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An experimental study of the mouse skin behaviour: Damage and inelastic aspects

M.J. Muñoz[a], J.A. Bea[b], J.F. Rodríguez[b], I. Ochoa[b], J. Grasa[b], A. Pérez del Palomar[b,*], P. Zaragoza[a], R. Osta[a], M. Doblaré[b]

[a]Lagenbio-Ingen, Aragón Institute of Engineering Research (I3A), University of Zaragoza, Spain
[b]Group of Structural Mechanics and Materials Modelling, Aragón Institute of Engineering Research (I3A), University of Zaragoza, Spain

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Abstract

Samples of male and female mice skin were tested under monotonic and cyclic loading to mechanically characterize the tissue for large deformations. Cyclic tests have shown a typical Mullins effect widely known for elastomers and other soft tissues. No statistical difference was found in the maximum stretch of the sample after the fifth loading cycle for male (1.26 ± 0.035) and female (1.18 ± 0.083). However, larger dispersion was obtained for the maximum stress for both genders, 0.61 ± 0.16 MPa for male and 0.78 ± 0.32 MPa for female. Results show the presence of inelastic strain and stress softening in the skin at large deformations. They also have shown how stress softening and residual strain change with the magnitude of the applied load. Good correlation was observed between the residual strain and the maximum strain previously attained by the sample during loading for all samples. However, the correlation was different between genders.

Keywords: Mouse skin behaviour; Mullins effect; Damage; Viscoelasticity

1. Introduction

Reconstructive surgery, from simple excision and closure to complex flap surgery, is performed daily to treat burn wounds, chronic lacerations, cancerous and non cancerous growths and birth defects (Gambarota et al., 2005). During surgery, skin may undergo large strain under cyclic loading, for which it is important to find the mechanical response of skin during this cyclic loading process. Skin poses a particular challenge in this regard due to its complex mechanical behaviour. It is an oriented fibrous structure that exhibits mechanical anisotropy, highly nonlinear stress–strain relationships, large deformations, viscoelasticity and strong axial coupling (Sacks, 2000). Skin has usually been treated, due to its hyperelastic behaviour, as rubber. However, the structures found within skin make the constitutive behaviour of the tissue and its microstructure more complex than for rubber. Skin comprises two main tissue layers: the dermis and the epidermis. In mammalian skin, the dermis is typically 20 times thicker than the epidermis (Shergold, 2004) and dominates the overall constitutive behaviour. The dermis is composed of a hydrated, gel-like ground substance with elastin fibres and collagen fibres embedded within it. The collagen fibres are the major structural component of the dermis, accounting for 60–80% of the dermis dry weight (Dombi and Haut, 1985; Reihsner et al., 1995), running along Langer's lines (Langer, 1978). In vivo experiments conducted by Ridge and Wright (1966) using a uniaxial extensometer have shown that tensile orientation of the Langer's lines was related to the orientation of the collagen network. Consequently, the constitutive behaviour of skin mainly depends upon the structure, density, and directionality (Nimni et al., 1966; Vogel, 1987) of the collagen fibres found within the dermal layer (Shergold et al., 2006).

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*Corresponding author. Tel.: +34 976761912; fax: +34 976762578.
E-mail addresses: amaya@unizar.es (A. Pérez del Palomar), mdoblare@unizar.es (M. Doblaré).

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Experimental investigations regarding mechanical behaviour of skin have usually been limited to uniaxial studies because of the difficulties in controlling two or three dimensional boundary conditions. In addition, most studies have focused on its response under monotonic loading (Sacks, 2000; Dombi and Haut, 1985; Reihsner et al., 1995). Lafrance et al. (1995) conducted quasi-static indentation tests on skin equivalents. They found three distinctive phases in the stress–strain response, a first phase distinguished by a low modulus, a second phase characterized by a lack of mobility of structural units with a considerable increase in modulus, and a third phase showing a rapid stiffening of the material. Del Prete et al. (2004) conducted creep and dynamic tests to characterize viscoelastic properties of skin in tight-skin (TsK) and Mov-13 mice. Edsberg et al. (1999, 2001) conducted uniaxial characterization of human skin which had been exposed to static and cyclic normal pressure, and compared the results with those obtained in human skin without any previous pre-loading history. They found that tissue subjected to pressure prior to the tensile tests was less stiff than control specimen tissue. This was evidence that damage had been induced by loading. On the other hand, they also found that the changes in mechanical properties due to only static pressure were more significant than those caused by cycling pressure. They also observed microstructural changes in the tissue. These changes were mostly related to collagen fibers realignment and matrix breakdown which could be a link to the strain softening behaviour observed. Sanders and Goldstein (2001) and Sanders et al. (2002) studied microstructural and morphological changes in pig skin in vitro and in vivo when subjected to cyclic compressive and shear loading. They observed no significant changes in skin morphology. However, stress samples were found to have significantly smaller collagen fibril density compared to unstressed samples.

Besides these efforts, little investigation has been devoted to the study of mechanical response of skin under cyclic loading and large deformations in order to fully understand the nonlinear behaviour of the tissue. In this regard, the study of Giles et al. (2007) on pig skin considered fully preconditioned samples under load control. They observed differences in the behaviour of the samples as compared with stretch controlled tests. This could be explained by nonlinear viscoelasticity. In their experiments, they also observed the typical softening in the unloading curve, but they did not report residual deformation in the samples during the test. This could be related to the small range of deformation involved in their study ($\lambda < 1.1$). The aim of this paper is to study the mechanical behaviour of skin for large deformations under monotonic and cyclic loading using an uniaxial test.

2. Materials and methods

All experiments and animal care were performed in accordance with the permission of the University of Zaragoza ethics commission and the international guidelines for the use of laboratory animals. Skin samples from the abdominal region of adult mice of the B6SJLF1/J strain were obtained by modification of the previously described procedure by Del Prete et al. (2004). The mice ($n = 6$) were kept alive and anesthetized with sodium pentobarbital (40 mg/kg) during the test. The fur was clipped and the margins of the skin were marked on the intact mouse. Four skin samples, 5 mm wide and 25 mm long approximately, were cut along the abdominal wall of each animal, with the long axis of the samples corresponding to the long axis of the animal. However, skin samples were harvested right before conducting the test and once the previous test had ended (a wet gauze was kept over the abdomen for protection of the samples and to prevent dehydration of the skin) (Fig. 1). Using this protocol, skin degradation was minimized. Two samples were used for the monotonic tensile test and the other two were used for cyclic testing. All samples were photographed after dissection and their dimensions recorded using a customized vision program. Sample thickness was estimated by placing the skin between two-glass plates measuring the distances with a Mitutoyo *Absolute Digimatic* micrometer which holds the measurement when the contact force reaches a value of 0.5 N. Before placing the sample in the testing machine, each rectangle of tissue was attached, using cyanoacrylate cement, to sandpaper tabs to avoid slippage between the tissue and the clamps. Two black ink markers were placed on the sample to allow strain measurements by means of a video-extensometer. All tests were carried out in a high humidity atmosphere, by means of an ultrasonic humidifier, to avoid over hydration of the sample. In this regard, the alternative of conducting the tests in a mineral oil bath was discarded due to the high risk of slippage of the sample. The cyclic test protocol was set in such a way that any test would not last more than 10 minutes.

Uniaxial tensile tests were performed under displacement control on an INSTRON 5848 microtester. Its configuration was a 5 N full scale load cell with a non-contact videoextensometer (INSTRON AVE). In order to avoid specimen drying, an ultrasonic humidifier was used. This provided a subcooling steam, allowing a constant temperature of 25 °C during the test. Two types of tensile tests, monotonic and cyclic with increasing amplitude, were performed. For the monotonic test, specimens were loaded at a displacement rate of 15 mm/min up to rupture or a maximum load of 5 N. Cyclic tests were performed following the profile shown in Fig. 2. A maximum of nine ramp cycles were applied, all of them under displacement control at the same displacement rate as for the monotonic test. Whenever a given force level is reached (see Fig. 2), unloading starts until reaching a force value close to zero at which point reloading starts. At the end of the ninth cycle, if the specimen still holds, a loading ramp is applied until the full displacement range of the testing protocol (set in 100 mm) is reached. Every specimen following either protocol, was subjected to an initial preloading of 0.1 N and four load cycles from 0 to 0.25 N in order to precondition the sample before the full test was carried out. We should point out that preliminary studies conducted on this type of skin showed that no more than four load cycles were needed for an adequate preconditioning of the tissue. The force, displacement and axial strain were recorded at a sample rate of 5 Hz.

Lilliefors' test (Lilliefors', 1967) was used to determine whether or not the data samples followed a normal distribution. Student's t-test was used to compare average values across groups with a p-value of 0.05 determining significance. A Wilcoxon signed-rank test was used as a non-parametric test of significance. All statistical analyses were performed in Matlab v.7.1. All values are reported in terms of the mean and the standard error of the mean (mean ± SEM).

3. Results

A total of 24 skin samples, from the abdominal region along the animal longitudinal axis, of approximately 25 mm length (gauge length of 11.36 ± 0.75 for videoextensometer measurements), 5 ± 0.72 mm width, and thickness of 0.58 ± 0.133 mm for males and 0.41 ± 0.026 mm for female, were harvested for monotonic and cyclic tensile