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The effects of cyclic tensile and stress-relaxation tests on porcine skin

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ABSTRACT

When a living tissue is subjected to cyclic stretching, the stress-strain curves show a shift down with the increase in the number of cycles until stabilization. This phenomenon is referred to in the literature as a preconditioning and is performed to obtain repeatable and predictable measurements. Preconditioning has been routinely performed in skin tissue tests; however, its effects on the mechanical properties of the material such as viscoelastic response, tangent modulus, sensitivity to strain rate, the stress relaxation rate, etc... remain unclear. In addition, various physical interpretations of this phenomenon have been proposed and there is no general agreement on its origin at the microscopic or mesoscopic scales. The purpose of this study was to investigate the effect of the cyclical stretching and the stress-relaxation tests on the mechanical properties of the porcine skin. Cyclic uniaxial tensile tests at large and constant strain were performed on different skin samples. The change in the reaction force, and skin's tangent modulus as a function of the number of cycles, as well as the strain rate effect on the mechanical behavior of skin samples after cycling were investigated. Stress-relaxation tests were also performed on skin samples. The change in the reaction force as a function of relaxation time and the strain rate effect on the mechanical behavior of skin samples after the stress-relaxation were investigated. The mechanical behavior of a skin sample under stress-relaxation test was modeled using a combination of hyperelasticity and viscoelasticity. Overall, the results showed that the mechanical behavior of the skin was strongly influenced by cycling and stress relaxation tests. Indeed, it was observed that the skin's resistance decreased by about half for two hours of cycling; the tangent modulus degraded by nearly 30% and skin samples became insensitive to the strain rates and accumulated progressively an inelastic deformation over time during cycling. Finally, the hysteresis loops became very narrow at the end of cycling and after relaxation process.

1. Introduction

The mechanical behavior of the skin has been investigated for a number of years, especially for clinical and cosmetic applications. The monotonic tensile test is one of many techniques used to characterize the skin (Groves et al., 2013; Lim et al., 2011; Ní Annaidh et al., 2012; Remache, 2013). The purpose of this test is to extract some classical mechanical parameters of the material (such as the elastic modulus and shear modulus), thereby enabling the development of numerical models to predict the mechanical behavior of the soft tissue (Bischoff et al., 2002; Lim et al., 2011; Remache, 2013; Shergold et al., 2006). However, the investigation of the skin mechanical behavior under cyclic tensile tests remains relatively unexplored despite some studies (Edsberg and Mates, 1999; Giles et al., 2007; Kang and Wu, 2011; Munoz et al., 2008).

It is known that, when soft tissue is subjected to cyclic uniaxial tensile tests, the obtained stress-strain curves form hysteresis loops,

which continually decrease and then stabilize (Xu and Lu, 2011). This phenomenon was first referred to by Fung (1993) as the preconditioning effect. Artery samples were preconditioned by Fung (1993) in order to obtain repeatable and predictable measurements, and then to introduce the concept of the pseudo-elasticity of soft tissues. The preconditioning phenomenon is well-known in filled rubbers and crystallizing pure gum (Diani et al., 2009) and is referred to as the Mullins effect (Mullins, 1969). However, it seems that the preconditioning phenomenon of the soft tissue is not well understood and, to our knowledge, no preconditioning protocol for the mechanical testing of skin tissue exists in the literature. Indeed, while Lanir and Fung (1974) found that a preconditioned soft tissue will return to its initial configuration after a large relaxation period, Vogel and Denkel (1985) found that only the skin in vivo can reach a full recovery after cyclic stretching. As well, while Kang and Wu (2011) did not reach a stabilization of the stress-strain curves for 100 cycles of stretching performed on porcine skin, Liu and Yeung (2008) reached this

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stabilization between 4 and 12 cycles. Furthermore, a wide range of loading cycles was reported in the literature (3 (Fung et al., 1972) to 100 cycles (Kang and Wu, 2011)). It appears thus that the preconditioning effect on the mechanical properties of skin tissue remains unclear (Cheng et al., 2009; Griffin et al., 2016). The preconditioning phenomenon was also observed in repeated stress-relaxation tests (Fung, 1993; Liu and Yeung, 2008). It was observed that, if a tissue is subjected to a repeated stress-relaxation test at constant strain, the obtained relaxation curves are seen to shift upward and the difference between these curves decreases as a function of the number of cycles (Fung, 1993). This change in the response of the material as a function of the number of cycles shows that the preconditioning test has a resulting effect on the mechanical properties of the material, such as viscoelastic response and the stress relaxation rate, etc.... The physical interpretations of this phenomenon remain unclear. In this study, we investigated the effect of the cyclical stretching and stress-relaxation tests on the mechanical properties of porcine skin.

2. Tools and methods

2.1. Samples preparation

The porcine skin was obtained directly from the butcher from different freshly slaughtered pigs. The animals should have roughly the same age, according to the butcher. Large porcine skin pieces were carefully excised from the abdomen in such a way that the fat is removed. This was achieved by the butcher. The obtained pieces had almost uniform thicknesses (about 2 mm). The samples were cut from the same zone and according to the direction of the porcine spine as shown in Fig. 1 (a). The samples were cut by a surgical scalpel according to an aluminum template whose dimensions are shown in Fig. 1 (b). For all tests, the samples were prepared then set up for testing within two hours at the latest to keep the freshness of skin tissue.

2.2. Experimental procedure

An Instron 8800 tensile testing machine with a load cell of 10 kN was used and all the tests were conducted at room temperature. Filtering frequencies from 20 to 2500 Hz were tested for two data

acquisition frequencies: 1 kHz and 5 kHz. The obtained results show that the load cell has a digital noise approximately 1 N. The tests were carried out only under displacement control because the output results under force control were too contaminated with noise.

One of the most important problems encountered during the tensile tests performed on the soft tissue is the fixation of the sample within the grips (Jacquemoud, 2007; Vescovo, 2002). Several grips such as for example elastomeric grips were tested. Only a developed screw grips with internal striated faces have completely prevented the samples from slipping for strains less than 30%. All the skin samples were therefore subjected to a maximum strain of 25%. The samples were placed in the grips, and then reference lines have been drawn at the ends of the samples to indicate a possible slip. A preload of 1 N was applied to each specimen, and then the machine load was set to 0 before starting each test. The preload was applied to the skin in order to get close to its natural state of pretension. The preload applied to the skin was taken from Bischoff et al. (2000). The strain rates and displacements were directly imposed through the machine crosshead. The obtained force was measured by the load cell. A lard layer was also applied on the surface of each sample in order to avoid the drying phenomenon. At the end of all the tests, no sliding of the tested samples was observed and no drying phenomenon was observed, at least to the naked eye. It should be noted that other tests (which we have not presented) carried out without the lard layer became dry at the end of the tests.

2.3. Experimental tests

Five types of tests were carried out. Type 1: a cyclic tensile test was performed on six skin samples at a constant strain rate, with a peak displacement of 25 mm and during two hours (Fig. 2). A strain rate of 1 mm/s was applied to the first three samples and a strain rate of 0.1 mm/s was applied to the other three samples. The number of cycles performed during two hours was calculated for each strain rate. We obtained 144 cycles for the strain rate of 1 mm/s and 14 cycles for the strain rate of 0.1 mm/s. Type 2: Two cyclic tensile tests were performed on a porcine skin sample according to the following steps: (1) the sample was loaded-unloaded 72 consecutive times up to 25 mm and at a strain rate of 0.5 mm/s; (2) the sample was then left in an unstressed phase for a resting period of two hours; (3) then, the sample was loaded-unloaded again three consecutive times up to 25 mm, at three different strain rates: 0.5 mm/s (the 73th cycle), 1 mm/s (the 74th cycle), 5 mm/s (the 75th cycle), respectively (Fig. 6). Type 3: a stress-relaxation test was performed on two skin samples (Fig. 7). Each skin sample was stretched up to 25 mm then was kept in the strained condition for two hours. The first sample was loaded at a strain rate of 0.1 mm/s and the second one at a strain rate of 1 mm/s. Type 4: a stress-relaxation test and a cyclic tensile test were successively performed on a porcine skin sample according to the following steps: (1) the sample was stretched up to 25 mm at a strain rate of 1 mm/s, then maintained in the strained condition for a period of two hours, (2) after the two hours in the strained condition, the sample was fully unloaded (i.e. the machine crosshead was reset to its initial position), (3) the sample was then subjected to three consecutive cyclic tensile tests up to a maximum stretch of 25 mm, at three different strain rates: 0.1 mm/s (cycle 1), 1 mm/s (cycle 2), 5 mm/s (cycle 3), respectively (Fig. 9). Type 5: a cyclic tensile test and a stress-relaxation test were successively performed on a porcine skin sample according to the steps: (1) the sample was subjected to cyclic tensile test for two hours, with a peak displacement of 25 mm, at a constant strain rate of 1 mm/s then, (2) the sample was stretched up 25 mm at a constant strain rate of 1 mm/s, and then maintained in a strained condition for a period of two hours (Fig. 10).

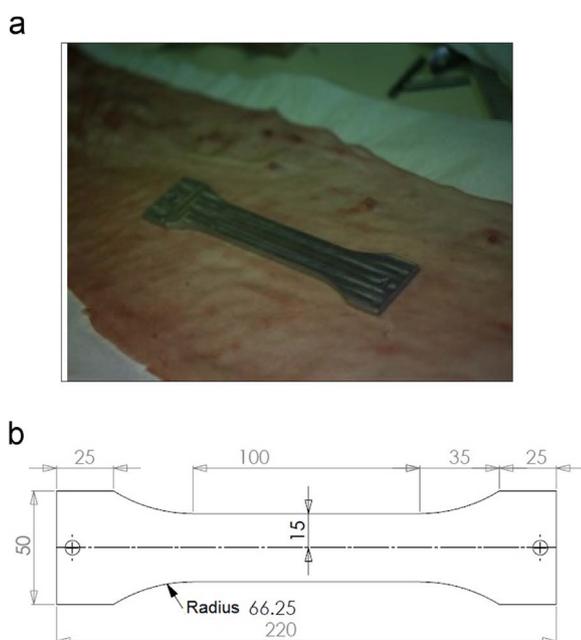


Fig. 1. (a) Template position showing the location where skin samples were cut. (b): Dimensions of an aluminum template used for cutting samples.

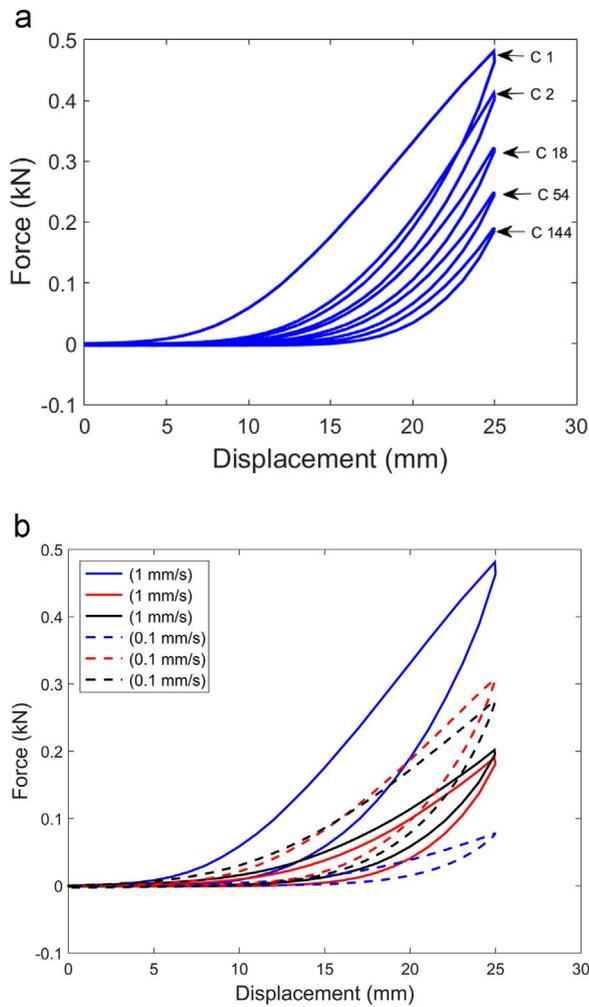


Fig. 2. (a) Hysteresis loops obtained from a cyclic tensile test performed on a porcine skin sample stretched up 25 mm, at a constant strain rate of 1 mm/s, and for two hours (which corresponds to 144 cycles). Only some hysteresis loops designated by their cycle numbers (Ci), are presented. (b) Six skin samples were subjected to cyclic tensile test, for each sample, only the first hysteresis loop is presented.

2.4. Analytical method

2.4.1. Variation in the peak reaction force

Several skin samples coming from different animals were subjected to a cyclic tensile test. The obtained reaction forces (i.e. skin's resistance) were measured as a function of the cycle number and as a function of the cycling time. The peak reaction force obtained in each cycle was extracted. In order to compare the results coming from different skin animals, the variation in the peak reaction force for each cycle with respect to the peak reaction force corresponding to the first cycle was calculated. The variation was then normalized by dividing it by the reaction force corresponding to the first cycle. The normalized variation is thus given by the following relationship:

$$R_c = \frac{F_{\max}(c_1) - F_{\max}(c_n)}{F_{\max}(c_1)} \quad (1)$$

where $F_{\max}(c_1)$ and $F_{\max}(c_n)$ are the maximum reaction force corresponding to cycle 1 and any cycle n, respectively.

2.4.2. Variation in skin's tangent modulus

In order to quantify the changes in the mechanical properties of the skin samples under cyclic tensile tests (type 1 of the tests) we introduced a parameter, D, calculated according to the following relationship :

$$D = \frac{G(c_1) - G(c_n)}{G(c_1)} \quad (2)$$

where $G(c_1)$ and $G(c_n)$ are the tangent moduli corresponding to the cycle 1 and any cycle n of the unloading curves, respectively; D represents the relative change in the tangent modulus of the unloading curves. The tangent moduli were calculated from linear regressions over the initial 3 and 20 data points for the strain rates of 1 mm/s and 0.1 mm/s, respectively.

2.4.3. Residual strain accumulation

During cyclic tensile tests performed on porcine skin, we observed an increase of gauge length of the skin samples as the number of cycles increased. This phenomenon, called the residual strain was quantified according to the following method. From the second cycle, at the beginning of each stretching phase, the machine crosshead moved a few millimeters with zero forces recorded by the machine. The skin showed thus a zero resistance to the stretch for the first few millimeters of each stretching. We considered that the displacement of the machine crosshead of zero forces (denoted by R_d in Fig. 5 (a)) was equal to the residual deformation accumulated by the skin during each cycle. The relative residual strain accumulated by a skin sample as a function of cycle number was calculated according to the following relationship :

$$R_s = \frac{R_d(c_n) - R_d(c_2)}{L_0} \quad (3)$$

where L_0 is the initial gauge length. $R_d(c_2)$ is the residual deformation accumulated in the second cycle; $R_d(c_n)$ is the residual deformation accumulated in any cycle n.

2.4.4. Variation in reaction force during stress relaxation

In type 3 of the tests, two porcine skin samples were subjected to stress-relaxation test (Fig. 7). The reaction force to the constant strain was measured as a function as relaxation time. The variation in the reaction force at each instant t (i.e. $F(t)$) with respect to the reaction force at the beginning of stress relaxation (i.e. $F(t_0)$) was calculated. Since the samples came from different animals, this variation was normalized by dividing it by $F(t_0)$ corresponding to each sample. The normalized variation in the reaction force denoted by $R(t)$ as a function of the relaxation time is thus given by the following relationship :

$$R(t) = \frac{F(t_0) - F(t)}{F(t_0)} \quad (4)$$

where $F(t_0)$ is the reaction force at the beginning of stress relaxation of the skin sample (i.e. the force corresponding to the maximum value of stretching) and $F(t)$ is the instantaneous reaction force.

2.5. Numerical approach

The stress response of the skin was assumed to be homogeneous and isotropic, and the material was assumed to be incompressible (Holzapfel, 2000). Two mechanical constitutive equations were separately used to calibrate the stress-relaxation test data using the Abaqus® calibration tool. An hyperelastic model was used to calibrate the non-linear portion of the material behavior, and a linear isotropic viscoelastic model was used to calibrate the viscoelastic portion. Two non-linear isotropic models were used for calibration; Ogden model (order 1) (Ogden, 1972) and Yeoh model (Yeoh, 1993). The form of the Ogden strain energy potential for incompressible material is expressed in Abaqus® as:

$$W = \sum_{m=1}^N \frac{\mu_m}{\alpha_m} (\lambda_1^{\alpha_m} + \lambda_2^{\alpha_m} + \lambda_3^{\alpha_m} - 3) \quad (5)$$

where λ_i ($i = 1, 2, 3$) are the principal stretches; α_m and μ_m are the material constants to be determined and N is a positive integer. The initial shear modulus is given by:

$$\sum_{m=1}^N \mu_m = \mu, \tag{6}$$

The Yeoh form (Yeoh, 1993) is viewed as a special case of the reduced polynomial with N=3:

$$W = \sum_{i=1}^3 C_{i0} (I_1 - 3)^i \tag{7}$$

where C_{i0} are the material constants and I_1 is the first invariant of the Cauchy Green tensor.

The linear isotropic viscoelastic model was represented in terms of the Prony series in which the relaxation functions are given by the following relationship :

$$G(\tau) = G_\infty + \sum_{i=1}^{N_G} g_i \exp(-\tau/\tau_i^G), \tag{8}$$

$$K(\tau) = K_\infty + \sum_{i=1}^{N_K} k_i \exp(-\tau/\tau_i^K), \tag{9}$$

where K_∞ and G_∞ represent the long-term bulk and shear moduli; g_i , k_i , τ_i^G , and τ_i^K are material constants.

The nominal stress-strain was calculated from the force-displacement data and the normalized stress as a function of relaxation time was set in Abaqus® as shear test data. The last value of the normalized stress (equal to 0.34) was set as the long term shear modulus. Time was selected as domain, and relaxation test data was selected as time.

3. Results and discussion

From a point of view of biomechanics, the skin tissue is a nonlinear, viscoelastic and anisotropic material (Bischoff et al., 2000; Edsberg and Mates, 1999; Fung, 1993; Groves et al., 2013; Jacquemoud, 2007; Khatyr et al., 2006; Lim et al., 2011; Ní Annaidh et al., 2012; Silver et al., 2002). In addition to this complex behavior, the mechanical properties of skin depend on several factors, such as, the tested species, sex (Agache, 2000; Cua et al., 1990; Diridollou et al., 2000; Remache et al., 2011), the anatomical body site (Diridollou et al., 2000; Hendley et al., 1982; Remache et al., 2011; Ryu et al., 2008), thickness (Remache et al., 2011; Takema et al., 1994), age (Ahn et al., 2007; Escoffier et al., 1989; Lanir et al., 1993; Ryu et al., 2008; Takema et al., 1994; Vogel, 1972), the hydration (Auriol et al., 1993; Hendley et al., 1982; Hendriks et al., 2004; Jemec and Serup, 1989; Yilmaz and Borchert, 2006), the relative humidity, temperature, blood flow (Xu and Lu, 2011) and other in vivo environmental factors. On the other hand, the measurement of the mechanical properties of skin depends heavily on the used technique of characterization (tensile, torsion, suction tests,..etc.), its boundary conditions, and the direction of loading and the loading rate.

In this study, in order to take into consideration at least some of the factors mentioned above, tensile tests were carried out on skin samples collected from pigs of the same age, from the same anatomical site, according to the same direction (direction of the porcine spine), and cut according to the same shape (Fig. 1). The results were then normalized since the samples came from different animals.

The slipping of the skin between the grips of the tensile testing machine constitutes one of the most important problems during the tensile test (Jacquemoud, 2007; Vescovo, 2002). In the present study, several grips were tested on samples for different levels of strains. Referring to reference lines drawn at the ends of each sample, a screw grips with internal striated faces have shown a good result for strains less than 30% (i.e. 30 mm of displacement). The drying phenomenon constitutes another problem encountered during tensile tests performed on skin samples. In order to avoid this problem, we applied a lard layer on the surface of each sample. The drying phenomenon was not observed (at least to the naked eyed) for at least for two hours.

3.1. Cyclic tensile tests

An example illustrating the results obtained on skin samples subjected to cyclic stretching is shown in Fig. 2 (a) (type 1 of the tests). From the figure, it can be seen that, in the loading phase, the force-displacement curves of the skin sample show the classic J-shape which is a characteristic of the mechanical behavior of many soft tissues (Daly, 1982). Based on microscopic observations (Brown, 1973), this observed behavior of the skin is characterized by three loading phases: in the initial linear phase, the extension of the skin is associated with the stretching of elastic fibers which are easily stretched. In the nonlinear phase (toe-region), the collagen fibers are progressively stretched and re-orientated in the load direction. In the final linear phase, the collagen fibers straighten and become aligned until the ultimate tensile strength is reached and the fibers begin to break.

The viscoelastic behavior of the skin is associated with the interaction of the collagen fibers with the proteoglycan matrix (Dunn and Silver, 1983; Lanir, 1979; Minns et al., 1973). The features of the viscoelasticity of materials are the hysteresis loops, stress relaxation, and creep (Fung, 1993). The viscoelastic behavior of the tested samples was manifested by the hysteresis loops (Fig. 2 (a)). However, as shown in Fig. 2 (a), the hysteresis loops shifted down and became increasingly narrow as a function of the number of cycles. This phenomenon can be related to one or several effects. Fung (1993) found that the internal structure of the tissue (i.e. change of collagen molecular structure and the fiber configuration) changes with the cycling and called this phenomenon: the preconditioning. While Auriol et al. (1993) found that the viscosity and hysteresis as well as elasticity of in vivo human skin were unmodified with increasing hydration, Jemec and Serup (1989) found that hydration of the skin increased the hysteresis significantly and reduced skin elasticity. Other works based on microscopic observations found that, during monotonic tension tests, a damage of collagen fiber bundles of skin tissue occurs (Edsberg and Mates, 1999; Kang and Wu, 2011). Overall, it appears that the viscoelastic aspect of the skin changed as a function of the number of cycles.

In type 1 of the tests, six samples were tested (Fig. 2 (b)). The samples showed a similar mechanical behavior shown in Fig. 2 (a). However, different peak forces were reached. Since the samples came from different animals, the disparity in the results showed that the skin elasticity depends on the animal, i.e. individual differences (Remache, 2013). In order to compare the results, the variation of the maximum reaction force (or skin's resistance to stretch) of each skin sample as a function of the cycle number was calculated according to the Eq. (1). The calculated values of R_c as a function of the cycle number of some cycles are shown in Fig. 3. As shown in the figure, the obtained curves showed similar trends for each of strain rates, and that for two hours of

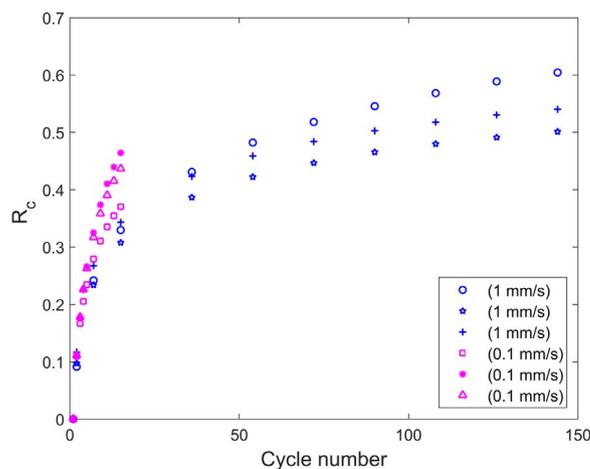


Fig. 3. The normalized variation in the peak reaction force (denoted R_c) of six skin samples as a function of the cycle number. R_c is presented only for some cycles.

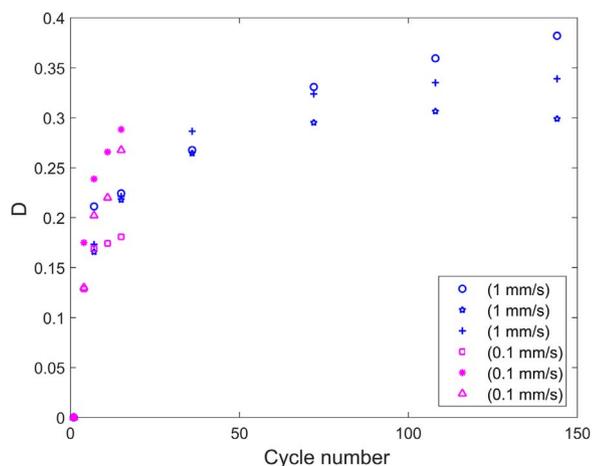


Fig. 4. The variation in skin's tangent modulus of the unloading curves (denoted D) of six porcine skin samples subjected to cyclic tensile tests. D was only calculated for some cycles.

repeated stretching, R_c increased as the number of cycles increased by nearly 50% and 60% for 0.1 mm/s and 1 mm/s of strain rate, respectively. Skin's resistance, thus, decreased by about half for two hours of cycling. To our knowledge, no viscoelastic material has shown such a continuous and great diminution of its resistance to stretch. Indeed, after a few cycles the response of the classical viscoelastic materials tends to stabilize. An apparent stabilization at the last cycles corresponding to the strain rate of 1 mm/s, (i.e. after at least about 140 cycles) was observed. It should also be noted that R_c increased faster with the smallest strain rate (i.e. 0.1 mm/s). It seems that, the more the material remains stretched, the more its resistance to stretch degrades. We assume that the skin accumulated damage with cycling in particular with the low strain rates.

3.1.1. Cyclic tensile effect on skin's tangent modulus

The variation in skin's tangent modulus was calculated (Eq. (2)) in order to quantify the degradation of the mechanical properties of the skin samples under cyclic tensile tests. Fig. 4 shows the calculated parameter D as a function of the cycle number of the tested skin samples. As shown in the figure, the parameter D increases as the number of cycles increases. This result showed that the tangent modulus degraded with the repeated cycling by nearly 30%. For the same number of cycles, the tangent modulus declined much more with the lowest strain rate, i.e. with the longest cycle. Once again, it seems that, the longer the material is stretched, the more its mechanical properties degrade. We assume that this degradation can be explained by the progressive failure of collagen fibers as found by Kang and Wu (2011) in monotonic tensile tests performed on porcine skin.

3.1.2. Residual strain accumulation

The residual strain accumulation during cyclic tensile tests was observed in several studies (Kang and Wu, 2011; Lanir and Fung, 1974; Munoz et al., 2008). In this study, this accumulation was measured as shown in Fig. 5 (a) and calculated according to the Eq. (3). Fig. 5 (b) shows the relative residual strain accumulated as a function of cycle number of two representative skin samples subjected to cyclic tensile tests at two different strain rates: 0.1 mm/s and 1 mm/s, respectively. As shown in the figure, R_s increased by about 15% as a function of the cycle number for the two skin samples. As found by Kang and Wu (2011), the results revealed that skin samples accumulated progressively an inelastic deformation over time during cycling. It was also found that the residual strain depends on the stress magnitude (Munoz et al., 2008).

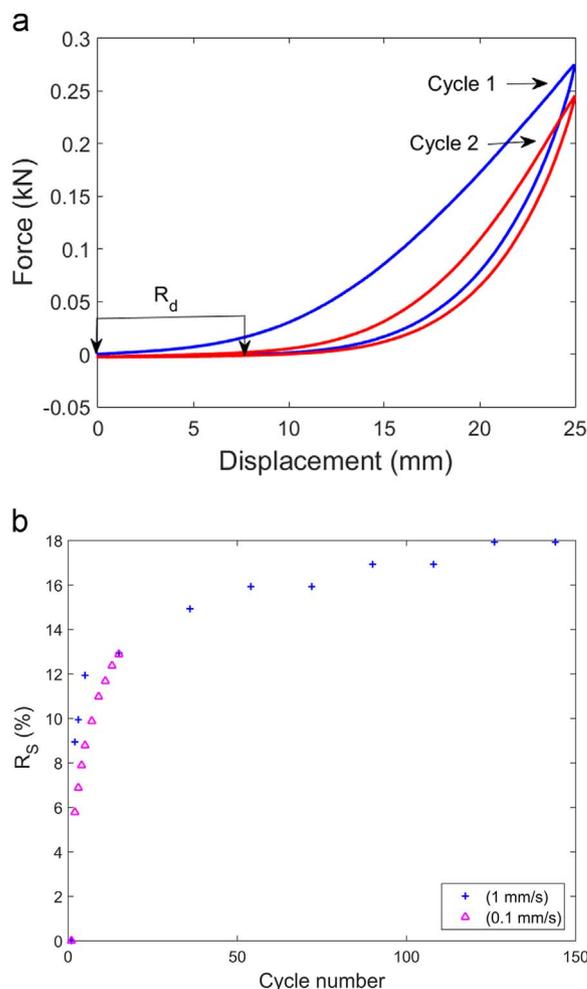


Fig. 5. (a) The residual deformation, Rd defined as the displacement of the machine crosshead of zero forces. The accumulated residual deformation was observed from the second cycle. (b) The relative residual strain accumulated by a porcine skin sample as a function of cycle number.

3.1.3. Strain rate effect

The strain rate effect on the skin mechanical behavior was investigated in monotonic tensile tests by several authors (Dombi et al., 1993; Edsberg and Mates, 1999; Fung, 1993; Hutton et al., 1975; Kang and Wu, 2011; Lanir and Fung, 1974; Lim et al., 2011; Shergold et al., 2006; Silver et al., 2002; Vogel, 1972; Xu and Lu, 2011). It was found that skin's resistance increases with the increasing strain rate in displacement controlled tests. However, to our knowledge, strain rate effect on the skin mechanical behavior has never been investigated in cyclic tensile tests. A cyclic-recovery-cyclic test was performed on a porcine skin (type 2 of the tests) according to the steps shown in Fig. 6. As was found in tests of type 1, the force-displacement curves showed a shift down and an increasingly narrowing of the hysteresis loops as a function of the number of cycles. From the figure, it can be seen that, the last three cycles are almost confused with the 72th cycle. This result showed that the initial mechanical response of the material was not recovered after a resting period of two hours, and that the material was insensitive to the strain rate after repeated cycling. This is in agreement with another study (Fung et al., 1972) that found that the hysteresis loop of most biological soft tissues is almost insensitive of the strain rate within several decades of the rate variation.

3.2. Stress relaxation tests

Stress relaxation tests were conducted on two porcine skin samples

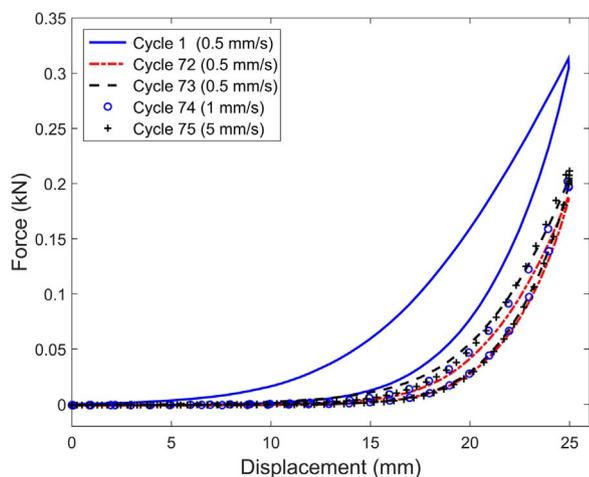


Fig. 6. Two cyclic tensile tests were performed on a porcine skin sample according to the following steps: (1) the sample was loaded-unloaded 72 consecutive times up to 25 mm and at a strain rate of 0.5 mm/s. Only the first cycle and the 72th cycle are presented, (2) the sample was then left in an unstressed phase for a resting period of two hours, (3) then, the sample was loaded-unloaded again three consecutive times up to 25 mm, at three different strain rates: 0.5 mm/s (the 73th cycle), 1 mm/s (the 74th cycle), 5 mm/s (the 75th cycle), respectively.

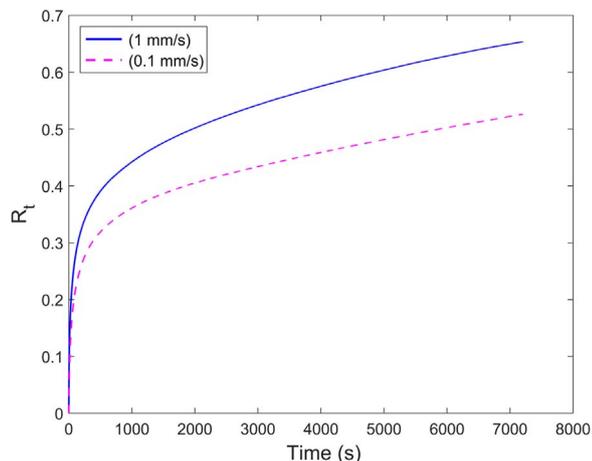


Fig. 7. Two skin samples were stretched up to 25 mm, at a strain rate of: 0.1 mm/s for the first sample and 1 mm/s for the second sample. Each sample was then kept in the strained condition for two hours. The variation in the reaction force at each instant t (i.e. $F(t)$) versus the reaction force at the instant t_0 (i.e. $F(t_0)$) was calculated and then normalized by dividing it by $F(t_0)$. The obtained values (denoted R_t) for each sample are presented as a function of the relaxation time.

at two different strain rates: 0.1 mm/s and 1 mm/s. The stress relaxation was considered as the change in force as a function of time. The variation in stress relaxation as function of relaxation time was calculated according to Eq. (4). Fig. 7 shows the variation in stress relaxation, R_t versus the time of the relaxation process. As shown in the figure, the expanded skin samples easily get relaxed, which obviously showed their viscoelastic behavior. The figure also shows that R_t -time curves increased exponentially and that the deviation between the two curves increased with the increase in maintaining duration. The skin response to a constant deformation depends thus on the skin sample. Otherwise, it seems that the curves have trend to continuously increase without stabilization, i.e. without a limiting value of force, which means, that the relaxation time was insufficient for the complete stress relaxation of the material, or there is another phenomenon responsible for this skin behavior. We assumed that, in addition to its viscous behavior, the skin accumulated damage with the increase in maintaining duration, i.e. more the material is expanded more the failure of collagen fibers are occurring (Arumugam et al., 1994). However, histological examination

Table 1
Summary of the identified material constants compared to some values from the literature.

Parameters	Ogen Model		Yeoh Model			Prony series			
	μ	α	C_{10}	C_{20}	C_{30}	g_1	g_2	τ_1	τ_2
This study (Flynn et al., 2011)	0.69 0.0096	23.27 35.9	0.26	15.5	1.75	0.34 0.34	0.32	52.6 1.68	2606
(Chanda et al., 2015)	-	-	0.95	4.94	0.01	-	-	-	-
(Remache, 2013)	0.02	29	-	-	-	-	-	-	-

μ , g_i and C_i are given in MPa, τ_i in second. $k_i = 0$.

is needed to validate this assumption and to quantify any damage due to stress relaxation tests.

3.2.1. Numerical analysis

The identified material constants of the hyperelastic and linear viscoelastic models obtained from the experimental data calibration compared to some values from the literature are summarized in Table 1. The material constants of the Ogden model are determined in Abaqus through a nonlinear least-squares-fit procedure, and those of Yeoh model are determined through a linear least squares fit for the reduced polynomial (Abaqus, 2014). The comparison of the stress-relaxation test data with numerical results is shown in Fig. 8. Yeoh model shows an accurately prediction of the nonlinear behavior of the tested skin (Fig. 8a). Fig. 8b shows the time-dependent behavior of the skin evaluated through a stress relaxation test at 25% strain. The viscoelastic behavior of the material showed that the mechanical properties degraded in 2 h to reach about 60% of the initial values. It seems that, according to the tendency of the experimental curve, the properties of the material continue to degrade.

3.2.2. Strain rate effect on the stressed, relaxed skin

In order to investigate the strain rate effect on a skin previously subjected to stress-relaxation test, a stress-relaxation test followed by a cyclic tensile test were performed on a skin sample according to the steps shown in Fig. 9 (type 4 of the tests). From the figure, it can be seen that, the obtained hysteresis loops are almost confused which means that the strain rate didn't have any effect on the material after a relaxation process. Similar results were found in Section 3.1.3. In addition, the three hysteresis loops, which are related to the dissipated energy of the viscoelastic material, are narrow. We assume that the material viscosity decreases with the increase in maintaining duration. This may be due to a modification in the internal structure of the material in time or with increasing hydration of the material. We assume therefore that the viscous property of the skin was also influenced by the stress relaxation process.

3.3. Cyclic-relaxation test

A cyclic tensile and a stress relaxation test were successively performed on a skin sample (Fig. 10; type 5 of the tests). The normalized variation in the maximum reaction force of the skin sample as a function of time cycling was calculated according to Eq. (1), and the normalized variation in the reaction force as a function of the time of the relaxation process was calculated according to Eq. (4). The obtained results for the two tests were compared in Fig. 10. The results showed an exponential increase of the normalized variation in the reaction force as a function of the cyclic time as well as the relaxation time. A strong stress relaxation was observed at the beginning of the relaxation process. The two curves followed, therefore a similar evolution. It appears that the material was subjected to the same phenomenon in cyclic

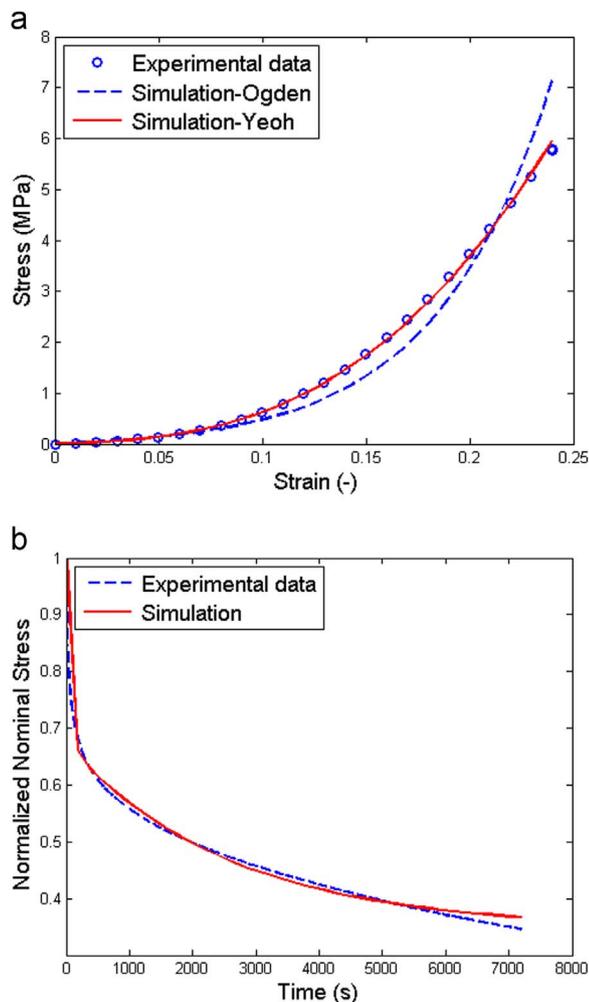


Fig. 8. Comparison between, numerical model and experimental test data; (a): tensile phase, (b): stress-relaxation phase.

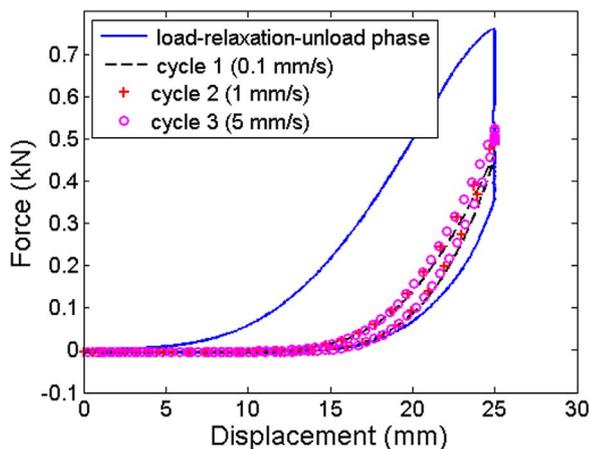


Fig. 9. A stress-relaxation test and a cyclic tensile test were successively performed on a porcine skin sample according to the following steps: (1) the sample was stretched up to 25 mm at a strain rate of 1 mm/s, then maintained for a resting period of two hours, then fully unloaded, (2) the sample was then loaded-unloaded three consecutive times up a stretch of 25 mm, at three different strain rates: 0.1 mm/s (cycle 1), 1 mm/s (cycle 2), 5 mm/s (cycle 3), respectively.

and relaxation tests. This particular test showed that, when a skin sample is stretched up a constant value, the response of the material as a function of time is the same in cyclic or relaxation tests. To our knowledge, this kind of tests was performed on skin for the first time in

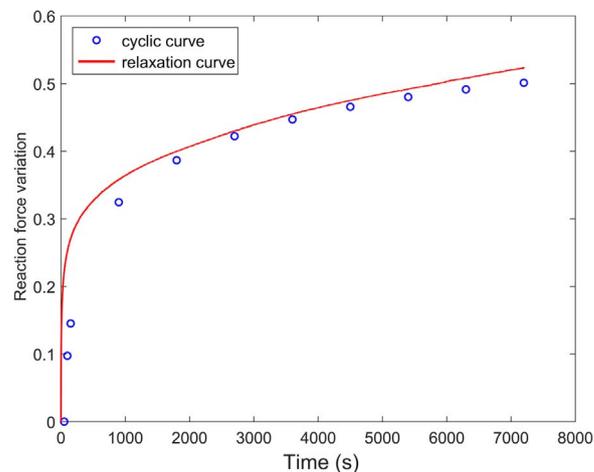


Fig. 10. A cyclic tensile test and a stress-relaxation test were successively performed on a porcine skin sample as follows: (1) the sample was subjected to cyclic tensile test for two hours, with a peak displacement of 25 mm, and at a constant strain rate of 1 mm/s, then (2) the sample was stretched up 25 mm at a constant strain rate of 1 mm/s, and then kept in the strained state for a resting period of two hours. The normalized variation in the maximum reaction force as a function of cyclic time and relaxation time, obtained from the two successive tests, is presented. For the cyclic test, only the normalized variation in the maximum reaction force of some cycles is presented.

this study. Other studies are needed to validate this assumption.

4. Conclusion

Porcine skin was subjected to cyclic uniaxial tensile tests at large and constant strain. The results showed that: (1) the force-displacement curves continuously shifted down with the increase in the number of cycles, (2) the initial mechanical response of the material was not time recovered after a resting period of two hours, (3) the material was insensitive to the strain rate after a certain number of cycles, and (4) the material accumulated progressively an inelastic deformation over time during cycling. Porcine skin was also subjected to stress-relaxation tests. It was observed: (1) an exponential degradation in reaction forces to the stress as a function of relaxation time, (2) the material was insensitive to the strain rate after a certain maintaining time. Furthermore, a cyclic tensile and stress relaxation tests were successively performed on a skin sample, and then the results were compared. It was found that both tests gave similar results. It appears that the same phenomenon occurred during continuous mechanical stress. Overall, the results showed that the mechanical properties of the porcine skin were strongly influenced by cyclic and stress relaxation tests. We assumed that the material accumulated viscoplastic strain or damage under continuous mechanical stress.

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