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Characterizing viscoelastic properties of breast cancer tissue in a mouse model using indentation

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ABSTRACT

Breast cancer is one of the leading cancer forms affecting females worldwide. Characterizing the mechanical properties of breast cancer tissue is important for diagnosis and uncovering the mechanobiology mechanism. Although most of the studies were based on human cancer tissue, an animal model is still describable for preclinical analysis. Using a custom-build indentation device, we measured the viscoelastic properties of breast cancer tissue from 4T1 and SKBR3 cell lines. A total of 7 samples were tested for each cancer tissue using a mouse model. We observed that a viscoelastic model with 2-term Prony series could best describe the ramp and stress relaxation of the tissue. For long-term responses, the SKBR3 tissues were stiffer in the strain levels of 4–10%, while no significant differences were found for the instantaneous elastic modulus. We also found tissues from both cell lines appeared to be strain-independent for the instantaneous elastic modulus and for the long-term elastic modulus in the strain level of 4–10%. In addition, by inspecting the cellular morphological structure of the two tissues, we found that SKBR3 tissues had a larger volume ratio of nuclei and a smaller volume ratio of extracellular matrix (ECM). Compared with prior cellular mechanics studies, our results indicated that ECM could contribute to the stiffening the tissue-level behavior. The viscoelastic characterization of the breast cancer tissue contributed to the scarce animal model data and provided support for the linear viscoelastic model used for in vivo elastography studies. Results also supplied helpful information for modeling of the breast cancer tissue in the tissue and cellular levels.

1. Introduction

Breast cancer is one of the leading cancers for females, with more appearing new cases than any other cancers (Jemal et al., 2011; Siegel et al., 2016). Understanding and uncovering the biological and physical mechanisms of breast cancer is of great importance for diagnosis and treatment (Ramião et al., 2016). It is known that mechanical properties of cancer tissues are closely related to the cancer pathology and development (Nia et al., 2016; Wirtz et al., 2011). Therefore, mechanical characterization of breast cancer tissues provides crucial information to uncover the mechanobiology mechanism of cancer (Jain et al., 2014; Stylianopoulos et al., 2012). In addition, quantifying the mechanical properties of the breast cancer tissue is also crucial for the construction of accurate physical models, which is needed for building finite element (FE) models (op den Buijs et al., 2011; Pathmanathan et al., 2008). Applications include biomechanical model based precise biopsy of the tumor (Moustris et al., 2011) and image registration (Hipwell et al., 2016). Recently, based on the mechanical properties of the breast cancer tissue, a palpation based automatic diagnosis system has also been proposed (Hosseini et al., 2010). A comprehensive review by Ramião et al. (2016) provides an excellent summary of the current development of biomechanical characterization of the breast tissue.

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Several mechanical testing methods have been used to characterize tumor tissues, such as biaxial test (Raghupathy and Barocas, 2010), rheometry test (Wex et al., 2014), and indentation test (Ahn et al., 2010). The biaxial test could provide homogeneous deformations that are ideal for characterizing soft tissues. However, special tissue fixing mechanism is needed that is challenging for small samples (Zhang et al., 2015). Similar problems also exist for rheometry test. However, indentation test has minimum requirements for tissue preparation and has been used to characterize many soft biological tissues such as brain (Budday et al., 2015; Feng et al., 2017b), kidney (Mattice et al., 2006), and meniscus (Han et al., 2017; Li et al., 2017). Therefore, indentation is especially suitable for testing relatively small tissues samples from small animal models. Moreover, the indentation technique is applicable to tissue tests at both macro- and micro- scales (Darling et al., 2006; Park et al., 2004; Toohey et al., 2016). Considering our experiment requirements and sample conditions, we choose indentation test for breast tissue characterization.

Although studies have investigated the mechanical properties of the breast cancer tissue, most of them focused on the elastic properties (Krouskop et al., 1998; Samani et al., 2003; Samani et al., 2007). Using indentation method and a hyperelastic model, Samani et al. (2003, 2007) characterized the elastic modulus of the breast cancer tissue. Using a novel tactile-guided detection device, Mojra et al. carried out viscoelastic characterizations of breast tissue in vivo (Madani and Mojra, 2017; Mojra et al., 2012; Mojra et al., 2011). It has been shown that the measured model coefficient was significantly different between the normal and tumor-included regions (Madani and Mojra, 2017). Nanoindentation of the breast cancer tissue showed the elastic modulus distribution of the tissue could be used to distinguish different cancer types (Plodinec et al., 2012). Magnetic resonance elastography (MRE) (Mariappan et al., 2009; Sinkus et al., 2005) and ultrasound (US) elastography (Chang et al., 2013; Coussot et al., 2009; Han et al., 2016; Ophir et al., 2000) were used to measure the elastic and viscoelastic properties of the breast cancer tissue. However, ex vivo measurements of the viscoelastic properties of the cancer tissue are still needed to guide and validate the tissue modeling. In addition, direct measurements of viscoelastic properties the breast cancer tissue could help biomechanical model based measurements and diagnosis in clinical applications (Griesenauer et al., 2017; Madani and Mojra, 2017).

For the indentation test of soft tissues, elasticity based formulation has been widely applied (Fischer-Cripps, 2000; Mattice et al., 2006; Oyen, 2005). By incorporating the classical elastic solutions with viscoelastic models, a variety of soft tissues were characterized (Budday et al., 2015; Darling et al., 2006; Ning et al., 2006; Qiang et al., 2011; Zhang et al., 2014). Specifically, Prony series based viscoelastic models are the most commonly used for characterizing the viscoelastic properties (Babaei et al., 2015a, 2015b; Wang et al., 2013). Therefore, we used a Prony series based viscoelastic model to characterize the breast cancer tissue.

In this study, we characterize the viscoelastic properties of the breast cancer tissue using a mouse model. To investigate the linear viscoelastic behavior of the tissue that was widely adopted for elastography studies, we compared the mechanical properties of the cancer tissues from two different cell lines at varied strain levels. The morphological structures of the tissue were also analyzed and compared. The results provide detailed viscoelastic characterizations of the breast cancer tissue and a validation for the viscoelastic characterization based on elastography, which could help tissue modeling and cancer diagnosis using biomechanical biomarkers.

2. Materials and methods

2.1. Sample preparation

Twelve healthy female nude mice aged 4 weeks were used in this study. All mice were raised in the Laboratory Animal Center of Soochow University (SPF grade, certificate No. SCXK 2002–0008). The mice were divided into 2 equal groups receiving 2 different tumor cell line injections, respectively. Two typically used breast cancer cell lines, 4T1 and SKBR3 (Shanghai Cell Bank of Chinese Academy of Sciences), were used for tumor implantation. The cells were re-suspended with phosphate-buffered saline (PBS) solutions at a concentration of 10^7 mL^{-1}. A cell suspension of 0.05 mL was injected subcutaneously into both sides of the rear legs of the mice. The mice were raised for 2–3 weeks until subcutaneous formations of palpable solid tumors with a diameter of 10–15 mm, at least on one of the rear legs (Fig. 1a). Then, the mice were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) for surgical removal of the solid tumors. Finally, the mice were euthanized by cervical dislocation after tumor resection. All of the animal procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Soochow University and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

A total of 7 solid tumor samples were harvested for each cell line (Fig. 1b). The sample thicknesses were 4.98 ± 0.55 mm and 3.60 ± 0.50 mm for the 4T1 and SKBR3 samples, respectively. Each sample was put in PBS solutions and transferred to the indentation tester immediately after the resection. The indentation tests were performed at room temperature (~24 °C).

2.2. Indentation test

A custom-built indentation device was used to measure the viscoelastic properties of the cancer tissue (Fig. 1c). We measured the indentation force and displacement using a load cell (GSO-10, Transducer Technique, USA) and a laser sensor (Model HG-C1050 30 mm, Panasonic, Japan). A linear actuator (43K4U-2.33-A07, Haydon Motion Solutions, Changzhou, China) was used to induce the indentation. The control of the actuator and the data acquisition were accomplished by a data acquisition board (PCI-1706U, Advantech Co., Ltd., China). Samples were placed on top of a translational adjustment unit. A custom-written LABVIEW (National Instruments, USA) program was used for indenter control and data acquisition.

We used a cylindrically shaped indenter with a diameter of 2 mm for the measurement (Fig. 1d). To investigate the linear viscoelastic behavior of the tissue that was adopted in elastography studies, especially MRE (Griesenauer et al., 2017; Sinkus et al., 2005), we indented each sample with five different strain levels that are 2%, 4%, 6%, 8%, and 10%. The indentation depth divided by the sample thickness defined the indentation strain (Feng et al., 2017a, 2017b, 2013). A ramp-hold testing protocol was implemented to characterize the viscoelastic properties (Elkin et al., 2011; MacManus et al., 2016; van Dommelen et al., 2010). In the ramp phase, a strain rate of ~0.1 s^{-1} was applied for each strain level. In the hold phase, the indenter was held for a relaxation time of 180 s. The indentation force-displacement data were acquired at a sampling rate of 1 kHz.

2.3. Viscoelastic parameter estimate

For isotropic elastic material, the analytical solution for the flat punch indentation is given by Fischer-Cripps (2000)

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