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Biomechanical properties and microstructure of human ventricular myocardium

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ABSTRACT

In the multidisciplinary field of heart research it is of utmost importance to identify accurate myocardium material properties for the description of phenomena such as mechano-electric feedback or heart wall thickening. A rationally-based material model is required to understand the highly nonlinear mechanics of complex structures such as the passive myocardium under different loading conditions. Unfortunately, to date there are no experimental data of human heart tissues available to estimate material parameters and to develop adequate material models. This study aimed to determine biaxial extension and triaxial shear properties and the underlying microstructure of the passive human ventricular myocardium. Using new state-of-the-art equipment, planar biaxial extension tests were performed to determine the biaxial extension properties of the passive ventricular human myocardium. Shear properties of the myocardium were examined by triaxial simple shear tests performed on small cubic specimens excised from an adjacent region of the biaxial extension specimens. The three-dimensional microstructure was investigated through second-harmonic generation (SHG) microscopy on optically cleared tissues, which emphasized the 3D orientation and dispersion of the myofibers and adjacent collagen fabrics. The results suggest that the passive human LV myocardium under quasi-static and dynamic multiaxial loadings is a nonlinear, anisotropic (orthotropic), viscoelastic and history-dependent soft biological material undergoing large deformations. Material properties of the tissue components along local microstructural axes drive the nonlinear and orthotropic features of the myocardium. SHG microscopy investigation revealed detailed information about the myocardial microstructure due to its high resolution. It enabled the identification of structural parameters such as the fiber and the sheet orientations and corresponding dispersions. With this complete set of material data, a sophisticated material model and associated material parameters can be defined for a better description of the biomechanical response of the ventricular myocardium in humans. Such a model will lead to more accurate computational simulations to better understand the fundamental underlying ventricular mechanics, a step needed in the improvement of medical treatment of heart diseases.

Statement of Significance

Unfortunately, to date there are no experimental data of human heart tissues available for material parameter estimation and the development of adequate material models. In this manuscript novel biaxial tensile and shear test data at different specimen orientations are presented, which allowed to adequately capture the direction-dependent material response. With these complete sets of mechanical data, combined with their underlying microstructural data (also presented herein), sophisticated material models and associated material parameters can be defined for the description of the mechanical behavior of the ventricular myocardium in humans. Such models will lead to accurate computational simulations to

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1. Introduction

Heart related diseases are the leading cause of mortality in the world [1], and it is estimated that Europe alone spends about €196 billion per year on heart related medical treatments [2]. To better understand heart related diseases such as ventricular fibrillation, there is an acute need for more fundamental research to be carried out not only on cardiac electrophysiology but also on cardiac mechanics. The propagation of an electrical potential in the heart generates an active contraction determined by the underlying mechanical structure. On the other hand, changes in the lengths of myocytes influence the membrane potential and the duration of the action potential, a phenomenon known as mechano-electric feedback. Mechanics and electrophysiology are thus interlinked, and greater knowledge and insight into the behavior of the passive myocardium is of utmost importance to accurately capture the mechanical behavior of the heart and to better understand the mechanisms of the heart and its diseases as well as the development and the improvement of medical health care. For example, diastolic mechanical properties of cardiac muscles are important determinants of cardiac function with distinct passive myocardial stiffness contributing to diastolic heart failure [3]. Such ventricular diastolic dysfunction in patients with heart failure is associated with significant morbidity and mortality [4]. Hence, the passive stiffness of the myocardium is a major determinant of the overall cardiac function [5,6]. It is further suggested that shearing of adjacent muscle layers in the left ventricular wall contribute substantially to wall thickening during systole [7], and wall thinning during diastolic filling [8].

Modeling the cardiac function by using the finite element method has turned out to be a powerful task to increase the understanding of the physiological response of the heart and to determine how the structural components of the heart influence its behavior [9–14]. However, the development of realistic finite element models of the mechanical behavior of both healthy and diseased myocardial tissues is highly dependent on the formulation of appropriate constitutive laws and the accurate identification of their material parameters [15]. In particular, a better understanding of the fundamental underlying ventricular mechanics relies on: (i) realistic description of the three-dimensional geometry and microstructure of the myocardium including boundary conditions; (ii) constitutive equations that characterize the material properties of the myocardium [16].

Moreover, tissue engineered cardiac grafts are a promising therapeutic technique to repair injured hearts and to restore cardiac function after myocardial infarction [17]. In previous applications, tissue engineered patches were implanted over a region of infarcted cardiac tissue in order to stimulate angiogenesis and attenuate a reduction in cardiac function [18,19,17]. Since stem cells differentiate into different lineages depending on the mechanical properties of the matrix material [20], an appropriate biomechanical microenvironment for stem cells might lead to right differentiated cells [21]. Matching of the mechanical properties would enable tissue constructs to respond synchronically with heart contraction and relaxation and, therefore, allows efficient mechanical signal transfer from the native myocardial environment to the stem cells [22,21]. Hence, it is of importance to have an engineered cardiac patch that matches the mechanical properties of the native myocardium [22,17].

In the literature, the passive biaxial extension properties of the myocardium were documented [23–28], which mainly come from equibiaxial extension tests on hearts from mongrel dogs; only Yin et al. [24] performed 'true' biaxial extension tests with different ratios (as distinct from equibiaxial tests) on mongrel dogs, a study performed more than 25 years ago. The biaxial data of dog hearts indicate highly nonlinear and anisotropic properties. For myocardial tissues, these tests do not yield sufficient quantitative information to formulate a reliable constitutive law. In fact such data may even suggest that the material is transversely isotropic [29]. Myocardial tissue, however, is not transversely isotropic, which has been clearly demonstrated by the results of simple shear tests in different directions on the passive ventricular myocardium from porcine hearts [30]. These tests highlight the orthotropic behavior of myocardial tissue. Moreover, myocardial tissues undergo complex patterns of tensile, compressive, and, in particular, shear deformations throughout the cardiac cycle. Hence, data from biaxial tests alone are not enough to characterize the passive response of an orthotropic material such as the myocardium [16]. Prerequisites to capture the direction-dependent nonlinear material response involve a combination of biaxial extension test data with different loading protocols, and shear test data at different specimen orientations [16]. To the authors knowledge, no studies have been documented which conduct biaxial extension, triaxial shear, or a combination of these tests on human myocardial tissues.

From a microstructural viewpoint, it is known that muscle fibers are not perfectly aligned in the myocardial tissue [31]. Furthermore, the angular dispersion of the fibers is changing and is associated with several cardiac diseases, including dilated cardiomyopathy and familial hypertrophic cardiomyopathy [32,31]. There is only one study available where a mechanical model accounts for the dispersion of fibers and sheets in the myocardial tissue [14], but corresponding data for human myocardial tissue are missing. Multi-photon microscopy is well suited for the three-dimensional visualization of soft tissues due to its sequential automated slicing ability yielding *z*-stacks [33]. Nonlinear imaging techniques like second-harmonic generation (SHG) further allow for labeling free imaging of certain proteins including collagen and collagen-enclosed structures such as myocardial fibers. When utilized in combination with optical tissue clearing, greatly enhanced penetration depths can be achieved, allowing for an automated determination of orientation and dispersion parameters throughout thicker (>1 mm) tissue samples [34,35].

The aim of the present study is to characterize the mechanical properties of the passive human myocardium through biaxial extension and triaxial shear testing, and to determine the three-dimensional microstructure of the tested myocardium through a combination of optical clearing, SHG microscopy and subsequent 3D image analysis. The data stated in this study are intended to be used for the determination of new constitutive descriptors and its related parameters for more accurate computational studies of the fundamental mechanisms of human heart mechanics. Furthermore, the data stated can be used as a first standard mechanical and microstructural data set of the human ventricular myocardium, e.g., for the design and development of an optimal engineered cardiac construct for cardiac tissue repair.