JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 54 (2016) 229–243

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm

Research Paper

Characterizing liver capsule microstructure via in situ bulge test coupled with multiphoton imaging

C. Jayyosi[a,b,c,*], M. Coret[d], K. Bruyère-Garnier[a,b,c]

[a]Université de Lyon, F-69622 Lyon, France
[b]Université Claude Bernard Lyon 1, Villeurbanne, France
[c]IFSTTAR, UMR-T9406, LBMC Laboratoire de Biomécanique et Mécanique des Chocs, F69675 Bron, France
[d]LUNAM Université, GEM, UMR CNRS 6183, Ecole Centrale de Nantes, Université de Nantes, France

ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form
23 September 2015
Accepted 24 September 2015
Available online 9 October 2015

Keywords:
In situ elliptic bulge test
Photobleaching
Strain measurement
Liver capsule
Multiphoton microscopy
Fibrous connective membrane

ABSTRACT

The characterization of biological tissue at the microscopic scale is the starting point of many applications in tissue engineering and especially in the development of structurally based constitutive models. In the present study, focus is made on the liver capsule, the membrane encompassing hepatic parenchyma, which takes a huge part in liver mechanical properties. An in situ bulge test experiment under a multiphoton microscope has been developed to assess the microstructure changes that arise with biaxial loading. Multiphoton microscopy allows to observe the elastin and collagen fiber networks simultaneously. Thus a description of the microstructure organization of the capsule is given, characterizing the shapes, geometry and arrangement of fibers. The orientation of fibers is calculated and orientation distribution evolution with loading is given, in the case of an equibiaxial and two non equibiaxial loadings, thanks to a circular and elliptic set up of the bulge test. The local strain fields have also been computed, by the mean of a photobleaching grid, to get an idea of what the liver capsule might experience when subjected to internal pressure. Results show that strain fields present some heterogeneity due to anisotropy. Reorientation occurs in non equibiaxial loadings and involves fibers layers from the inner to the outer surface as expected. Although there is a fiber network rearrangement to accommodate with loading in the case of equibiaxial loading, there is no significant reorientation of the main fibers direction of the different layers.

1. Introduction

The characterization of soft biological tissues is of many interests since it has a lot of applications, one of them being the development of constitutive models. Structural constitutive models are of crucial importance because they can help to identify the functional parameters that are essential to tissue functions, making them valuable for tissue engineering

*Corresponding author at: IFSTTAR, UMR-T9406, LBMC Laboratoire de Biomécanique et Mécanique des Chocs, F69675 Bron, France. Tel.: +33 478656861.
E-mail address: charles.jayyosi@ifsttar.fr (C. Jayyosi).

http://dx.doi.org/10.1016/j.jmbbm.2015.09.031
1751-6161/

applications (Sacks, 2003). In order to build realistic and functional models the study of soft tissue microstructure has been an active research area. In particular, many studies aim to understand the extension and reorganization mechanisms of collagenous structures on different tissues such as tendons (Sasaki and Odajima, 1996; Screen et al., 2004), skin (Brown, 1973), connective tissue (Liao et al., 2005; Purslow et al., 1998), or arteries (Keyes et al., 2013; Rezakhaniha et al., 2012; Wang et al., 2013). Indeed, the changes in the microstructure at the fiber level can impact greatly the mechanical properties of the tissue and thus its functional purpose, affecting cell environment and behavior, leading to diseases or disorders (Engler et al., 2006; Koch et al., 2014; Pizzo et al., 2005; Robertson et al., 2012).

The identification of the relationship between microstructure and mechanical behavior needs coupling mechanical tests with microstructural observation which turns out to be challenging. Uniaxial tensile tests are often carried out and several studies present their implementation and association with a microscopic imagery technique. Therefore, uniaxial tensile tests have been used to investigate the strain and reorganization of collagen fibers in tendons (Goulam Houssen et al., 2011; Lake et al., 2010; Screen et al., 2004), but also the behavior of fetal membrane microstructure (Mauri et al., 2013, 2015) and liver capsule (Jayyosi et al., 2014). However, uniaxial tension loading might be quite far from what the tissues can experience in vivo. Röhrnbauer et al. (2013) highlighted the fact that biaxial and uniaxial loading leads to very different behaviors of the tissue and thus it is recommended to test the biological materials with a type of loading that this given tissue could be subjected to in vivo.

Biaxial testing applies a stress state that is closer from in vivo loading for some tissues, and thus experiments coupling biaxial testing and microstructure observation have been designed. Liao et al. (Liao et al., 2005) for example, applied a biaxial stretch to native bovine pericardium samples and investigated collagen fiber/molecular kinematics using a small angle light scattering and small angle X-ray scattering systems. They also highlighted the difference between the behavior of the tissue when subjected to uniaxial and biaxial loading. Keyes et al. (2011) developed a micro biaxial optomechanical device allowing to pressurized tubular samples while imaging their microstructure thanks to a multiphoton microscope. They were able to record the microstructural organization of mouse aorta under different pressured states and measured fiber orientations and composition of the arterial wall.

At the macroscopic scale, bulge test turns out to be a good solution to test biological materials since it applies a biaxial stress state, if radial stress is negligible before in-plane stress. It is usually used to test thin metallic or polymer membranes (Çakmak et al., 2014; Rivlin and Saunders, 1951; Tsakalakos and Jankowski, 1986), calculating stress by assuming a spherical deformation cap. The same process has been transposed to test many biological tissues such as skin (Tonge et al., 2013), fetal membrane (Joyce, 2009; Perrini et al., 2013), pericardium (Hildebrandt et al., 1969), sclera (Myers et al., 2010), arteries (Drexler et al., 2007), but also liver capsule (Brunon et al., 2011). For capsules encompassing pressurized organs such as liver or kidney, bulge test is particularly fitted to determine mechanical properties. Indeed, in case of impact or compression on such organs, an increased pressure arises (Sparks, 2007), submitting the surrounding membrane to a stress state very close from what bulge testing induces.

However, among the studies implementing bulge test on biological tissues, few take into account considerations about the impact of this mechanical test on the microstructure. Tonge et al. (2013) used the bulge test to assess the anisotropy in human skin. The minor axis of the ellipsoid, formed by inflation of a circular sample, is associated to the stiffest direction of the sample, which corresponds to a preferred collagen fiber orientation. Therefore they managed to get insight into the material micro organization without imaging directly the microstructure during loading.

The use of several imaging technique have been reported in order to assess tissues microstructure. Multiphoton microscopy is one of the most efficient ones for tissue composed of elastin and collagen fibers because it combines fluorescence and Second Harmonic Generation (SHG). Therefore, it allows taking advantage of the autofluorescent properties of elastin and the organization of collagen molecules that generates second harmonics (Campagnola et al., 2002; Goulam Houssen et al., 2011; Zoumi et al., 2002) to image these two different fiber networks with a single two-photon excitation microscope, while making the distinction between them (Zoumi et al., 2004, 2002). Consequently, multiphoton microscopy is very convenient compared to classic histology techniques because the sample does not need any preparation or fixation protocol before imaging, thus preventing additional tissue alteration.

The liver capsule known as Glisson's capsule is a fibrous connective membrane mainly composed of collagen and elastin fibers. It encompasses the hepatic parenchyma, to which it adheres more or less depending on the location, while remaining clearly dissociable from it for human livers. From a structural point of view, capsule makes the whole organ stiffer considering its different mechanical properties from parenchyma (Brunon et al., 2010). Since it contributes greatly to the global mechanical behavior of human liver, and it is involved in extern livers injuries, liver capsule mechanical behavior has been studied from a macroscopic point of view (Brunon et al., 2011, 2010; Dan, 1999; Hollenstein and Mazza, 2012; Valtorta and Mazza, 2005).

In a previous paper (Jayyosi et al., 2014), we already used multiphoton microscopy to assess local strain fields in liver capsule subjected to uniaxial tensile test. We took advantage of a side effect of microscopy fluorescence known as photobleaching to position markers on the fibrous structure. Following these markers during loading, we were able to measure displacement fields and the local strain field on the tissue via a finite element method interpolation. To assess local strain, other experiments have used the track of markers that are usually stained cell nuclei (Bruehlmann et al., 2004; Mauri et al., 2015; Screen and Evans, 2009).

Therefore, the aim of this study was to use the method developed in Jayyosi et al. (2014) to characterize the liver capsule microstructure, assessing the local strain field and fiber orientation, when it is subjected to a bulge test. To this end, a protocol of in situ bulge testing coupled with multiphoton microscopy imaging was developed. Consequently,