Finite element modeling of hyper-viscoelasticity of peripheral nerve ultrastructures

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\begin{abstract}
The mechanical characteristics of ultrastructures of rat sciatic nerves were investigated through animal experiments and finite element analyses. A custom-designed dynamic testing apparatus was used to conduct in vitro transverse compression experiments on the nerves. The optical coherence tomography (OCT) was utilized to record the cross-sectional images of nerve during the dynamic testing. Two-dimensional finite element models of the nerves were built based on their OCT images. A hyper-viscoelastic model was employed to describe the elastic and stress relaxation response of each ultrastructure of the nerve, namely the endoneurium, the perineurium and the epineurium. The first-order Ogden model was employed to describe the elasticity of each ultrastructure and a generalized Maxwell model for the relaxation. The inverse finite element analysis was used to estimate the material parameters of the ultrastructures. The results show the instantaneous shear modulus of the ultrastructures in decreasing order is perineurium, endoneurium, and epineurium. The FE model combined with the first-order Ogden model and the second-order Prony series is good enough for describing the compress-and-hold response of the nerve ultrastructures. The integration of OCT and the nonlinear finite element modeling may be applicable to study the viscoelasticity of peripheral nerve down to the ultrastructural level.
\end{abstract}

1. Introduction

Understanding the biomechanical properties of peripheral nerves is essential to improve the repair and regeneration process of the nerve after injury (Sunderland, 1978; Wall et al., 1991). Most past studies have been focused on the longitudinal traction of peripheral nerve and the tensile behavior was dominant by perineurium (Sunderland, 1978); a rabbit tibial nerve was tested and it was found that the nerve exhibits elastic behavior (Haffek, 1970); the relaxation test of a tibial nerve was performed and different peak stress at strain levels of 6%, 9%, and 12% was related to the structural distribution of the nerve (Wall et al., 1991). Recently, few studies have examined the transverse compressive mechanical properties of peripheral nerves. The hyperelastic properties of median nerve were estimated by using a finite element model to fit the experimental data obtained using a platen compression apparatus (Main et al., 2011). In vitro parallel compression test was used to estimate the elastic properties of rabbit sciatic nerves (Ju et al., 2004). After that, in situ circular compression test integrated with laser Doppler flowmetry was utilized to estimate the transverse elastic modulus of rabbit sciatic nerve and to quantify the allowable strain before declination of blood flow (Ju et al., 2006). Using the circular compression test, the transverse Young’s modulus of diabetic rat sciatic nerve was found to be two-fold higher than that of the normal group and the relaxation time of the diabetic was longer than that of the normal nerve (Chen et al., 2010a, 2010b). However, in above studies, the whole nerve was postulated as an isotropic material. From a structural point of view, the peripheral nerve was mainly consisted of three ultrastructures, namely perineurium, endoneurium and epineurium (Sunderland, 1978). There is a need to study the mechanical properties of individual ultrastructure of the nerve.

Optical coherence tomography (OCT) is a non-invasive technology to image biological tissue at micrometer scale (Huang et al., 1991). Several studies have utilized OCT to obtain two- or three-dimensional structural images by scanning the optical beam across the human skin (Welzel et al., 1997), neural retina (Hee et al., 1995; Swanson et al., 1993), arterial wall (Chau et al., 2004), and nerves (Brezinski et al., 1997; Schuman et al., 1995). Up to now, there is no study that integrates OCT and compression testing to investigate
the deformation and mechanical properties of ultrastructures of peripheral nerves.

The main goals of the current study are twofold. The first was to integrate a custom-designed parallel compression device and an OCT for concurrently measuring mechanical response and acquiring structural images. The second was to build a mechanical model of the nerve based on the OCT image and to estimate the material properties of the ultrastructures using the inverse finite element analysis.

2. Methods

2.1. Experimental setup

Fig. 1(a) and (b) shows picture of the custom-designed transverse compression apparatus and schematic diagram of the setup. The apparatus was integrated with a transverse compression part and an OCT (OCS1300SS, Thorlabs). The compression apparatus consists of a specimen fixture, a uni-axial force transducer (LTS-100GA, KYOWA), a DC servo motor (SGAM 20-35, SIGMA), and two glass plates (Fig. 1(b)). The upper glass plate was mounted on the platform and the bottom glass plate was placed against the force transducer which was installed with the servo motor. The up-and-down movement of the bottom glass plate was controlled by the motor. The device was designed to compress the nerve and simultaneously measure force response of the nerve. In particular, the OCT was used to acquire the cross-sectional images of the nerve. The scanning range of OCT was 10 mm × 10 mm × 3 mm with a longitudinal resolution of 9 μm in the liquid phase. The scanning rate was 24 frames/s. Both the force history and the image were digitized and stored in two computers for off-line analyses.

2.2. Experimental procedures

The research protocol was approved by the animal ethics committee of the National Cheng Kung University. Six normal Wistar rats (ranging in age of 6–8 weeks, 300–350 g) were used in this experiment. Before the experiment, the rat was anesthetized with an intraperitoneal injection of 7% chloral hydrate solution (0.45 mg/100 g). A segment of sciatic nerve of 15 mm long was dissected from the lower limb of the rat. The nerve was held at ends by the epineurium and the glass was assumed to be frictionless except that node A of epineurium (as shown in Fig. 2) was fixed at the bottom glass plate. The upper glass plate was fixed and the bottom plate had the same movement as the force transducer. The FE modeling of this study was reduced to solve a plane strain compression problem. The finite element simulation of the compression was displacement-driven from the bottom glass plate and the total reaction force on the upper plate was calculated. All the ultrastructures were assumed as isotropic, homogeneous, and incompressible materials. The perineurium, endoneurium, and epineurium were postulated as isotropic, hyper-viscoelastic materials. The nonlinear elasticity of each ultrastructure was described by a first-order Ogden hyperelastic equation which was commonly used in nerves and tendons (Main et al., 2011; Natali et al., 2006; Shreehari Elangovan and Odegard, 2009). The relationship between the principal Cauchy stress σi and stretch λi could be expressed as follows (Ogden, 1972, 1984):

\[ \sigma_i = \lambda_i \frac{\partial W}{\partial \lambda_i} - p \quad (i = 1, 2, 3) \]  

where \( p \) was an arbitrary hydrostatic pressure. Assuming that each ultrastructure was incompressible, the strain energy density function \( W \) could be described as a function of deviatoric principal stretches \( (\lambda_i, i = 1, 2, 3) \) (Ogden, 1972):

\[ W(\lambda_1, \lambda_2, \lambda_3) = \frac{2\mu}{\alpha} (\lambda_1^p + \lambda_2^p + \lambda_3^p - 3) \]  

\[ \dot{\lambda}_1 \dot{\lambda}_2 \dot{\lambda}_3 = 1 \]  

where \( \mu \) and \( \alpha \) were the material parameters that reflected the slope at low strain and curvature at hyper-strain of the stress-strain curve respectively.

The hyper-viscoelastic strain energy density functions of the three ultrastructures could be expressed as the following convolution integral (Miller and Chanze, 2002; Sparrey and Keaveny, 2011):

\[ W(t) = \frac{2\mu_0}{\alpha} \int_0^t \left[ \mu(t - \tau) \frac{d}{dt} (\dot{\lambda}_1^p + \dot{\lambda}_2^p + \dot{\lambda}_3^p - 3) \right] d\tau \]

\[ i = 1, 2, 3 \]

\[ (1 - \text{epineurium}, 2 - \text{perineurium}, 3 - \text{endoneurium}) \]  

\[ \mu(t) = \mu_0 \left[ 1 - \sum_{j=1}^{3} g_j (1 - e^{-t/\tau_j}) \right] \]  

where \( \mu(t) \) was the reduced relaxation response described by the 2nd order Prony series, \( t \) was time (s), \( \alpha_i \) was the material parameter, \( \mu_0 \) was the instantaneous shear modulus and it was used for comparing elasticity of the three ultrastructures, \( g_j \) was Prony constant, \( \tau_j \) was Prony retardation time constant. For simplification, the relaxation responses of the three ultrastructures were assumed to be similar.

Fig. 1. (a) An enlarged photograph of the compression testing apparatus; (b) lateral schematic drawing of the whole compression device; (c) block diagram of the experimental setup.
Fig. 3 shows the flow chart of the inverse FE analysis in this study. First, the initial values of all material coefficients were loaded into FE input file. Then, the compressive force/displacement data obtained after the FE simulation were extracted from FE output files. The parameters $\mu_0$ ($i=1,2,3$), $\alpha$ ($i=1,2,3$), $g_j$ ($j=1,2$), and $\tau_j$ ($j=1,2$) were determined by using the optimization algorithm of simulated annealing (Kirkpatrick et al., 1983) in MATLAB (The Mathworks). The model parameters were updated until the objective function converged to the value of $1 \times 10^{-3}$.

2.4. Statistical analysis

The total number of nerve samples was six. One-way analysis of variance (ANOVA) method was used to analyze the difference between instantaneous shear moduli of the epineurium, perineurium, and endoneurium. The $p$-value was set as 0.01.

3. Results

A total of six samples of sciatic nerves were tested and inverse FEM analyses were performed. Fig. 4(a) compares the typical force history obtained from the finite element simulation with that of the experiment (nerve P3). The relaxation response was simulated well. The root-mean-squared error was 0.072 N and the $R$-square value was 0.991. The peak forces of the experimental curve and the simulation curve were 0.277 N and 0.267 N respectively. The forces of the experimental and the simulation curves at the final time were 0.156 N and 0.161 N respectively. Fig. 4(b) shows the FE model of the ultrastructures of nerve P3, which consists of 4277 quadratic-triangular elements and 19,739 nodes. It also depicts the deformed fascicles and distribution of normal stress ($\sigma_{yy}$) of the ultrastructures. Higher level of normal compressive stress appears on the right fascicle which has larger cross-sectional area than the left fascicle. The left and right-outer boundaries of epineurium were subjected to normal tensile stress. Higher level of normal tensile stress appears at the middle region between the two fascicles. Both fascicles were deformed from the initial shapes obtained by the OCT (Fig. 2(a)). The thickness difference of perineurium and endoneurium between the un-deformed and the apparent state ranged between 20% and 35% from the FE simulation.

Table 1 shows the material parameters of the first-order Ogden model obtained from the inverse finite element analyses for the six samples. The coefficients of 2nd order Prony series are also listed in Table 2. The mean instantaneous shear modulus of perineurium $\mu_{02}$ was greater than those of epineurium and endoneurium. The mean shear modulus of epineurium $\mu_{01}$ was the smallest among the ultrastructures. For all ultrastructures, the material parameter $\alpha$ was higher than the parameter $\mu$ by two.
orders. The coefficient of variations (CV) of the parameter $\mu_0$ was higher than that of the parameter $\alpha$. The mean of time constant $\tau_2$ was higher than that of $\tau_1$. The means and standard deviations of instantaneous shear modulus $\mu_0$ of the epineurium, perineurium, and endoneurium were compared (Fig. 5). ANOVA analyses showed that the instantaneous shear modulus, $\mu_0$, of each ultrastructure of the nerve was significantly different ($p < 0.01$).

4. Discussion

Accurate mechanical properties of ultrastructures of nerves are helpful for better understanding of mechanical behavior of peripheral nerve in physiological environment. In this study, in vitro compressive response of rat sciatic nerve was measured and simulated by using hyper-viscoelastic finite element model.

It was found that the ultrastructures of three layers of a nerve could be clearly delineated by using the OCT. The average diameter of six sciatic nerves was $\sim 1$ mm, which was consistent with other studies (Brezinski et al., 1997; Layton et al., 2004; Sunderland, 1978). Another approach to build the FE model of the nerve was to utilize the histological images of the nerve. Although a FE model can still be built from a histological image, the structural artifact from histological processing was a challenging problem. In previous study, an OCT based and histological image based FE models of arterial wall were compared and it showed similar stress distribution but the histological artifacts can cause stress concentration at the edge during FE simulation (Chau et al., 2004). In this study, the acquired OCT images were free of artifacts and the stress concentration at edge did not occur.

Table 1
Summary of parameters of ($\mu_0$ and $\alpha$, $i=1,2,3$) of first-order Ogden model for the six rat nerves. Subscript ‘1’ for epineurium, ‘2’ for perineurium, and ‘3’ for endoneurium.

<table>
<thead>
<tr>
<th></th>
<th>$\mu_{01}$ (MPa)</th>
<th>$\mu_{02}$ (MPa)</th>
<th>$\mu_{03}$ (MPa)</th>
<th>$\alpha_1$</th>
<th>$\alpha_2$</th>
<th>$\alpha_3$</th>
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<tbody>
<tr>
<td>P1</td>
<td>0.028</td>
<td>0.105</td>
<td>0.070</td>
<td>9.142</td>
<td>9.043</td>
<td>6.941</td>
</tr>
<tr>
<td>P2</td>
<td>0.023</td>
<td>0.088</td>
<td>0.061</td>
<td>8.351</td>
<td>8.988</td>
<td>6.912</td>
</tr>
<tr>
<td>P3</td>
<td>0.021</td>
<td>0.080</td>
<td>0.052</td>
<td>8.313</td>
<td>8.995</td>
<td>6.968</td>
</tr>
<tr>
<td>P4</td>
<td>0.014</td>
<td>0.074</td>
<td>0.036</td>
<td>8.392</td>
<td>8.921</td>
<td>6.947</td>
</tr>
<tr>
<td>P5</td>
<td>0.013</td>
<td>0.071</td>
<td>0.034</td>
<td>8.342</td>
<td>8.949</td>
<td>6.941</td>
</tr>
<tr>
<td>P6</td>
<td>0.012</td>
<td>0.119</td>
<td>0.077</td>
<td>7.834</td>
<td>9.056</td>
<td>6.987</td>
</tr>
<tr>
<td>Mean</td>
<td>0.022</td>
<td>0.089</td>
<td>0.055</td>
<td>8.396</td>
<td>8.992</td>
<td>6.949</td>
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<tr>
<td>SD</td>
<td>0.007</td>
<td>0.019</td>
<td>0.018</td>
<td>0.421</td>
<td>0.052</td>
<td>0.026</td>
</tr>
<tr>
<td>CV (%)</td>
<td>31.8</td>
<td>21.3</td>
<td>32.7</td>
<td>5.0</td>
<td>0.5</td>
<td>0.3</td>
</tr>
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Table 2
Summary of 2nd order Prony series coefficients ($g_j^p$ and $\tau_j$, $j=1,2$) for the six rat nerves.

<table>
<thead>
<tr>
<th></th>
<th>$g_1^p$</th>
<th>$g_2^p$</th>
<th>$\tau_1$ (s)</th>
<th>$\tau_2$ (s)</th>
</tr>
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<tr>
<td>P1</td>
<td>0.124</td>
<td>0.347</td>
<td>31.295</td>
<td>39.912</td>
</tr>
<tr>
<td>P2</td>
<td>0.270</td>
<td>0.439</td>
<td>20.928</td>
<td>38.428</td>
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<tr>
<td>P3</td>
<td>0.241</td>
<td>0.171</td>
<td>41.986</td>
<td>63.082</td>
</tr>
<tr>
<td>P4</td>
<td>0.174</td>
<td>0.215</td>
<td>38.706</td>
<td>51.706</td>
</tr>
<tr>
<td>P5</td>
<td>0.312</td>
<td>0.316</td>
<td>21.808</td>
<td>74.958</td>
</tr>
<tr>
<td>P6</td>
<td>0.273</td>
<td>0.339</td>
<td>19.793</td>
<td>34.704</td>
</tr>
<tr>
<td>Mean</td>
<td>0.232</td>
<td>0.305</td>
<td>29.086</td>
<td>50.465</td>
</tr>
<tr>
<td>SD</td>
<td>0.070</td>
<td>0.097</td>
<td>9.692</td>
<td>15.908</td>
</tr>
<tr>
<td>CV (%)</td>
<td>30.2</td>
<td>31.8</td>
<td>33.3</td>
<td>31.5</td>
</tr>
</tbody>
</table>

Fig. 4. (a) Typical experimental force history of the nerve P3 (black dot) compared with the optimized FE results (solid line). (b) Deformed nerve ultrastructures and distribution of normal stress ($\sigma_{yy}$) of the same nerve.
It is known that nerve tissues were anisotropic and exhibit strain-stiffening behavior (Chau et al., 2004; Fung, 1993). In this study, only the transverse mechanical characteristics were accounted by using three different isotropic hyper-viscoelastic ultrastructures. The FE simulations showed the epineurium, the perineurium, and the endoneurium of the six sciatic nerves were mostly undertaken compressive load. The maximum normal compressive stress appeared at the larger fascicle, which indicates that the larger one bears the load before the smaller one. Both fascicles were found to enlarge toward the right and left sides during the compression test. Since all ultrastructures were assumed incompressible and tightly connected, the tensile stress occurred at the right and left sides of the nerve model and the maximum tensile stress occurred at the middle region between the two fascicles. There were some blood vessels that have been found inside the nerve ultrastructures (Sunderland, 1978). The blood vessels within the high compressive stress region may be damaged when the nerve is subjected to long duration of compression.

The hyper-viscoelastic properties of each ultrastructure of six nerves were determined by using the inverse FE method. Because the apparent strain in the test reaches as high as 0.35 so instead of linear elastic model the hyperelastic Ogden model was adopted to deal with the large deformation of the nerve ultrastructures. The coefficient of variations of parameter $\alpha$ were smaller than those of parameter $\mu_0$, which may due to the fact that stress is more sensitive to parameter $\alpha$ than $\mu_0$. The instantaneous shear modulus of perineurium, $\mu_{0\alpha}$, was found higher than those of the other two ultrastructures, which was consistent with the results of tensile tests (Georgeu et al., 2005; Sunderland, 1978). From histological point of view, the stiff perineurium was mainly composed of dense collagen fibrils and lamellar layers (Sunderland, 1978). For the endoneurium, its main structure was the longitudinal myelinated nerve fibers (Lundborg, 2004; Sunderland, 1978). On the contrary, the epineurium was found to have the smallest stiffness which may due to the fact that it is composed of loose connective tissues and some aligned collagen fibrils (Sunderland, 1978). It has been found that the perineurium of rat sciatic nerve can bear higher tensile force before damaged (Georgeu et al., 2005) and similar function of perineurium was found from transverse elasticity in this current study. Together, the epineurium and the perineurium may provide protection against compression load. The epineurium may serve like a buffer against the external load. It has been proved that the whole nerve behaves like hyper-elastic material by some studies (Main et al., 2011; Shreehari Elangovan and Odegard, 2009). However, in this study, the viscous properties of the nerve ultrastructures were also characterized. The retardation time constants might be used to characterize the patho-biomechanical property of diseased nerves.

4.1. Limitation

One limitation of this study was that the three ultrastructures were assumed to have the same relaxation behavior. Although the fitting to the hyper-viscoelastic model had R-square values ranged between 0.989 and 0.993, the maximum residual error occurred at the time of peak force. Although each ultrastructure can be assumed to have different viscous properties, the number of material constants would be increased and the analyses become very time-consuming.

Compared with the largest retardation time constant, the whole relaxation time of 190 s may not be sufficient for full relaxation of the nerve tissue under the compress-and-hold test. However, the surface of the sciatic nerve would be dry at the room temperature of 25 °C when the relaxation time was set longer than 190 s. An environmental chamber may be used to cover the apparatus to solve this problem in the future.

Another limitation was that the FE results were two-dimensional and plane strain was postulated. The assumption was valid if the nerve was constrained longitudinally or if the longitudinal dimension of nerve was large enough to neglect longitudinal strains. It has also been found that the number and size of fascicle varied at given levels for different specimens of the same nerve (Lundborg, 2004; Sunderland, 1978). All the six nerves of this study were controlled to have the same number of fascicles. The future work will be to scan the nerve longitudinally and construct a three-dimensional FE model. The same limitation has been mentioned for building an OCT based FE model for atherosclerotic plaque study (Chau et al., 2004). However, to the best of our knowledge, this study was the first to build a structure-based FE model to analyze viscoelastic characteristics for the rat sciatic nerve.

5. Conclusion

The compressive force response and the cross-sectional images of rat sciatic nerves could be acquired using the custom-designed compressive apparatus and OCT. The two-dimensional OCT based FE model was successfully employed to estimate the hyper-viscoelasticity of the ultrastructures of the nerve. The inverse FE results show the shear stiffness of the nerve tissues in decreasing order of perineurium, endoneurium, and epineurium. The epineurium and perineurium could give protection to the nerve fibers against compressive loads. Further work will involve an extension to a three-dimensional model and to compare the structural mechanical properties of nerves of normal and diabetic rats.

Conflicts of interest

We certify that all four authors did not have any financial and personal relationships with other individuals or organizations that could inappropriately influence (bias) this work.

Acknowledgments

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**Fig. 5.** Average instantaneous shear modulus $\mu$ for the epineurium, perineurium, and endoneurium of the six sciatic nerves. Results were shown as mean ± SD. The instantaneous shear modulus of each ultrastructure was significantly different ($p < 0.01$) and in descending order of perineurium, endoneurium, and epineurium.
References


