Strain rate sensitivity of skin tissue under thermomechanical loading

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There have been limited studies addressing the thermally dependent mechanical properties of skin tissue, although this can contribute to a variety of medical applications. To address this, an experimental study on the tensile behaviour of pig skin tissue under different thermal loading conditions and different mechanical stretching rates was performed. The results indicate that there is a significant variation among skin tensile behaviours under different temperatures and loading rates, which is correlated with dermal collagen denaturation. The Ogden model was used to summarize the effect of the strain rate and the temperature upon the measured constitutive response through two parameters ($\alpha$ and $\mu$). These results can be used in future models to improve clinical thermal treatments for skin tissue.

Keywords: skin tissue; thermal denaturation; thermomechanical behaviour; tension

1. Introduction

Advances in laser and similar techniques have led to recent developments in thermal treatments of diseased and injured skin tissue, involving either a raising (Kono et al. 2006) or lowering (Spencer 2004) of the temperature in a targeted skin area to kill the necrotic cells, but without affecting the surrounding healthy tissue. A detailed understanding of the coupled biological–mechanical response under thermal loading can contribute to the design, characterization and optimization of these treatments for delivering better medical treatments.

When skin tissue is heated, collagen (major component of skin) inside the tissue undergoes a transition from a highly organized crystalline structure to a random, gel-like state (denaturation process; Flory & Garrett 1958) due to the breaking of heat-labile intramolecular cross-links. This results in the shrinkage of collagen (Flory & Garrett 1958; Arnoczky & Aksan 2000) and thus marked changes in tissue mechanical properties (Chen et al. 1997, 1998a,b; Chen & Humphrey 1998; Chen et al. 1999).
This thermal effect depends on several factors, including the collagen content (Chvapil & Jensovsky 1963), the maximum temperature reached and exposure time (Allain et al. 1980) and the mechanical stress applied to the tissue during heating (Chen et al. 1998a, b). The degree of thermal damage to collagenous tissue can be correlated with collagen denaturation as calculated using the Arrhenius burn integration, defined as follows (Henriques & Moritz 1947):

\[
\text{Deg}(t) = \frac{C(0) - C(t)}{C(0)} = 1 - \exp[-\Omega(t)],
\]

where \(C(0)\) and \(C(t)\) are the initial concentration and the concentration remaining at time \(t\) of undenatured collagen, respectively; \(\Omega = \int_0^t A \exp(-E_a/RT)\) with \(A, E_a\) and \(R\) being the material parameter (frequency factor), the activation energy and the universal gas constant, respectively.

The mechanical properties of skin tissue under normal physiological conditions have been studied extensively, where the mechanical behaviour is found to depend on the loading rate (Finlay 1978; Potts et al. 1983). For example, Vogel & Hilgner (1979) observed an increase in stiffness of rat skin tissue with an increased strain rate of uniaxial stretching. Shergold et al. (2006) studied the uniaxial compressive responses of pig skin over a wide range of strain rates (0.004−4000 per second) and found pig skin stiffens and strengthens with increasing strain rate. Similar results were also reported for biaxial testing (Lanir & Fung 1974). However, most of these investigations have been done without taking the effect of temperature into account, which is often pertinent in medical applications. This is addressed in the present study. Here, we have experimentally investigated the relationship between the loading rate on the skin tensile behaviour and the corresponding thermal damage. The paper is organized as follows. Firstly, the skin sample preparation and experimental test methods are presented, followed by the modelling of the observed behaviour. Secondly, the obtained experimental and theoretical results are presented and discussed.

2. Sample preparation and testing methods

(a) Sample preparation

Pig skin is used here to avoid the ethical and immunological issues associated with human skin testing. It also offers the benefit of a high degree of structural and functional similarity to human skin (Shergold & Fleck 2005; Shergold et al. 2006). The samples were prepared according to the standard organ procurement protocol, as described in our previous studies (Xu et al. 2009a, b), and is briefly summarized here. Pig belly skin to a depth of the subcutaneous fat was procured and fast-chilled to 4°C in a pre-gassed (95%O2, 5%CO2) Krebs–Henseleit Ringer solution (KHR; pH 7.4). Subcutaneous fat was then removed from the samples by wet/fast dissection on a bed of ice. Rectangular skin specimens were cut using a customized cutter. After placing the sample between glass slides in the undeformed state (before testing) the thickness, width and length of each skin sample were measured using a micrometer (with an accuracy of 0.01 mm). Five measurements were performed at different locations of each sample and the average was recorded.
(b) Procedure for thermomechanical testing

We have designed and built a custom-made hydrothermal tensile testing system in the laboratory. The system has been described in detail in our previous study (Xu et al. 2009a,b). The skin sample was placed at the centre of a test chamber filled with KHR in a controlled thermal environment (e.g. temperature, heating rate). This thermal environment was achieved through a circulation system connected to a thermostat.

Thermal loading. The skin sample was quickly heated/cooled to the target temperature by pumping KHR at a desired temperature into the chamber and the sample temperature was maintained with bath circulation. A T-type thermocouple near the sample was used to inform the controlling software, via a PC, the exact time at which the experiment started.

Mechanical loading. Before starting each test, mechanical preconditioning was performed in KHR at 37°C to ensure a reproducible response to the following test: skin samples were subjected to a displacement controlled sinusoidal excitation with a maximum stretch ratio of 1.05 and frequency of 0.05 Hz. After the preconditioning cycles, the samples were loaded to a stress of 10 kPa to mimic the initial stress of skin in vivo (Diridollou et al. 2001). Tensile testing with different loading rates (0.25, 0.5, 1, 2.5, 5 and 10% s⁻¹) was performed. The thermal and mechanical responses were recorded using the LabVIEW program for analysis.

3. Constitutive modelling

Here, we used a constitutive model to summarize the effect of the strain rate upon the measured constitutive response. A number of strain energy functions have been proposed to describe the nonlinear elastic behaviour of skin tissue (Fung 1968, 1981), in view of the similarity in deformation characteristics between skin and rubber (Shergold et al. 2006). Among these, the Ogden model is widely used for characterizing the nonlinear elastic responses of isotropic materials (assumed here for skin tissue) and is given as follows (Wu et al. 2002):

\[
W = \sum_{i=1}^{N} \frac{2\mu_i}{\alpha_i} \left( \lambda_x^{-\alpha_i-1} + \lambda_y^{-\alpha_i-1} + \lambda_z^{-\alpha_i-1} - 3 \right) + \sum_{i=1}^{N} \frac{1}{D_i} (J - 1)^2, \tag{3.1}
\]

where \(\lambda_i (i = 1, 2, 3)\) are the principal stretches; \(\overline{\lambda}_i = J^{-1/3} \lambda_i (i = 1, 2, 3)\) are the deviatoric principal stretches; \(J = \lambda_1 \lambda_2 \lambda_3\); and \(\alpha_i\) and \(\mu_i (i = 1, \ldots, N)\) are the material parameters to be determined.

Note that skin can be approximated as incompressible (North & Gibson 1978) and therefore in the strain energy function the latter term \(J - 1\) will be zero. Consequently, the stress can be rewritten as

\[
\sigma_z = \sum_{i=1}^{N} \frac{2\mu_i}{\alpha_i} \left( \lambda_z^{-\alpha_i-1} + \lambda_z^{-1-\alpha_i/2} \right), \tag{3.2}
\]

where the material parameters (i.e. \(\mu_i\) and \(\alpha_i\)) were determined by fitting the experimental data using the nonlinear least squares method. It was found during the data analysis that one term (i.e. \(N = 1\)) in equation (3.1) is sufficient.
for the purposes of fitting. In this case $\alpha$ and $\mu$ have the physical meaning of the strain hardening exponent and the shear modulus under infinitesimal straining, respectively. We hypothesize that these two parameters are temperature dependent.

4. Results and discussion

(a) Stress versus stretch relationship

A typical measured uniaxial tensile stress versus principal stretch response of pig belly skin obtained at body temperature ($T = 37^\circ\text{C}$) is shown in figure 1 (filled circles). The stress–stretch relationship exhibits three-stage strain hardening behaviour: a toe region with low stiffness at low stretches, a transition region from low to high stiffness and a high stiffness region at large stretches. Transition from low to high stiffness occurs at a uniaxial stretch of around 1.4. Similar results have been reported by other researchers (Wu et al. 2007).

The Ogden model is also included in figure 1 as a solid line, with the parameters $\alpha = 9.16$ and $\mu = 0.00136$ as obtained by the curve fit. The discrepancy between the experimental result and the fitted curve is slightly large in the toe region and gradually reduces with increased stretch. Good agreement is achieved when $\lambda$ is more than 1.9.

(b) Effects of temperature and loading rate

(i) Temperature

Figure 2 shows the influence of temperature on the stress–stretch behaviour of pig belly skin under uniaxial tension at a loading rate of 1\% s$^{-1}$. The stress needed to achieve the same strain state decreases with increased temperature, covering both the hyperthermal and hyperthermic temperature range. Skin tends to be softer at the same stress level with increased temperature and the transition region changes as well. Stress at a high stretch value under low temperature is much higher than that under high temperature; however, the discrepancy at the low stretch region is not that large.

There are several possible mechanisms responsible for the observed thermal effect. (i) The first one is the denaturation of collagen during the loading procedure under hyperthermal temperatures. Collagen provides the principal structural and mechanical support for skin tissue and its denaturation will affect the mechanical behaviour of skin. During denaturation, the structure as well as the hydration of collagen changes, which may involve an initial liberation, but subsequent absorption, of water via water bridges. Thermal denaturation of collagenous tissue can lead to remarkable changes in its thermal (Davis et al. 2000), mechanical (Chen et al. 1997; Chen & Humphrey 1998) and optical properties (Bosman 1993; Thomsen & Vijverberg 1993; Jun et al. 2003). Increased extensibility of soft tissues due to thermal treatment has been observed in both uniaxial (Lennox 1949; Chachra et al. 1996; Chen & Humphrey 1998) and biaxial studies (Harris et al. 2003; Wells et al. 2004). From our previous studies (Xu et al. 2009a,b) we can see that collagen in skin is damaged to a higher degree with increased heating time and increased temperature. Moreover, the longer the time of heating the severer is the skin burn damage. In order to investigate the degree.
Figure 1. Typical stress–stretch curves for pig belly skin and the Ogden curve fit result (filled circles, experiment; solid line, model).

Figure 2. Stress–stretch relations for skin sample under hyperthermal and hyperthermic temperatures, loading rate $\gamma = 1\% s^{-1}$ (temperature: solid line, 10°C; dashed line, 15°C; dotted line, 40°C; dash-dotted line, 50°C; dash-double dotted line, 60°C).

of thermal damage during the loading procedure under different temperatures, we plotted the degree of thermal damage versus stress (loading rate $1\% s^{-1}$; figure 3a). The parameter was adopted directly from the literature (Xu et al. 2009a,b). These results show the degree of thermal damage occurring in skin under different stress values and can tell us the amount of collagen that works in skin, noting that collagen will denature under severe temperature. (ii) The second possible mechanism is the changed hydration induced by heating. The influence of the change of water content on the viscoelastic behaviour of skin has been

*Phil. Trans. R. Soc. A* (2010)
much reported. Note that the viscoelastic behaviour of soft tissue is related to interactions between collagen, proteoglycans and water molecules (Humphries & Wildnauer 1971; Miller & Wildnauer 1977). There is an inward or outward flux of interstitial fluid due to heating (Humphrey 2003) and the viscoelastic properties of soft tissues can be changed by altering their water content (Chimich et al. 1992). Baek et al. (2005) pointed out that faster relaxation in thermally treated tissue is due to faster and greater water loss. It is not difficult to comprehend that the inward and outward flux of water also influences the mechanical behaviour of skin in our experiment. (iii) The third possible mechanism is cell viability in skin. Yip et al. (2007) studied the effect of in situ fibroblast viability on the mechanical properties of rat back skin and found that there is greater stress relaxation for skin tissue strained in normal Kreb’s solution than that in Kreb’s solution with poison. This change of viability will influence the interaction between collagen and cells of skin, which in turn will have an influence on the mechanical behaviour of skin in the loading process.
Strain rate sensitivity of skin tissue

(ii) Loading rate

We investigated the loading rate effect (0.25, 0.5, 1, 2.5, 5 and 10% s\(^{-1}\)) on skin (figure 4). We can see that skin has great strain rate sensitivity, which is in accordance with reported results (e.g. Jamison et al. 1968; Shergold et al. 2006). The figure clearly shows that with an increased loading rate the stress–stretch curve shows a tendency of hardening. The obvious discrepancy among curves can be observed in both low- and high-strain states, which implies that both the important components of skin, elastin (responsible for the response at low-strain state) and collagen (responsible for the response at high-strain state), are rate-dependent materials. The transition region from low to high stiffness is also observed to shift to a lower stretch region with increased loading rate. This indicates that both the elastin and collagen are loading rate sensitive materials and their stiffness increases with the loading rate.

Since collagen fibres comprise bundles of collagen fibrils aligned parallel to a hyaluronic acid chain, and linked to the hyaluronic acid by proteoglycan side-chains, it is probable that the strain rate sensitivity is attributable to viscous losses from the interaction of the ground substance with the collagen fibres at the macroscopic scale (Cohen et al. 1976) and at the microscopic scale (Haut 1983). Much of the sensitivity to the strain rate has been attributed to the uncoiling and aligning movement of collagen (Lanir 1979), while Daly (1982) believed that the strain rate sensitivity of skin occurred at all structural levels of collagen and is due in part to bonds between fibrils and fibres. From the modelling results of Shergold et al. (2006), we can also get the same explanation: the strain rate sensitivities are well modelled by increasing the shear modulus with an increase in strain rate, with no attendant change in the strain hardening exponent. Arumugam et al. (1994) also attribute the higher stress to higher frictional force at higher strain rates. There might be some other explanations: Edsberg et al. (1999) attributed...
the decrease in stiffness seen with an increase in the strain rate to the Mullins effect, which is the stress–strain response in filled rubbers that typically depends on the maximum loading previously encountered. The movement of fluid in the hydrated matrix may also play an important role (Li et al. 1983).

The loading process was performed under constant temperature. During the loading procedure, thermal damage will occur under severe temperature and collagen will be damaged to a certain level that is dependent upon the exposure time and temperature. Thermal damage to the sample in the loading procedure can thus be calculated according to the exposure time. Figure 3b shows the relationship of the degree of thermal damage and stress during the loading process under different loading rates. When the temperature is below body temperature, no thermal damage occurs in the skin tissue, but as the temperature goes up to a value higher than body temperature, damage occurs in the skin tissue.

Figure 5. Change of Young’s modulus of pig belly skin with (a) temperature at loading rate $\gamma = 1 \text{s}^{-1}$ (filled square, low; filled circle, high) and (b) loading rate at $T = 45^\circ$C (filled square, low; filled circle, high).
Table 1. Material parameters of the one-term Ogden model for pig belly skin obtained for various loading rates and temperatures—influence of loading rate at $T = 45^\circ C$ and influence of temperature at $\gamma = 1\% s^{-1}$.

<table>
<thead>
<tr>
<th>loading rate or temperature</th>
<th>$\alpha$</th>
<th>$\mu$</th>
</tr>
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<tbody>
<tr>
<td>influence of loading rate ($% s^{-1}$)</td>
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<td></td>
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<tr>
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<table>
<thead>
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<td>8.582</td>
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</tr>
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</tr>
<tr>
<td>60</td>
<td>9.677</td>
<td>0.000368</td>
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</table>

The higher the temperature the severer the thermal damage. From figure 3b we can evaluate the role of thermal damage in stress–stretch relaxation in the loading procedure under different temperature environments and different loading rate conditions.

(iii) Temperature- and strain rate-dependent parameters

As one of the most important factors that influence the mechanical properties of a material, Young’s modulus, which characterizes the relationship between stress and stretch of a material, has been studied extensively. From the stress–stretch relationship of skin we can see that Young’s modulus of skin is not a constant value; but it can be divided into two parts: low-value region and high-value region (Pan et al. 1998). In this paper, the influences of the temperature and the loading rate on Young’s modulus were investigated. Figure 5a shows Young’s modulus of skin tissue under different temperatures. Young’s modulus decreased for both the low-stretch region and the high-stretch region with increased temperature, which indicated that the structure of collagen has been damaged. From figure 5b, we see that Young’s modulus apparently increases with the loading rate, indicating that skin becomes stiffer as the loading rate increases. Noting that collagen is responsible for the skin’s high Young’s modulus, we can infer that collagen stiffness also increases with the loading rate.

Table 1 shows the effect of the loading rate and the temperature on fitting results for $\alpha$ and $\mu$ of the Ogden model (equation (3.2)). From table 1, $\alpha$ increases significantly with both increased loading rate and increased temperature. This is understandable in view of the fact that $\alpha$ has the physical meaning of the strain hardening exponent. However, no obvious tendency towards change can be observed for the shear modulus ($\mu$), which was regarded as a stiffening parameter.

Phil. Trans. R. Soc. A (2010)
5. Conclusions

In summary, this study measured the tensile behaviour of pig skin tissue under different temperatures and loading rates. The tissue mechanical behaviour at the macroscale level was correlated to microscale level structural change, i.e. the thermal damage of collagen within the skin tissue. The Ogden model was used to summarize the effect of the strain rate and the temperature upon the measured constitutive response through two parameters ($\alpha$ and $\mu$). The significant variation of the skin tensile behaviour with temperatures and loading rates means that these effects should be considered when designing thermal treatments. For this, more experiments are needed to better understand these phenomena and to quantify the variation of skin properties with temperature and corresponding collagen denaturation, so that these properties can be more accurately determined.

This work was supported by the National Natural Science Foundation of China (10572111, 10632060), the National Outstanding Youth Foundation (10825210) and the National Basic Research Program of China (2006CB601202).

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