Rheological Behavior Analysis of Liver Fibrosis in Rats

Ying Zhu, Yuanyuan Shen, Xin Chen, Haoming Lin, and Siping Chen

***Abstract*—The process of liver fibrosis changes rheological properties of tissue. This study characterizes and compares two stages of liver fibrosis in rats. Two rheological models—Voigt model and Zener model are applied to the measured data. The experimental results demonstrate that Zener model is preferred to Voigt model for describing rheological properties of liver fibrosis stages F0 and F2 in rats.**

***Index Terms*—Liver fibrosis, rheological properties, voigt model, zener model.**

I. Introduction

Liver fibrosis is the widespread disease worldwide. The liver have been invaded by various pathogen, causing liver damage and inflammation, at the same time the immune system of liver tissue is activated. Liver fibrosis is a repair process of the damaged tissue, which refers to the accumulation of extracellular matrix(ECM) proteins. Currently liver biospy is still the gold standard for the diagnosis of liver fibrosis. Fibrosis grading has been evaluated semiquantitatively according to the METAVIR scoring system: F0, no fibrosis; F1, portal fibrosis without septae; F2, portal fibrosis and few septae; F3, numerous septae without cirrhosis; F4, cirrhosis [1]. The alterations of tissue pathological status means its biomechanics properties are changed. Feng [2] regarded viscoelasticity as the best indicator of soft tissue mechanics properties. As everyone knows, obtaining viscoelaticity parameters quantificationally depends on the appropriate rheological model of describing soft tissue. Generally, Voigt model is often used to describe rheological properties of normal soft tissue [3]-[9], which has one elasticity parameter and one viscocity parameter. However, due to pathological changes, does Voigt model explain appropriately rheological behavior of liver fibrosis at different stages? So far, there are no studies reported in liteature on rheological properties of liver fibrosis. This study performed rheological mechanical experiments to confirm appropriate rheological model for fibrosis stages F0 and F2 in rats.

II. Theory And Experiments

Rheology experiments describe the dynamic mechanical behavior of biological tissue. A sinusoidal shear strain $\varepsilon(t)=\varepsilon_0 e^{i\omega t}$ is imposed on the tissue, then a sinusoidal shear stress $\sigma(t)=\sigma_0 e^{i(\omega t+\delta)}$ is induced at the same frequency. The ratio of sinusoidal strain and sinusoidal stress is represented by the complex shear modulus $G^*(\omega)$

$$G*(\omega)=\frac{\sigma_0 e^{i(\omega t+\delta)}}{\varepsilon_0(t)e^{i\omega t}}=\frac{\sigma_0}{\varepsilon_0}e^{i\delta} \\ =\frac{\sigma_0}{\varepsilon_0}(\cos\delta+i\sin\delta)=G_1(\omega)+iG_2(\omega). \quad (1)$$

where ε_0 is shear strain amplitude, σ_0 is shear stress amplitude, ω_0 is angular frequency, δ is a phased-shifted angle. $G_1(\omega)$ is the storage modulus, $G_2(\omega)$ is the loss modulus, the magnitude of $G^*(\omega)$ is

$$|G(\omega)|=\sqrt{G_1^2(\omega)+G_2^2(\omega)}. \quad (2)$$

Voigt model and Zener model are used in this study, as shown in Fig. 1 (a) and Fig. 1 (b).Voigt model is a common rheological model of soft tissue, which consists of an elastic spring E_1 and a viscous damper η connected in parallel. Zener model consists of an elastic spring E_2 and a viscous damper η connected in series, then with spring E_1 in parallel. Their complex shear moduli are respectively expressed as

$$G_V^*(\omega)=E_1+i\omega\eta. \quad (3)$$

$$G_Z^*(\omega)=(E_1+\frac{\omega^2\eta^2E_2}{E_2^2+\omega^2\eta^2})+i\frac{\omega\eta E_2^2}{E_2^2+\omega^2\eta^2} \quad (4)$$

Thus the magnitude of the complex shear modulus can be expressed as

$$|G_V^*(\omega)|=\sqrt{E_1^2+\omega^2\eta^2}. \quad (5)$$

$$|G_Z^*(\omega)|=\sqrt{E_1^2+\frac{(2E_1+E_2)E_2\omega^2\eta^2}{E_2^2+\omega^2\eta^2}}. \quad (6)$$

In this experiments, 16 Male Sprague-Dawley rats (provided by Guangdong Medical Laboratory Animal Center, Foshan, Guangdong) weighing 180-270g were used. These rats were randomized into 2 groups with 6 members in the control group and 10 members in the model group inducing liver fibrosis. About 50% carbon tetrachloride (CCl_4) in olive oil was injected subcutaneously in 10 rats twice a week with 0.3 ml/100g weight. The concentration was doubled at the

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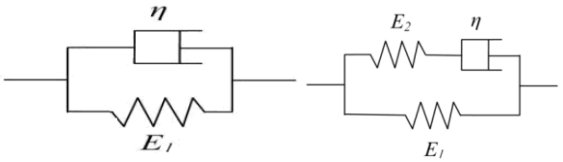
first time and adjusted according to the rat weight for the rest of time. After the 5th-8th weeks, rats in liver fibrosis stage F2 were obtained. Fibrosis grading for each rat was identified by pathological section and trichrome masson staining. The modeling of liver fibrosis stage F2 were successful in 8 members of 10 rats, the rest were for stage F1. All the procedures of studies were approved by Animal Care committee of Shenzhen University and Guangdong Medical Laboratory Animal Center.



(a) (b)

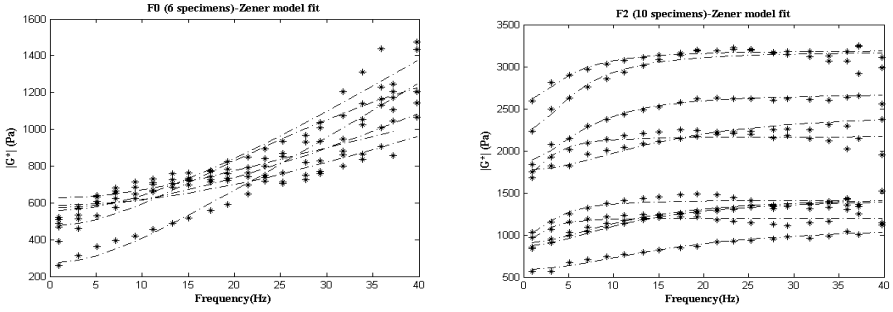
Fig. 1. (a) Voigt model. (b) Zener model.

To quantify the dynamic mechanical behavior of the rats' liver, rheometer tests were performed in sequence of fibrosis stages F0 and F2. The tests in small deformations (linear domain) were carried out at room temperature(23 ± 1℃) using a strain-controlled rheometer (AR1000, TA Instruments, New Castle, DE,USA) using its 25mm- diameter parallel plates configuration. The livers were harvested after euthanasia for the rats. Each rat liver was extracted to 1 or 2 specimens, thus total liver samples were 16 pieces, including 6 pieces for F0, 10 pieces for F2. The specimens, which were 4 ± 1mm thickness, were placed between the plates and the edges were carefully trimmed with a scalpel. First, the tissue linear behavior domain was determined by performing strain sweep oscillation tests at 1Hz and 40Hz respectively with the strain amplitude increasing from 0.01% to 2%. Then frequency sweep oscillations were carried out at the fixed strain 0.5% from 1Hz to 40Hz. Finally, the magnitude of complex moduli $|G^*(\omega)|$ values was obtained.

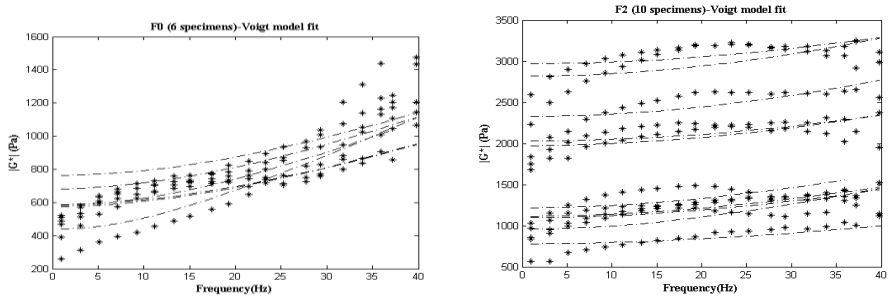
III. Results and Discussion

Fig. 2 (a) shows $|G^*(\omega)|$ values for rat livers in fibrosis stages F0 and F2, represented by symbol "*", meanwhile, the $|G^*(\omega)|$ values are fitted to Zener model using Levenberg-Marquardt method for nonlinear least squares fitting and are represented by the dashed line. The $|G^*(\omega)|$ data are also fitted to the conventional Voigt model by the dashed line, shown in Fig. 2 (b). Viscoelasticity parameters of the models and the coefficient of determination R^2 are given in table Ⅰ. R^2 is ratio of regression deviation and total deviation which is regarded as evaluation indicator for goodness of fit.

Considering vertical coordinate in Fig. 2 (a) and (b), $|G^*(\omega)|$ is ranged from 200Pa to 500Pa, which indicated liver rheological properties are changed with fibrosis stages. According to R^2 in Table I, it is obvious that Zener model provides better regression than Voigt model for stage F2, though the two models are equal regression for stage F0. Equation (6) is approximately equal to (5) due to $E_2 >> E_1$ in stage F0, thus the two models can both explain the rheological behavior of rats liver in stages F0.However, in stage F2, the order of magnitudes of E_2 is close to E_1, so (6) is not approximately equal to (5) any more, which depended on the relationship of E_1 and E_2. The change of ratio E_2 / E_1 reveals that liver elasticity changed from stages F0 to F2.Voigt model is not appropriate for describing rheological properties for stage F2. But better agreement between fit and experiments is achieved by using Zener model for stages F0 and F2. In conclusion, the experimental results demonstrate that Zener model can describe tissue rheological behavior of rats' liver in fibrosis stages F0 and F2 successfully.



(a)



(b)

Fig. 2. The experiments results of stages F0 and F2, fitted to (a)Zener model (b) Voigt model.

TABLE I: THE VISCOELASTICITY PARAMETERS ACCORDING TO THE MODELS

Stage	Specimen	Zener model				Voigt model		
		E_1 (Pa)	E_2 (Pa)	η (Pa s)	R^2	E_1 (Pa)	η (Pa s)	R^2
F0	1	272.245	7.89E+06	4.858	0.9453	272.244	4.859	0.9453
	2	626.201	982.169	2.532	0.9160	638.537	3.332	0.9160
	3	558.042	9.14E+06	5.019	0.9199	558.042	5.020	0.9199
	4	474.575	1378.9	4.495	0.9773	517.875	4.742	0.9633
	5	573.790	7.35E+06	3.673	0.8530	573.790	3.674	0.8530
	6	587.495	3.47E+05	3.029	0.9022	587.215	3.036	0.9022
F2	1	2591.6	602.399	18.319	0.9483	2984.5	5.386	0.3266
	2	590.952	524.008	4.228	0.9612	690.776	3.573	0.8503
	3	1692.4	477.381	24.523	0.9105	2114.3	0.898	0.1263
	4	1771.2	693.606	6.484	0.8599	1926.1	6.484	0.7566
	5	1872.4	813.114	16.740	0.9158	2294.2	6.607	0.4912
	6	2233	952.026	23.384	0.9294	2813.9	6.852	0.3154
	7	985.000	430.156	18.115	0.9552	1328.1	2.029	0.2786
	8	923.198	279.004	17.233	0.9277	1151.8	1.652	0.2701
	9	907.822	541.779	6.572	0.9526	1080.7	4.335	0.7497
	10	871.412	563.783	6.612	0.9243	1047.6	4.360	0.7139

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