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Short communication

Effect of ligation on the viscoelastic properties of liver tissues

Dong Liu [a,1], Guo-Yang Li [b,1], Chen Su [a], Yang Zheng [b], Yu-Xuan Jiang [b], Lin-Xue Qian [a,*], Yanping Cao [b,*]

[a] Department of Ultrasound, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, PR China
[b] Institute of Biomechanics and Medical Engineering, AML, Department of Engineering Mechanics, Tsinghua University, Beijing 100084, PR China

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ABSTRACT

It has been reported that *ex vivo* viscoelastic properties of liver tissues usually differ from those measured in *in vivo* state due to the reasons such as the effects of perfusion, temperature, and native pre-stress. Therefore, the development of an appropriate *ex vivo* protocol, which enables the measurement of liver mechanical properties close to those *in vivo*, is of great importance and has been pursued over the years. In this paper, we propose a simple protocol by ligating the liver when performing *ex vivo* indentation relaxation tests. Our results show that the viscoelastic kernel function, which measures the intrinsic time-dependent mechanical behavior of a viscoelastic material, determined with the present protocol can describe the *in vivo* viscoelasticity of liver tissues well in comparison with the *ex vivo* result measured on a liver without ligation and that obtained *in vitro*. The performance of the protocol reported here is similar to the *ex vivo* perfusion system developed by Kerdok et al. (2006). However, the present experimental set-up is much easier to realize.

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1. Introduction

The characterization of *in vivo* mechanical properties of liver tissues has found a wide variety of applications including surgical planning, computer-aid surgical training, tissue engineering and diagnosis of liver diseases (Basdogan et al., 2007; Chen et al., 2013; Griffith and Swartz, 2006; Marchesseau et al., 2010). In liver-related surgical planning and computer-aid surgical training, understanding the deformation behaviors of livers to external stimuli plays an essential role (Gao et al., 2010; Jordan et al., 2009; Marchesseau et al., 2010; Schwartz et al., 2005), which requires the determination of *in vivo* mechanical properties of liver tissues as accurate as possible. In tissue engineering, an engineered liver tissue should not only have good biocompatibility but also be mechanically compatible with surrounding soft tissues. It has been recognized that liver diseases can alter liver mechanical properties; therefore, characterizing the mechanical parameters of liver tissues *in vivo* may provide valuable information for the diagnosis and therapy of these diseases, e.g., staging liver fibrosis (Chen et al., 2013; Ferraioli et al., 2015; Nightingale et al., 2015).

Given the importance in determining the *in vivo* mechanical parameters of liver tissues, much effort has been made over the years. Not only linear elastic but also viscoelastic properties of liver tissues have been investigated in previous studies (Kerdok et al., 2006; Mattei et al., 2014a, 2014b; Mazza et al., 2007; Nava et al., 2008; Sakuma et al., 2003). However, the existing data regarding the viscoelastic properties of liver tissues exhibit a large scatter because the measurement results are sensitive to many factors. According to the comprehensive review of Mattei and Ahluwalia (2016), such scatters mainly stem from (1) the testing and analysis variables, e.g., testing methods and conditions and mechanical models used to interpret the data; and (2) the sample variables, including animal types, testing temperature, post-mortem time, the physical and pathophysiological conditions, and so on.

Among these factors, the perfusion is of great importance for determining the viscoelastic properties of livers, and therefore is a key factor responsible for the significant difference between *ex vivo* and *in vivo* results. Liver has rich vasculatures and is full of bloods (Selle et al., 2002). In most cases, *ex vivo* and *in vitro* experiments are much easier to perform in comparison with *in vivo* measurements. However, both *ex vivo* measurements and *in vitro* measurements on excised section of liver tissues may change the distribution of bloods and the viscoelasticity of the matrix from their *in vivo* states, and thereby alter the creep and relaxation behavior of liver tissues. In a previous study, Brown et al. (2003) used a self-developed motorized endoscopic grasper (MEG) to characterize the *in vivo* and *ex vivo* liver tissues and found the *in vivo* data is significantly different from the *ex vivo* data. Later on, Kerdok et al. (2006) developed an *ex vivo* perfusion system

* Corresponding authors.
E-mail addresses: qianlinxue2002@163.com (L.-X. Qian), caoyanping@tsinghua.edu.cn (Y. Cao).
[1] D. Liu and G.-Y. Li made equal contribution to this study.

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which can maintain the mechanical integrity of the whole liver. Interestingly, they reported that an unperfused liver is stiffer and more viscous than that in *in vivo* state, whereas an *ex vivo* perfused liver has very similar viscoelastic properties to those of an *in vivo* liver. Their study indicates that maintaining the perfusion state of an *ex vivo* liver can reasonably mimic *in vivo* livers. However, establishing a perfusion system to maintain the pressure of the hepatic artery and portal vein, and the blood circulation is by no means trivial in an *ex vivo* measurement.

Bearing this challenge in mind, in this paper we propose a simpler experimental protocol in which both the hepatic artery and portal vein of the *ex vivo* liver are ligated instead of establishing a perfusion system, and then an indentation relaxation test is conducted to measure the viscoelastic properties of liver tissues. Our results show that the intrinsic viscoelastic properties of *ex vivo*-ligated livers are similar to those of *in vivo* livers, indicating that the present protocol is a simple and more convenient way to evaluate the *in vivo* viscoelastic characteristics of liver tissues through *ex vivo* experiments.

2. Materials and methods

The study was approved by Beijing Friendship Hospital Capital Medical University Animal Care and Use Committee. A total of 12 heathy male Sprague-Dawley rats, weighted 200 ± 15 g, were used. The environment temperature and humidity during the experiments were approximately 25 °C and 50%, respectively.

2.1. Equipment

Indentation tests were conducted with the ElectroForce® 3100 (Bose) test instrument. The capacity and accuracy of the force sensor are 2 N and 0.001 N, respectively. Spherical indenter with tip radius of 3.5 mm was used (see Fig. 1b). The resolution of the indentation depth is approximately 1 μm.

2.2. In vivo measurements

After given intrapretoneal aneasthesia with 10% choral hydrate in dose of 0.3 ml/100 g by body weight, the rats were fixed on supine positions and the midline laparotomy was performed on each subject. After that the stomach, spleen and intestine dial were put left side to expose the liver, as shown in Fig. 1(a).

In the *in vivo* experiments, the indentation relaxation tests were conducted on the left lateral lobe (LLL) of the exposed liver, as shown in Fig. 1(b). According to Martins and Neuhaus (2007), the LLL is flattened and comprises approximately 30% of the liver weight. We also found it convenient to expose this lobe in the *in vivo* measurement.

2.3. Ex vivo measurements with ligation and without ligation

After *in vivo* experiments, the hepatic artery, portal vein, prehepatic and posthepatic posterior vena cava of the liver were ligated. Once the ligation process was finished, the liver was

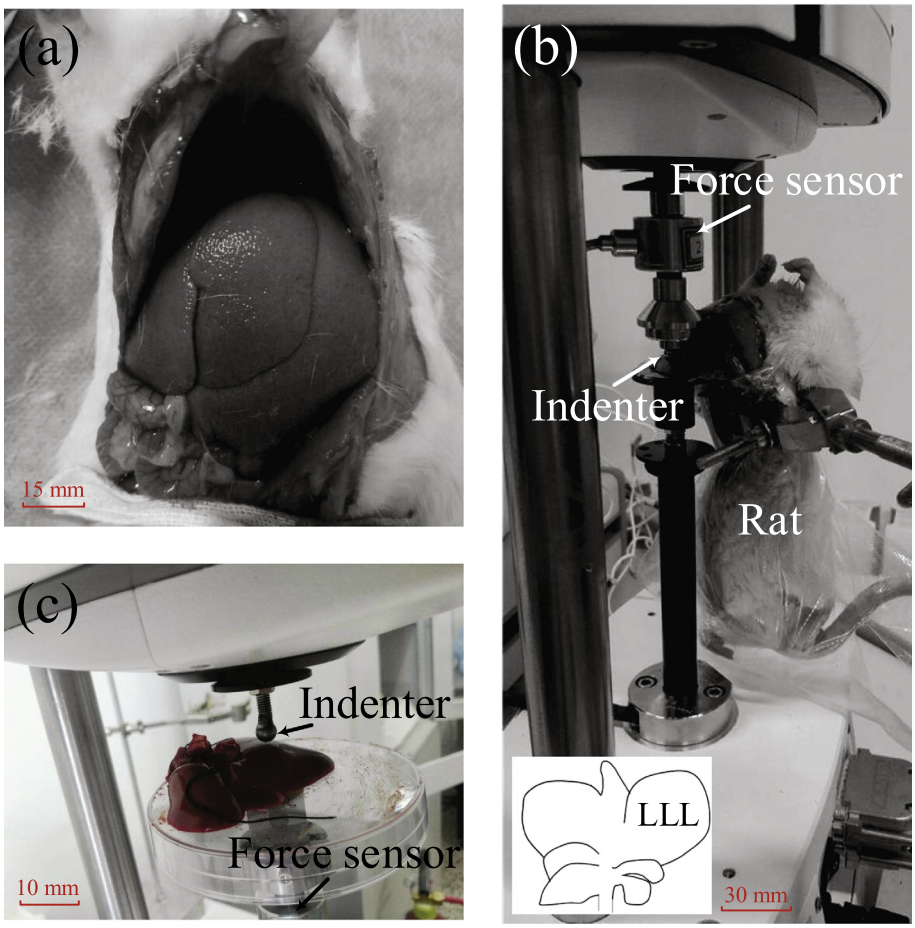

Fig. 1. (a) The exposed liver tissues and (b) *in vivo* indentation test. (c) The indentation test of a ligated liver. The lower left corner of (b) shows the schematic of the rat liver. Indentation tests were performed on the left lateral lobe (LLL) of the liver.

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