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The Preconditioning and Stress Relaxation of Skin Tissue

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Abstract

This paper reports our recent study on the preconditioning effects and stress relaxation behaviour of fresh swine skin. Uniaxial cyclic tensile loading and stress relaxation tests were performed with swine skin samples in two different directions to study the effects of fibre direction on mechanical properties. With stress relaxation tests, skin samples were loaded to different strains to observe its effects on stress relaxing over time. Mathematical modelling was performed to compare the data obtained from stress relaxation tests. Fibre direction effects were demonstrated in results of cyclic preconditioning as well as stress relaxation tests. The stress relaxation experimental results indicated that strain and time independency only appears at certain strain levels. The skin tissue has stress relaxation characteristics at strain of 15% and below. Under such strain amplitudes, the samples exhibited greater stress decaying rate in the first 200 seconds of the relaxation period, and began to approach steady decay after 200 seconds. With quasi-linear viscoelastic theory and reduced relaxation function, the modelling has adapted the experimental data sufficiently. This study is a significant step towards understanding skin tissue rheological behaviour and its multidiscipline properties.

Keywords: Skin, Preconditioning, Stress relaxation, Soft Tissue

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1 INTRODUCTION

The mechanical properties of biological soft tissues have been widely investigated [1-3]. These tissues exhibit viscoelastic behaviour, which could be a result of fluid flow [4] or structural interactions within the extracellular matrix of the tissue [5] during mechanical loading. Biological skin is a type of soft tissue which exhibits load-history dependent behaviour. The epidermis and dermis of skin consists largely of collagen (about 75% of dry weight), and elastin (4% of dry weight) fibres embedded and mobile in a gel-like ground substance [6]. The interlacing network of collagen and elastin fibres provides the skin with extensibility and elasticity [7], hence displaying a viscoelastic nature.

Due to the structural composition of biological soft tissue, the resultant viscoelastic capability produces unequal mechanical results when subjected to a repeat mechanical loading. Preconditioning, therefore, is considered as a necessary step towards overcoming the effects of soft tissue handling and thus establishes a repeatable set of experiments. Such a procedure orientates the molecular structure of tissues to its natural in vivo alignment, allowing tissues to gradually adapt to loading and hence resulting in more consistent data from mechanical testing [5]. Uniaxial cyclic loading have been used to demonstrate the preconditioning effect in various soft tissues [8].

Another viscoelastic property of soft tissue is stress relaxation behaviour， which describes the time-dependent decay of stress as the applied strain is held constant. The quasi-linear viscoelastic (QLV) constitutive model is one of the methods to characterise stress relaxation behaviours of skin[9-11]. The responses of many types of soft tissue including porcine anterior cruciate ligament [12], goat medial collateral ligament [13], human [14], sheep digital extensor tendon [15], porcine aortic valves [16], and rat bladder [17] can be captured by using the QLV mode. According to QLV theory [5], the general form of stress response is the hereditary integral equation [18].

This study investigates the rheological properties of different fibre orientations in skin tissues quantitatively through experimentation. The QLV model is an adaptation of linear viscoelasticity models that is appropriate for nonlinear materials, such as soft tissues, which do not reside in the small strain regime [5]. The hypothesis of this study is that the QLV model can be used to adapt the skin experimental data. The results will indicate the time and strain rate dependency behaviour by stress relaxation after preconditioning.

The preconditioning effects were investigated by loading samples of fresh swine skin in a uniaxial cyclic tensile manner. Experimental tests also were focused on stress relaxation with different strain levels. The preconditioning and stress relaxation were investigated in both parallel and perpendicular directions of tissue fibre. The stress relaxation behaviour was modelled by using QLV theory, together with the reduced relaxation function [19]. The modelling results were then used to compare with the experimental data.

2 MATERIAL AND METHODS

2.1. Sample Preparation

This study used fresh swine skin for the experiments. Swine skin is a well established replacement for human skin in many scientific studies, such as photoprotection studies, pigment induction and modulation [20], and DNA uptake and gene expression [21]. This is due in part to the fact that swine skin has a similar mechanical response as that of human skin. Moreover, the thickness of the human and pig dermis are similar [22].

Skin samples from a swine's abdominal region were obtained from a local wholesaler within 24 hours after the animal was slaughtered. All the samples were excised from the same part of the animals. Six pigs were employed for this research. The weights of those pigs were between 60kg to 70kg. Rectangular-shaped samples with similar skin fibre lengths were selected to enhance the investigation of the effects of tissue fibre direction. A skin sample of 100mm x 50mm was obtained from the animal prior to the actual size of test samples. The fascia and most of the hypodermis of swine skin were removed using a surgical scalpel. Three groups of samples were cut with the long axis parallel to the long axis of the extracted piece of skin, which were referred to as parallel samples. And the other three groups of samples were cut with the long axis perpendicular to the piece of skin, which were referred to as perpendicular samples. The fibres of all the samples were parallel to the native swine skin. The sample dimensions were measured using a digital calliper in three places along the strip. The measured dimensions were 10mm long, 9.4±0.04mm wide, and 2.15±0.03mm thick. Skin samples were bathed in normal saline solution with temperature of 4^{o}C for about two to three hours prior to testing.

2.2. Experimental Method

The mechanical tests of the skin samples were conducted using a Dynamic Mechanical Analyser (DMA 2980, TA Instruments USA), along with the built-in software. Samples were clamped in Film Tension mode with a gauge length of 13.5±0.2mm. Sandpaper was used to enhance grip on the samples. Each test was set be preloaded with 0.01N under ambient temperature, and samples were hydrated using normal saline solution throughout testing. Experimental results were recorded every two seconds over the duration of the relaxation experiment to avoid unnecessarily excessive data file size.

2.2.1. Preliminary Test and Preconditioning

Following a standard calibration, the DMA machine was preset to the Controlled Force setting. Preliminary uniaxial tensile tests were conducted to identify the best load and loading rate. Figure 1 is a typical result of the uniaxial tensile tests under 3N/min loading rate. The result demonstrated the linear region of skin tissue under the certain loading force and rate. Distending the skin sample beyond the point of structural failure was rendered superfluous, due to the limitations of the DMA test machine. The result also illustrated that the force-strain curve decreased linearly in the domain where the applied force was less than 3N. A load of 1.25N and rate of 3N/min were optimal in reaching the repeatable state within the aforementioned linear region.

In order to minimize any damage to the sample, the applied force, loading rate and cycles for preconditioning were chosen as small as possible. Likewise, the loading cycles are diminished. The preconditioning effect was examined using six groups of samples. There were at least five samples in each group with identical conditions, and the average values of experimental results were recorded for analysis. A controlled force value of 1.25N was loaded to each sample at a low loading rate of 3N/min, and then unloaded to the preload force of 0.01N at the same rate as the loading force. Loading and unloading under the same constant rate was repeated until the stress-strain loop of sample appeared to be periodic. The recovery time between each cycle was five minutes.

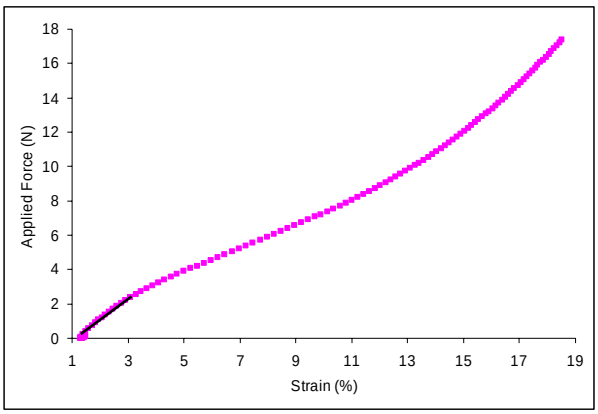


Figure 1: A typical uniaxial tensile test result to identify the linear region of skin sample.

2.2.2. Stress Relaxation

The DMA machine was preset to the Stress Relaxation mode. Each skin sample was preconditioned using the method mentioned above, before the uniaxial stress-relaxation tests were performed. Each sample was equilibrated for five minutes before rapid ramping to the same strain rate as the preconditioning. Constant strain amplitude of 5%, 10%, or 15% were applied respectively to different samples for each strain amplitude. The tests were carried out in groups

separated by strain over a 1200 second period to observe stress relaxation behaviour. The tests were repeated five times per strain amplitude, each time with a new sample. Testing details are shown in Table 1.

Table 1: Sample groups for stress relaxation tests

Cut Direction	Sample Group No.	Strain (%)
Parallel	1	5
	2	10
	3	15
Perpendicular	4	5
	5	10
	6	15

2.3. Modelling

The QLV constitutive model and the reduced relaxation function were employed for modelling in this study. The reduced relaxation function is defined as:

$$G(t) = \frac{\sigma(t)}{\sigma(0)} \tag{1}$$

where $\sigma(t)$ is the stress at time t. $\sigma(0)$ is the initial stress when the sample is firstly ramped up to designated strain. This reduced relaxation function is the viscoelastic constitutive function of the sample, which has the condition,

$$G(0) = 1 \tag{2}$$

The instantaneous stress follows the equation,

$$\sigma(t) = G(t) \times \sigma^{e}(\varepsilon) \tag{3}$$

where $\sigma^{e}(\varepsilon)$ is the stress at instantaneous strain. The convolution integral that describes the stress history of the sample is then represented by

$$\sigma(t) = \int_{-\infty}^{t} G(t-\tau)\frac{\partial \sigma^{e}(t)}{\partial \varepsilon}\frac{\partial \varepsilon}{\partial \tau} \tag{4}$$

where $\frac{\partial \sigma^{e}(t)}{\partial \varepsilon}$ is the instantaneous elastic response, and $\frac{\partial \varepsilon}{\partial \tau}$ is the strain history of the sample. The reduced relaxation function is

$$G(t) = ae^{-bt} + ce^{-dt} + ge^{-ht} \tag{5}$$

where a, b, c, d, g, h are constants, which could be determined empirically by experimental data. Together with the nonlinear elastic representation of the instantaneous stress response,

$$\sigma^{e}(\varepsilon) = A(e^{B\varepsilon} - 1) \tag{6}$$

where A and B are constants determined by using experimental data, the reduced relaxation function can model the stress relaxation responses. Experimental data obtained from stress relaxation tests were used to determine coefficients A, B, a, b, c, d, g and h from (5) and (6). Optimisation was performed using MATLAB to generate model functions for each sample.

3 RESULTS

3.1 Preconditioning

Figure 2 illustrates a typical change between the stress-strain loops during preconditioning. This stress-strain curve shows repeatability starting from the 4th cycle of uniaxial tensile loading. Parallel samples required 4-6 cycles before reaching the preconditioned state, and the perpendicular samples required slightly more repeated loading cycles of 8-12. The regularity of cycles required for the preconditioning of skin samples was higher in the parallel samples compared to the perpendicular samples. Details of the preconditioning cycles required for each group for both the parallel and perpendicular fibre directions at a low constant loading rate are shown in Table 2. The repeated cyclic loading indicated stress-strain loops moving towards the right, demonstrating preconditioning phenomenon of skin tissue.

Table 2: Cycles required for preconditioning of samples

Cut Direction	Sample Group No.	Cycles Required
Parallel	1	4
	2	7
	3	6
Perpendicular	4	10
	5	8
	6	12

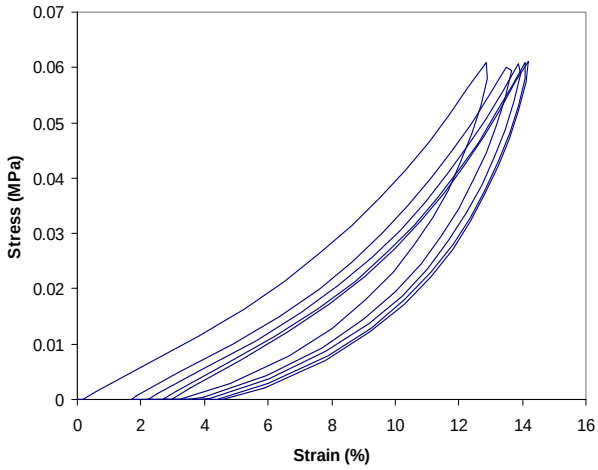
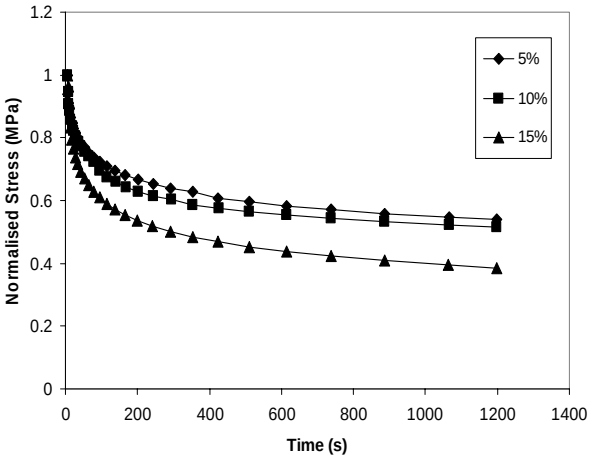


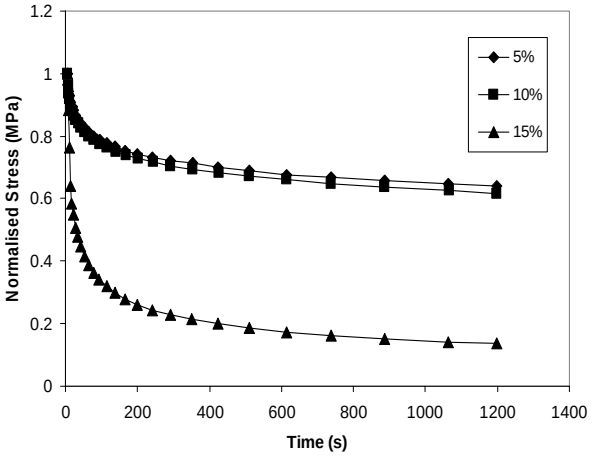
Figure 2: Preconditioning result of a typical swine skin sample.

3.2. Stress Relaxation

Both parallel and perpendicular skin samples were raised to 5%, 10% or 15% strain individually before the stress relaxing. Figure 3(a) shows the normalised stress against time of parallel samples. The strains of 5% and 10% displayed a similar stress relaxation trend, whereas the strain of 15% displayed relatively rapid relaxation for the first 200 seconds, but the decaying rate was reduced after that and reached a similar level to the other two strains. Both 5% and 10% strains of the samples decayed to a normalised stress of approximately 0.6MPa, whereas the 15% strain of samples decayed to a normalised stress of approximately 0.4MPa. Figure 3(b) shows the stress relaxation behaviour of perpendicular samples under the same strain range. Decaying trends are similar to those of parallel samples, perpendicular samples of 5% and 10% strains relaxed to just above a normalised stress of 0.6MPa, and the 15% strain of sample relaxed to below 0.2MPa.



(a)



(b)

Figure 3: Stress relaxation trend of different strains in, (a) parallel samples with normalised stress versus time, (b) perpendicular samples of normalised stress versus time

3.3 Modelling

Calculation of the coefficients, A, B, a, b, c, d, g and h were carried out by using MATLAB. Results of values obtained for coefficients are shown in Table 3 and Table 4. Graphs of model and experimental data are shown in Figure 4, where instantaneous stress $\sigma(t)$ is plotted against time for each graph. With Figure 3, the test data was normalised so that it is easier for comparison of different rates. However, for the groups of data individually in Figure 4, normalisation is not required. Figure 4 (a), (b) and (c) are the graphs of parallel samples, and Figures 4 (d), (e) and (f) are of perpendicular samples.

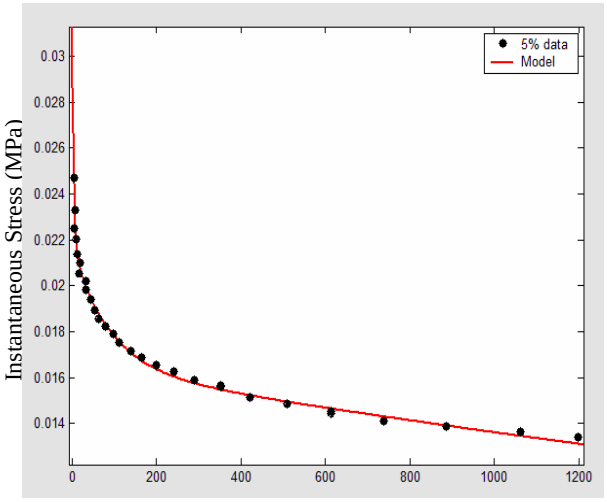
The QLV model is suitable to describe the elastic component *(A* and *B)* and the viscous component *(a ,b, c, d, e, f, g and h*) of the material. The skin tissue has viscoelastic behaviour with both parallel and perpendicular orientations which were well modelled by QVL. Comparing both fibre orientations under the same strain, the elastic components A of parallel orientation were greater than perpendicular orientation. Conversely, the components B were reversed. The results show the value of both A and B increasing slightly with the strain. Larger values of A and B would indicate higher peaks in stress and higher equilibriums in stress for the same level of extension.

Table 3: Coefficient values of parallel samples

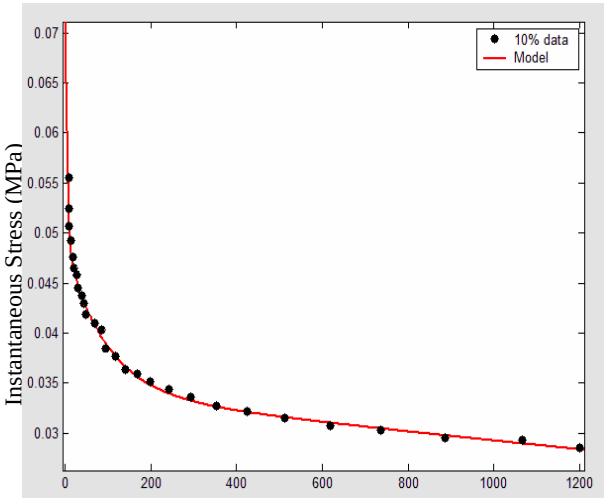
	Parallel Sample Groups		
	1	2	3
Strain	5%	10%	15%
A	0.6486	0.6383	0.8051
B	0.5557	0.56	0.7587
a	0.2765	0.3344	0.6063
b	0.2021	0.2027	0.1096
c	0.8883	0.9194	0.4475
d	0.0001882	0.0001523	0.00882
g	0.2765	0.3843	0.9133
h	0.01128	0.01058	0.0002477

Table 4: Coefficient values of perpendicular samples

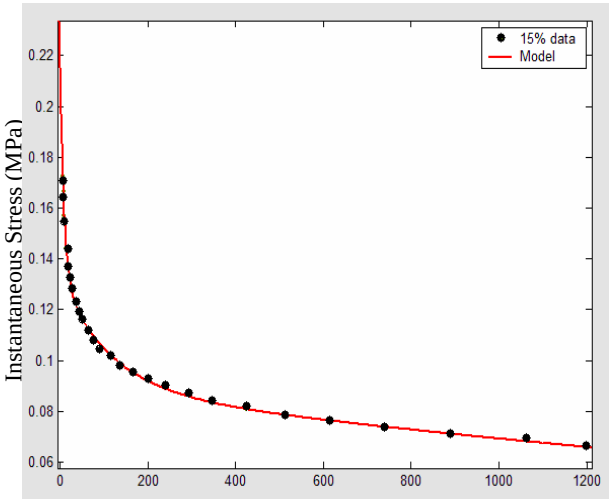
	Perpendicular Sample Groups		
	4	5	6
Strain	5%	10%	15%
A	0.5383	0.5946	0.6514
B	0.5611	0.6533	0.839
a	0.1372	0.1731	1.483
b	0.2801	0.1537	0.1787
c	0.8276	0.7994	0.5766
d	0.0001634	0.0001415	0.0006151
g	0.2006	0.1862	0.7228
h	0.01743	0.01091	0.01399



(a)



(b)



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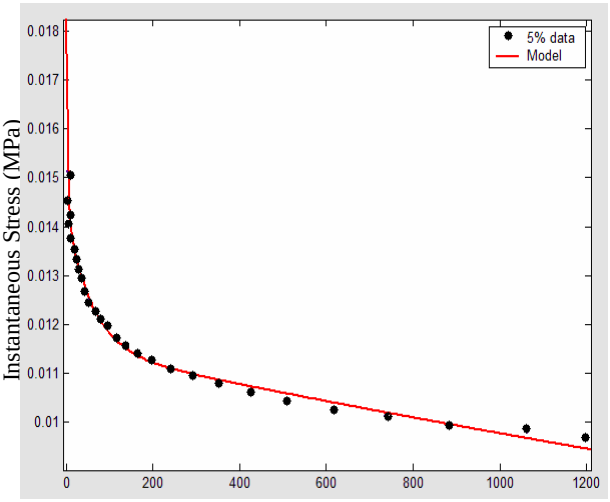
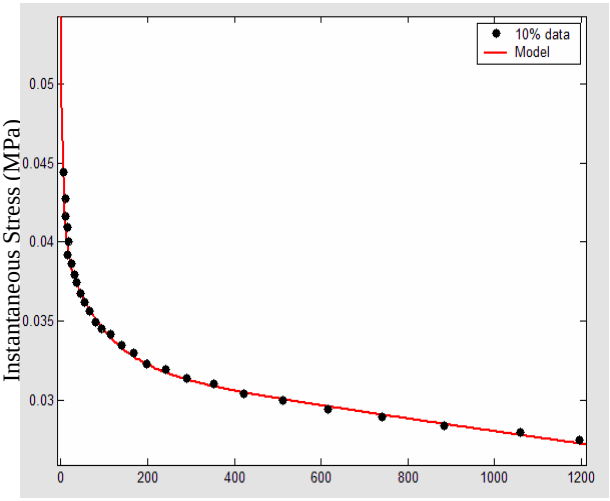
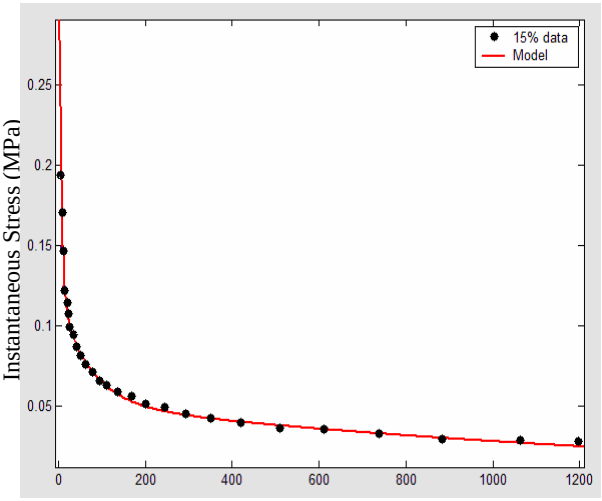


(d)



(e)



(f)

Figure 4: Comparison of modelling with experimental data: (a) parallel with 5% strain, (b) parallel with 10% strain, (c) parallel with 15% strain, (d) perpendicular with 5% strain, (e) perpendicular with 10% strain, and (f) perpendicular with 15% strain.

4 DISCUSSIONS

The different directions of cutting on the samples were considered as an indication of the effect of fibre orientation on viscoelastic properties. During preconditioning, the parallel samples required fewer cycles to reach steady state consistently comparing with the perpendicular samples. This indicates that the viscoelastic properties are dependent upon fibre direction, and that the regular fibre realignment process only occurs when mechanical loading is exerted in a particular fibre direction. Fibre direction also seemed to affect the stress relaxation behaviour of the samples. The parallel samples to which were applied the 5% and 10% strains had a greater decaying rate in the first 200 seconds compared with the perpendicular samples. However, an application of 15% strain showed a greater relaxation rate in perpendicular sample than in the parallel sample, possibly indicating that the strain-independence of stress relaxation phenomenon only applies to certain strain levels. Further studies are encouraged for a clearer explanation of such behaviours. Experimental results from the stress relaxation tests showed relaxation rate approaching steady state after 200 seconds of strain displacement across all the samples, demonstrating the time dependent nature of the tissue.

Due to the variation in each skin sample, it is acceptable the experimental output were slightly different within the same group. The number of cycles required for preconditioning only slightly differs from sample to sample in the same group. The results shown in Table 1 indicate the strain magnitude for both preconditioning and the afterward stress relaxation test for each group. It is clear that preconditioning cycles required for different group were caused by the strain magnitude. It is possible that some damages on the extracellular matrix proteins were caused by the high strain rate. However, the loading rate of 3N/mim for all the tests should be small enough to avoid such damages.

In this study, the modelling of stress relaxation behaviour was performed using MATLAB based on the QLV theory with the reduced relaxation function. The success of this model is linked both to its ability to accurately capture experimental data as well as its ease in fitting material parameters because of the separation of the elastic response and time-dependent viscoelastic effects. Results of modelling show a close fit with fresh skin stress relaxation data. The benefit of using this reduced relaxation function is that it is less complicated compared to the most extensively used equation by Fung [5] and its modified version used by other researchers [14].

5 CONCLUSION

This study investigated preconditioning and stress relaxation effects of fresh swine skin. The results indicated that the fibre direction of skin samples affected the preconditioning cycles required, the consistency of viscoelastic response, and the stress relaxation rate at the beginning of the relaxation period. Skin tissue has similar stress relaxation behaviour characteristics of strain at 5% and 10%. However, it appeases different behaviours with much slower stress relaxation rate when the strain reaches 15%. This stress relaxation phenomenon implies the strain-independency only appeared in samples below 15% strain. All samples exhibited greater stress decaying rate in the first 200 seconds of the relaxation period, and began to approach steady decay after. The QLV theory and reduced relaxation function can be used to model the stress relaxation tests data of skin tissues.

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