxial loading does not reflect in vivo deformations, which leads to a reorientation of collagen fiber orientations and often to softer stress-strain curves compared to data obtained from biaxial testing. In fact, our method is not contradictory to the aforementioned conclusion: we took uniaxial extension test in the perpendicular direction as a specialized biaxial extension test, i.e., there is deformation but no force in the direction perpendicular to extension, and this has been considered in estimating material parameters. Therefore, the prior constitutive model can be employed to determine the material mechanical properties for those that are not amenable to biaxial extension test by our method. Finally, the residual stress in our study has not been taken into account, which should be incorporated to reflect the full mechanical state of the tissue.

**Conclusions**

This study identified the material coefficients of the rat and mice skin tissues in different anatomical locations of the body, such as the abdomen and back, using histostructural and uniaxial

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**Table 1.** The identified polynomial parameters of rat and mice skins at different anatomical locations, i.e., back and abdomen

| Skin type      | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ | $\beta_1$ | $\beta_2$ | $\beta_3$ | $\beta_4$ | $\beta_5$ |
|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Rat abdomen    | -2.432     | 14.013     | -64.275    | 149.052    | -129.214   | -1.502     | 2.306      | -1.950     | 0.849      | -0.147     |
| Rat back       | -1.164     | 1.389      | 0.961      | -3.136     | 1.545      | -0.907     | 0.953      | 0.815      | -1.389     | 0.452      |
| Mice abdomen   | -1.340     | 26.534     | -125.518   | 410.016    | -995.296   | -0.671     | 1.561      | -6.574     | 9.493      | -5.678     |
| Mice back      | -1.321     | 0.903      | 2.684      | -5.414     | 2.793      | 2.166      | -8.832     | -49.205    | 34.869     | 0.304      |
extension test through the fiber family based constitutive equation proposed by Holzapfel. The results regardless of the skin type revealed the anisotropic mechanical behavior of the skin tissue. The fiber angle which was determined by optimization method was in good agreement with that of histological data. These results may have implications not only for capturing the directional mechanical properties of the skin tissues but also for providing more information for orthopedic surgeries, cosmetic surgeries, and any type of skin related surgeries as well as biomechanical modeling.

### Materials and Methods

#### Experimental testing

The sample preparation, experimental setup, and stress-strain analysis were described comprehensively in our previous studies. Eight male rats and 8 male mice aged from 8–10 weeks and weighing between 260–280 and 20–25 g, respectively, were used. All sixteen murine were humanely killed at the animal facility (according to the ethics regulations) with overdose ketamine hydrochloride and their abdomen and back skins' hairs were carefully removed from the samples using a surgical scalpel. The abdomen and back skins were excised together in the axial and circumferential directions with panniculus having length and width of 20 and 20 mm, respectively. The width and length of the skin tissues were measured precisely using digimatic ruler having a resolution of 0.005 mm ± 0.05% (Insize, Vienna, Austria). Samples were preserved in 0.9% normal saline solution with a temperature of 4°C right before the mechanical measurements. The preliminary test results (data not reported) showed that the conditioning of the tissue can be fully reached after 10 cycles. The tensile test was performed using a uniaxial tensile test apparatus adapted for testing biological specimens used in our previous studies. All tests were performed at 25°C and each sample was tested only once. A low strain rate of 5 mm/min which is typical for surgical procedures and gives more insight into the skin tissue behavior was employed by the action of an axial servo motor. Moreover, rough sandpaper was used between the jaw and sample to assure no slip boundary. The sample’s length was measured after the application of the preload. The curves of stress/strain that occurred in the meantime were obtained.

#### Constitutive model

The passive mechanical behavior of the skin is characterized by a strain-energy function per unit reference volume ($\Psi$) as follows,

$$
\Psi = \Psi_{iso}(E) + \Psi_{ortho}(E)
$$

where $\Psi_{iso}$ is an isotropic contribution to $\Psi$, which mostly considers the initial stiffness of the skin tissue represented by the elasticity of the non-fibrous substances. The strain energy $\Psi_{ortho}$ stands for an orthotropic contribution which governs the much higher stiffness at large strains represented by the randomly oriented collagen and aligned components of collagenous fibers.

As a particular choice for $\Psi$ a combined polynomial-exponential form of this constitutive equation has been proposed. The Neo-Hookean model $\Psi_{iso} = C(I_1 - 3)/2$ for the isotropic contribution has been suggested with the stress-like material parameter $C > 0$ (the shear modulus). The strain energy function can be expressed as following by considering the 4-fiber family of collagen:

$$
W = \frac{C}{2} (I_1 - 3) + \sum_k \frac{C_k^e}{4C_2^e} \left( \exp \left[ \frac{C_k^e}{4C_2^e} \left( \frac{\lambda_{(k)^2} - 1}{2} \right)^2 - 1 \right] \right)
$$

$$
\lambda_{(k)^2} = \left( \lambda_1 \cos \theta_{(k)} \right)^2 + \left( \lambda_2 \sin \theta_{(k)} \right)^2
$$

which is a form of strain-energy function, a straightforward extension of the Holzapfel model, where $I_1$ is the first invariant of the right Cauchy-Green tensor ($C$).

$$
I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2
$$

$C$ (kPa) is also an associated material parameter. Superscript $k$ denoting the $k$th fiber family, $\lambda_{(k)}$ is the stretch of the $k$th fiber family, $\theta_{(k)}$ is the angle between the fiber direction and the axial direction. Fiber families $k = 1$ and 2 denote axially ($\theta_{(1)} = 0^\circ$) and circumferentially ($\theta_{(2)} = 90^\circ$) oriented collagen fibers, respectively, whereas families $k = 3$ and 4 denote diagonally ($\theta_{(3)} = -\theta_{(4)} = \theta_{(5)}$) oriented collagen fibers. Positive numbers $C^e_k$ (kPa) and $C^e$ (dimensionless) are associated material parameters. If we assume that the material parameters for the diagonal fibers become equal ($C^e_3 = C^e_4$ and $C^e_5 = C^e_2$), then it leads to the 4-fiber

### Table 2. The identified material parameters of rat and mice skins at different anatomical locations, i.e., back and abdomen

| Skin type          | $C$ (kPa) | $C_1^e$ (kPa) | $C_2^e$ (1) | $C_3^e$ (kPa) | $C_4^e$ (1) | $C_5^e$ (kPa) | $C_6^e$ (1) | $C_{theoretical}$ (experimental) | $R$ |
|--------------------|-----------|---------------|-------------|--------------|-------------|--------------|-------------|----------------------------------|-----|
| Rat abdomen        | 63.911    | 1.183         | 1.668       | 0.497        | 0.344       | 119.831      | 0.241       | 41.711 (47.321 ± 3.521)          | 0.999 |
| Rat back           | 2.196     | 0.095         | 0.245       | 0.002        | 0.341       | 0.121        | 0.832       | 13.121 (14.102 ± 1.205)          | 0.998 |
| Mice abdomen       | 0.922     | 1.845         | 1.262       | 6.640        | 0.391       | 3.066        | 1.559       | 48.352 (52.256 ± 2.852)          | 0.999 |
| Mice back          | 1.210     | 0.326         | 0.139       | 1.103        | 1.189       | 0.115        | 0.093       | 28.591 (29.101 ± 1.987)          | 0.999 |
family 8-parameter model,37
\[
W = \frac{C}{2} \left(2(E_{11} + E_{22}) - 1 + \frac{1}{(1 + 2E_{11})(1 + 2E_{22})} \right) \\
+ \frac{C_1}{4C_2} \left(\exp \left[C_1^2(2E_{11})^2\right] - 1 \right) + \frac{C_2}{4C_3} \left(\exp \left[C_3^2(2E_{22})^2\right] - 1 \right) \\
+ \frac{C_1^2}{4C_2} \left(\exp \left[C_1^2(2E_{11}\cos^2\theta + 2E_{22}\sin^2\theta)^2\right] - 1 \right)
\]  
\tag{5}
Which \( E_{ii} \) is the component of the Green-St. Venant strain tensor, and is computed by:
\[
E_{11} = \frac{1}{2}(\lambda_1^2 - 1), \quad E_{22} = \frac{1}{2}(\lambda_2^2 - 1) \tag{6}
\]

The relationship between \( E_{11} \) and \( E_{22} \) and the relationship between the stress and strain in uniaxial tests

Imagine that planar load is applied to the material, without loss of generality, by the application of chain rule derivatives, the second Piola-Kirchhoff stresses can be written as:
\[
S_{11} = S_1(E_{11}, E_{22}) = C \left[1 - \frac{1}{(1 + 2E_{11})(1 + 2E_{22})}\right] \\
+ C_1 E_{11} \exp \left[C_1^2(2E_{11})^2\right] \\
+ C_2 E_{11} \exp \left[C_2^2(2E_{22})^2\right] \\
+ C_3 E_{11} \exp \left[C_3^2(2E_{11}\cos^2\theta + 2E_{22}\sin^2\theta)^2\right] \\
(11)
\]
\[
S_{22} = S_2(E_{11}, E_{22}) = C \left[1 - \frac{1}{(1 + 2E_{11})(1 + 2E_{22})}\right] \\
+ C_1 E_{11} \exp \left[C_1^2(2E_{22})^2\right] \\
+ C_2 E_{11} \exp \left[C_2^2(2E_{11}\cos^2\theta + 2E_{22}\sin^2\theta)^2\right] \\
(12)
\]  
For uniaxial loading, the component of the stress tensor in the transverse (circularmerential) direction of the tensile axis is zero. From \( S_{22} = 0 \) when tension is in \( x_1 \)-direction, we obtain:
\[
E_{22} = \alpha(E_{11}) = \alpha_1 E_{11} + \alpha_2 E_{11}^2 + \cdots + \alpha_5 E_{11}^5 \tag{9}
\]
Substitute the above equation into Equation (7), we obtain:
\[
S_{11} = \xi(E_{11}) = S_1(E_{11}, \alpha(E_{11})) \tag{10}
\]
Similarly, considering tensile in the \( x_2 \)-direction:
\[
E_{11} = \beta(E_{22}) = \beta_1 E_{22} + \beta_2 E_{22}^2 + \cdots + \beta_5 E_{22}^5 \tag{11}
\]
and
\[
S_{22} = \eta(E_{22}) = S_2(\beta(E_{22}), E_{22}) \tag{12}
\]  
Here, we note that \( \alpha(\cdot) \) and \( \beta(\cdot) \) give a description of the relationship between \( E_{11} \) and \( E_{22} \) and that \( \xi(E_{11}) \) and \( \eta(E_{22}) \) are the second Piola-Kirchhoff stresses in uniaxial extension tests.

**Histology**

After all tests, the strip samples were inserted into a 4% buffered formaldehyde solution (pH 7.4) for fixation and further histological investigation. Specimens were embedded maintaining their planar geometry and sectioned serially at 5 and 2 \( \mu \)m in tangential orientation for the rat and mice skin tissues, respectively, hence the fiber orientations in the \( (x_1, x_2) \)-plane were seen on the histological images, where \( x_1 \) and \( x_2 \) denote the circumferential and the axial directions. A skilled histopathologist measured the orientation of at least 38 representative collagen fibers in the skin tissue per specimen from the histological images. Mean (fiber) angles and standard deviations were determined numerically from the data by assuming normal distribution and symmetrical arrangement with respect to the circumferential direction.

**Estimation of the material parameters**

The material parameters can be estimated by optimizing (minimizing) the stress-based nonlinear function
\[
f_s = \sum_{i=1}^{n} \left(\xi(E_{11}^{(i)}) - S_{11}^{(i)}\right)^2 + \sum_{j=1}^{m} \left(\eta(E_{22}^{(j)}) - S_{22}^{(j)}\right)^2 \tag{13}
\]
in which \( n \) and \( m \) are the number of experimental data in \( x_1 \)-direction and \( x_2 \)-direction uniaxial tensile tests, respectively. In Equation (13), \( S_{11}^{(i)} \) and \( S_{22}^{(j)} \) are second Piola-Kirchhoff stresses, and \( E_{11}^{(i)} \) and \( E_{22}^{(j)} \) are the components of the Green-St. Venant strain tensor, all of which can be calculated directly from the \( i \)th and \( j \)th experimental data point, respectively. However, the analytical expressions of \( \xi(\cdot) \) and \( \eta(\cdot) \) are not determined yet as \( \alpha(\cdot) \) and \( \beta(\cdot) \) are not given. On the other hand, a variety of numerical simulations reveal that we always get better fits (a polynomial of degree 5) to the data generated by \( S_{22} = 0 \) and \( S_{11} = 0 \). Therefore, the following optimization problem is needed to be solved:
\[
\min f_s = \sum_{i=1}^{n} \left[ \frac{1}{2} \sum_{p=1}^{5} \alpha_p (E_{11}^{(i)})^p - S_{11}^{(i)} \right]^2 + \sum_{j=1}^{m} \left[ \frac{1}{2} \sum_{p=1}^{5} \beta_p (E_{22}^{(j)})^p - S_{22}^{(j)} \right]^2 \tag{14}
\]
\[
g_s = \sum_{i=1}^{n} \left[ \frac{1}{2} \sum_{p=1}^{5} \alpha_p (E_{11}^{(i)})^p \right]^2 + \sum_{j=1}^{m} \left[ \frac{1}{2} \sum_{p=1}^{5} \beta_p (E_{22}^{(j)})^p \right]^2 < \delta \tag{15}
\]
where \( \delta \) is a given sufficiently small positive number. The goal
now is to minimize the nonlinear function \( f_\ell \) with respect to material coefficients and parameters \( \alpha_p \) and \( \beta_p \) \((p = 1, \ldots, 5)\), which should be subjected to the positivity of material parameters and to the inequality constraints:

\[
0 \leq \theta \leq 90^\circ; \quad -0.5 \sum_{p=1}^{5} \alpha_p (E_1^{(i)})^p, \quad \sum_{p=1}^{5} \beta_p (E_2^{(i)})^p \leq 0 \quad (i = 1, \ldots, n; j = 1, \ldots, m)
\]

(16)

In order to compare the goodness-of-fit, the traditional coefficient of determination \( R^2 \) was computed.

**Statistical analysis**

Data were first analyzed by analysis of variance (ANOVA); when statistical differences were detected, student’s t-test for comparisons between groups was performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, United States).48,49 Data are reported as mean ± std at a significance level of \( P < 0.05 \).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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