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Biaxial quantification of passive porcine myocardium elastic properties by region

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A B S T R A C T

Considering accurate constitutive models is of the utmost importance when capturing the mechanical response of soft tissue and biomedical materials under physiological loading conditions. This paper investigated the behaviour of porcine myocardium in passive rested hearts. This was done by applying biaxial loads on the myocardium. The main objective of this research was to investigate the cardiac mechanics of various regions of a healthy passive rested porcine heart. The biaxial mechanical properties of myocardial tissue samples were captured using a biaxial testing system. The porcine heart was divided into three regions, namely, left ventricle (LV), septum and right ventricle (RV). In these regions, 18 × 18 mm2 equal samples were cut from six porcine passive hearts. For the LV sample, the biaxial elastic modulus in the fibre direction was 33.3% larger than in the cross fibre direction, for the mid-wall sample it was 18.8% larger, and for the RV sample it was 33.3% larger. It was concluded that the cardiac mechanics of LV, septum and RV exhibit mechanical behaviour that differs considerably. In developing adequate computational models, these data could be applied to estimate the material parameters of the myocardium.

1. Introduction

The relationship between the biomechanical behaviour of different regions of passive porcine hearts is still not fully understood. In order to develop clinically acceptable computational models, there is a need to understand fully the mechanical behaviour of passive myocardium in various regions of the heart. As the mechanical properties of passive myocardium are largely assumed to be constant throughout the regions, the study of cardiac mechanics in these regions remains valid and relevant for ensuring that accurate computational models are generated. Accurate computational models with full understanding of mechanical properties may assist in the prediction of tissue replacement suitability. It may sometimes be vital to study the mechanics of the right ventricle (RV) to understand the underlying mechanisms of heart disease (Buckberg and Nanda, 2015).

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The majority of research devoted to simulating the behaviour of the heart has preferred to concentrate on the left ventricle (LV) (Wise et al., 2016, Sirry et al., 2016). In some cases, RV mechanics have been simulated using the LV material properties (Masithulela, 2016a, Masithulela, 2015). In reality, however, LV material properties may differ significantly from those of the RV. The LV is divided into two main regions, namely, the free wall and the septal wall. It is argued that even though these regions are situated in the same LV, they could exhibit different mechanical properties. This is evident because most computational studies have assumed the same material parameters (free wall and septum – those of the LV) to study the mechanics of the whole heart. Although this approach has provided some acceptable computational results, there is a need to consider the mechanical behaviour of each region in order to improve the accuracy of computational models of the heart. The experimental mechanics of various regions of the heart, such as the septum and the RV, have clearly not been adequately studied and remain poorly understood. Generally, materials constants obtained from biaxial testing of the LV are applied through heart models. This is done with the assumption that, passively, the materials constants of the other regions including the RV and the septum are similar to those of the LV. Therefore, this study aimed to develop and generate experimental results for the LV, septum and RV in the passive porcine heart. This was done by conducting biaxial testing of porcine hearts in a controlled environment. The cardiac mechanics of the LV, septum and RV were then compared to each other in order to have a full understanding of the porcine heart. These data could also be used in generating material parameters that can be applied in various computational heart models in the future. The finite element method is a powerful tool for modelling cardiac function (Masithulela, 2016a, Masithulela, 2015). In this study, this was done in order to increase and determine the physiological response of the heart by understanding how the structural components of the heart may influence its behaviour. The formulation of accurate and appropriate constitutive laws is vital in identifying the material parameters.

The function of the heart is similar to that of a pump where pathological conditions are analogous to mechanical issues that alter its efficiency. Myocardium exhibits large deformations under comparatively small forces and then undergoes proportionally smaller deformations under larger forces. This mechanical response to loading, typical of soft biological tissue in general, is dependent on the direction of the load. Planar biaxial testing has been used for decades, resulting from the understanding, grounded in continuum mechanics, that uniaxial testing is insufficient when one's goal is to characterise anisotropic materials. Planar biaxial testing makes it possible to explore a wide range of loads, understood here as forces or displacements, applied at once in two orthogonal directions. The loads applied in both directions may the same (equibiaxial loading), may be kept in constant proportion between both directions (proportional loading), or may be independent from each other (general biaxial loading). With the advent of commercially available, integrated biaxial testing equipment specially designed for biological soft tissues, such as the Biotester (CellScale, Waterloo, Canada) used in this study, it is timely to revisit the practice of planar biaxial testing. First, we will go over the details of the materials experimental behaviour of different regions in the heathy porcine heart and then will describe and discuss our findings in the contexts of the different mechanics of the LV, septum and RV. Heart research is highly multidisciplinary in its approach. It is thus vital to identify precisely the material properties of the myocardium of resting hearts. To fully understand the extremely nonlinear mechanics of complex structures such as the passive myocardium under different loading conditions, a rationally based material model must be developed. Thus, utilising new cutting-edge equipment, planar biaxial tension tests were performed to determine the biaxial expansion properties of the inactive porcine myocardium.

Heart-related infections are the main source of mortality across the globe, and it is estimated that Europe alone spends about €196 billion every year on heart-related medicinal treatments (Løgstrup and O'Kelly, 2012). To better comprehend heart-related diseases, for example ventricular fibrillation, there is an intense requirement for more basic research to be completed on cardiovascular electrophysiology as well as on cardiac mechanics. The mechanics of the passive myocardium are important for accurately capturing the mechanical behaviour of the heart and for better understanding the mechanisms of the heart and heart diseases, as well as the development and improvement of medical health care. The diastolic mechanical properties of cardiac muscles are important determinants of cardiac function with distinct passive myocardial stiffness contributing to diastolic heart failure. Such ventricular diastolic dysfunction in patients with heart failure is associated with significant morbidity and mortality. Hence, the passive stiffness of the myocardium is a major determinant of the overall cardiac function. The point of the present paper is to describe the mechanical properties of the inactive porcine myocardium in various areas including the LV, septum and RV through biaxial testing. It is planned that the information provided by this study will be used to determine new constitutive descriptors and their related parameters for more precise computational modelling research of the major mechanisms of heart mechanics. Moreover, the data presented in this study may be used as a first standard mechanical data collection of the porcine ventricular myocardium, which could possibly be extrapolated for use in the development and construction of cardiac tissue.

Material and methods

2.1 Tissue preparations

Eight fresh porcine hearts were obtained from the local abattoir within a few hours of slaughter and stored in saline solution at 4 °C. The porcine tissues were harvested from adult pigs weighing about 104 kg. All the samples were dipped into 0.6% glutaradehyde for 20 minutes before testing. Initially, the heart was divided into LV, septum and RV. The ventricles were then cut into 18 × 18 mm2 squares in order to be tested in the biaxial tester.

2.2 Equipment and experiment protocol

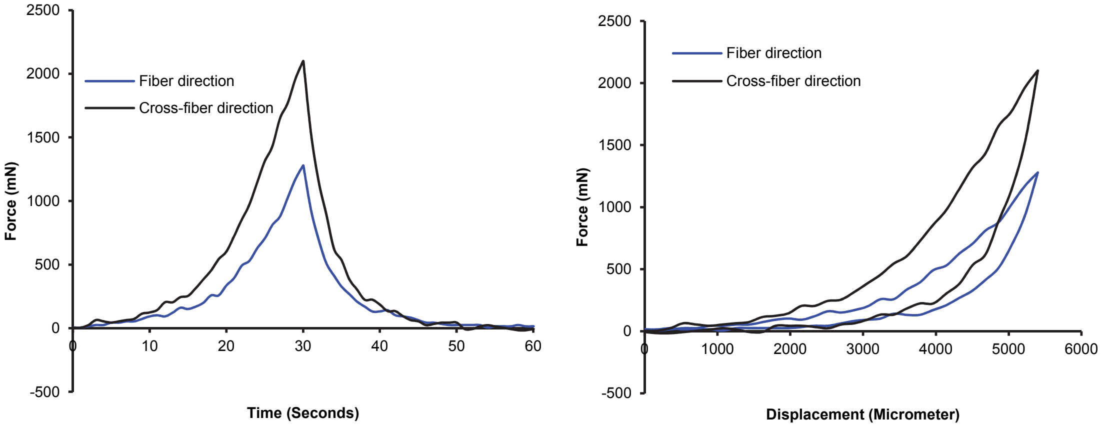
The thickness of each sample was evaluated by taking three measurements at different locations using an electronic thickness gauge. The samples were mounted on the 23N capacity biaxial testing equipment and then immersed in a saline solution in a bath with a temperature control. The temperature of the saline solution was set at 37 °C for 15 minutes before testing. During testing, the samples were taken out of the solution to avoid reflections impairing the image tracking. The Biotester comes with an integrated software interface, LabJoy, whose data collection module allows the user to set the parameters for the test phases (preloading, stretching, holding, recovering and resting) of load cycles gathered into a test sequence, as well as the saline bath temperature and image acquisition frequency (here, 5 Hz) (see Figure 1). Biaxial testing procedures have previously been described in detail (Abbasi & Azadani, 2015).


(a) (b)

Fig. 1. Biaxial testing equipment used for testing resting porcine myocardium

2.3 Biaxial tensile testing

The biaxial mechanical properties of myocardial tissue samples were captured using a biaxial testing system (Grashow et al., 2006). The length, width and thickness of samples were measured using a Vernier calliper and recorded prior to testing. Force and displacement data were collected at a rate of 10 Hz in both fibre and cross-fibre directions. The force–time relationship curve was plotted to demonstrate their relationship (see Fig. 2 (a)). The thickness of the tissue and the original length of the sample were used to calculate the cross-sectional area. For a single sample, three thickness measurements were made and the average was taken. All biaxial tests were performed with tissue samples completely submerged in 37 1C PBS. Biaxial tensile load was applied along the cardiac circumferential and longitudinal directions of the sample at 0.5 mm/s. To quantify the elastic modulus and stiffness in the two material axes, fibre and cross-fibre were calculated from the steep region of the equibiaxial stress–strain curve of each sample. Force displacement relationships were also plotted for each direction (fibre and cross-fibre) (see Fig. 2 (b)). The sample was prepared by carefully cutting the LV, RV and septal wall from the heart into $20 \times 20\ \text{mm}^2$ pieces. The fibre orientations of the myocardium were carefully inspected to ensure that the fibre direction was properly aligned to the axis of the equipment (see Fig. 3).



(a) (b)

Fig. 2. (a) Force-time curve of the sample LV; (b) Typical sample LV force-displacement curve and direction of loading

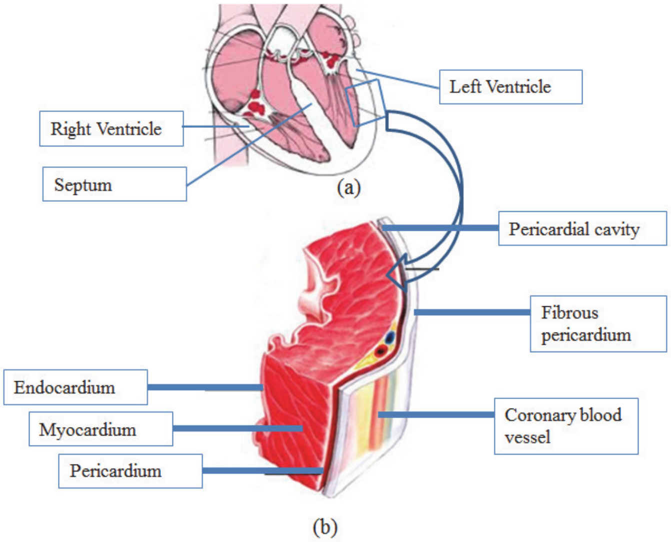


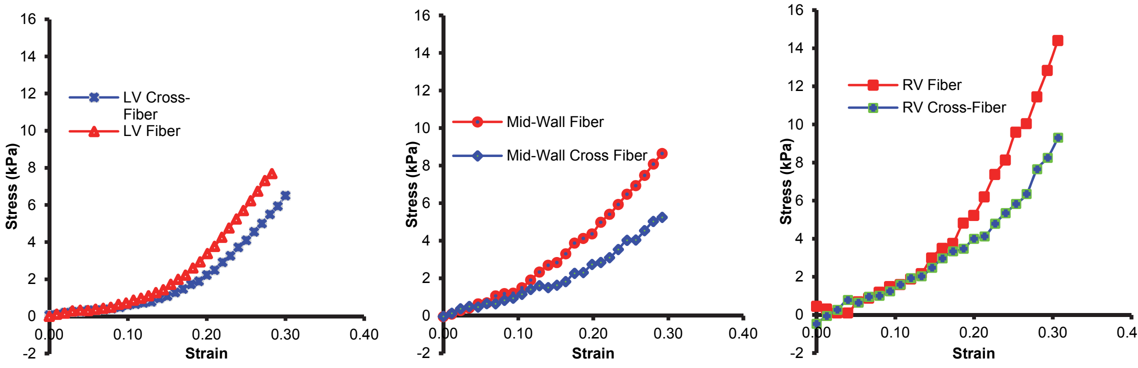
Fig. 3. (a) Cross-sectional porcine heart, (b) Isolated heart LV myocardium to be tested. The same dimension of the RV and septum have been cut and tested by biaxial Biotester machine

2.4 Image and data processing for stress-strain plots

No significant change was observed in the pre/post measurements of specimen cross-sectional thickness. It is well known that soft tissue or biomaterial samples may be subjected to less strain than calculated from the grip displacements due to attachment site effects and potential tissue tearing. This motivated the use of the image-tracking module in LabJoy to determine the actual strain distribution within the sample. Given the large number of protocols (hence, images) to be processed, only the loading portion of the cycles was of interest (because the loading and unloading paths were relatively close to each other, as can be appreciated in Fig. 2)

3. Results

The mean thickness of the LV, mid-wall and RV samples was 10.4 mm, 4.2 mm and 2.2 mm, respectively (see Table 2). After taking measurements of all the samples, it was observed that all regions had similar thickness and were measurable. Therefore, the method used in this experiment can be repeated elsewhere. The average stress–strain curve of the LV, mi-wall and RV in both the fibre and cross-fibre direction is shown in Figure 4. Figure 5 shows the average stress–strain curve with selected regions where moduli were determined.



(a) (b) (c)

Fig. 4. An average experimental stress-strain curves in (a) LV, (b) Septum and (c) RV walls in the fibre and cross-fibre directions.

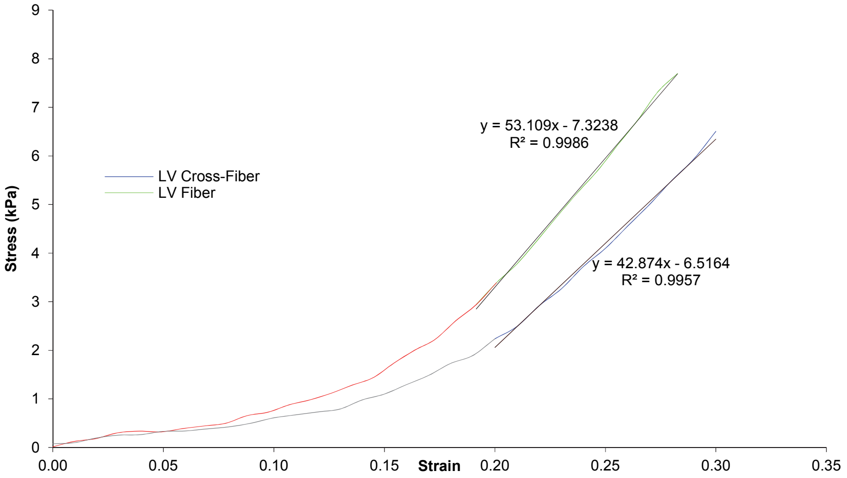


Fig. 5. Representative average stress-strain plot for the left ventricle (LV) detailing linear region with applied linear regression, from 0.24 to 0.3 strain

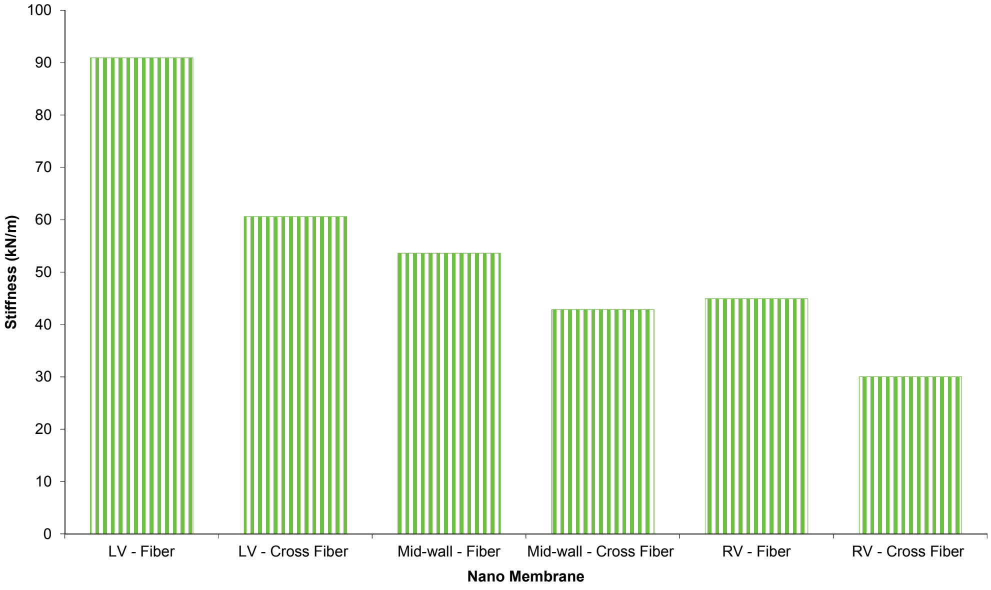


Fig. 6. Average stiffness results separated by considered regions (LV, mid-wall and RV)

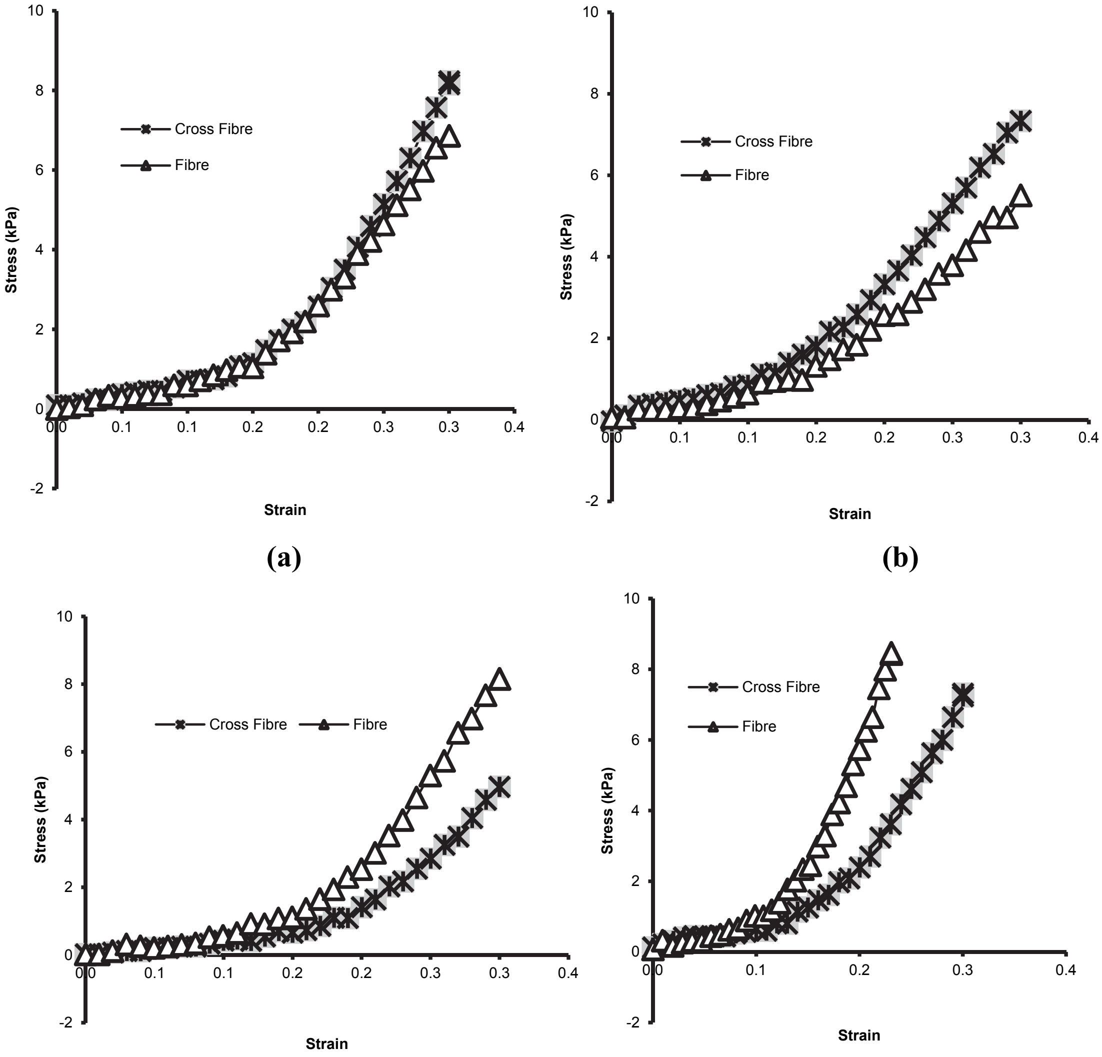
The highest elastic modulus of 90 kPa was observed in the LV region in the fibre direction. The elastic modulus in the RV region of the fibre direction was significantly smaller than both the LV and RV regions. For the LV sample, the biaxial elastic modulus in the fibre direction was 33.3% larger than in the cross-fibre direction, for the mid-wall sample it was 18.8% larger and for the RV sample it was 33.3% larger (see Figure 6). When comparing the LV and the mid-wall samples in terms of the fibre direction, the LV elastic modulus was 50% higher than the mid-wall and 40% higher than the RV in the fibre direction. The individual stress–strain for each sample on the LV, septum and RV is plotted in Figures 7, 8 and 9, respectively. The stress of the LV sample is lower than that of the RV and the septum. The highest stress was found in the RV sample (see Figure 9). Table 2 lists the thicknesses of all the materials tested and the number of samples retained for determining the material constants. Indeed, as a result of either tearing near the tines or image-tracking issues, some samples had to be discarded.

Table 1. Thickness of various samples (LV, Septum and RV) tested

Material	LV Sample1	LV Sample2	LV Sample3	LV Sample4
N	4	4	4	4
Thickness	10.6	10.3	10.2	10.6
Strain in fibre direction @ 5.2 kPa	0.259889	0.289944	0.249833	0.19344
Strain in cross-fibre direction @ 5.2 kPa	0.249833	0.240167	0.3	0.206279
Material	Septum Wall1	Septum Wall2	Septum Wall3	Septum Wall4
N	6	6	6	6
Thickness	4.2	4.3	4.4	4.2
Strain in fibre direction @5.2 kPa	0.229833	0.209778	0.386556	0.253
Strain in cross-fibre direction @ 5.2 kPa	0.27022	0.29022	0.186667	0.2
Material	RV wall1	RV wall2	RV wall3	RV wall4
N	5	5	5	5
Thickness	2.2	2.25	2.3	2.4
Strain in fibre direction @5.2 kPa	0.239667	0.226277	0.1466111	0.253
Strain in cross-fibre direction @ 5.2 kPa	0.25344	0.2	0.173278	0.280111

Table 2. Strain range of considered linear region for all considered heart regions (LV, mid-wall and RV)

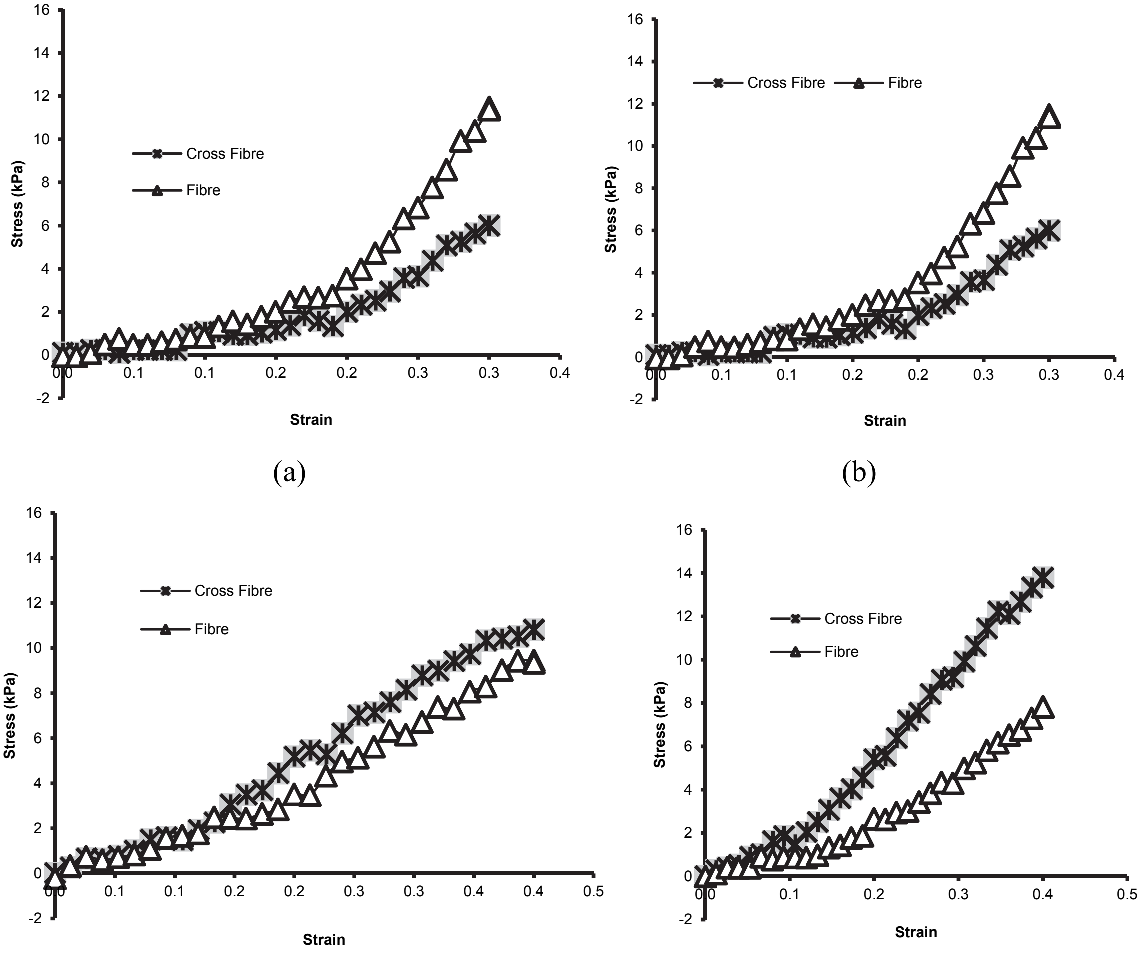
Region	Direction	Regression	Strain range
Left Ventricle (LV)	Fibre Direction	0.9829	0.24-0.3
	Cross Fibre direction	0.9787	0.24-0.3
Septum (Mid-wall)	Fibre Direction	0.9988	0.21-0.29
	Cross Fibre direction	0.9957	0.21-0.29
Right ventricle (RV)	Fibre Direction	0.9983	0.19-0.28
	Cross Fibre direction	0.9857	0.19-0.28



(a) **(b)**

(c) (d)

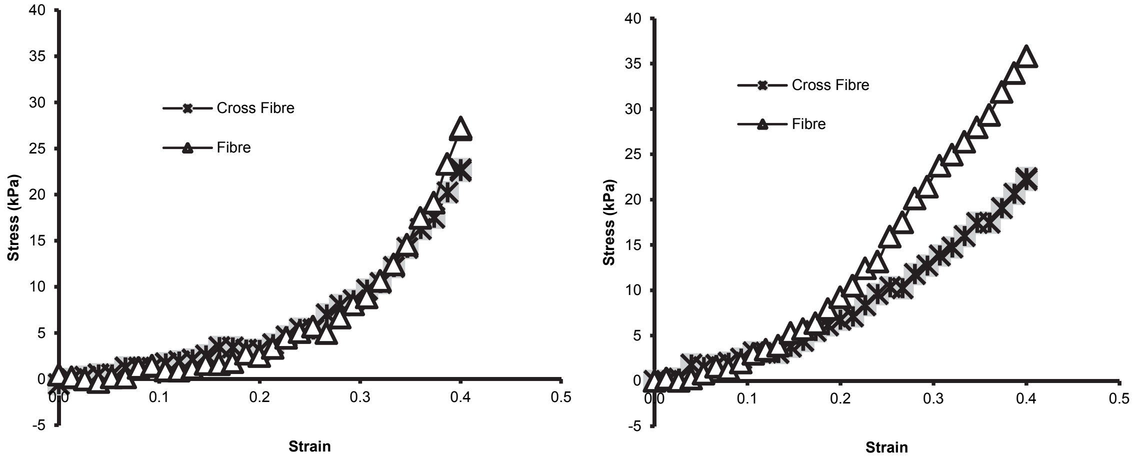
Fig. 7. Experimental results of stress-strain curve of left ventricle (LV) at 30% strain biaxial stretching (a) Sample 1, (b) Sample 2, (c) Sample 3 and (d) Sample 4



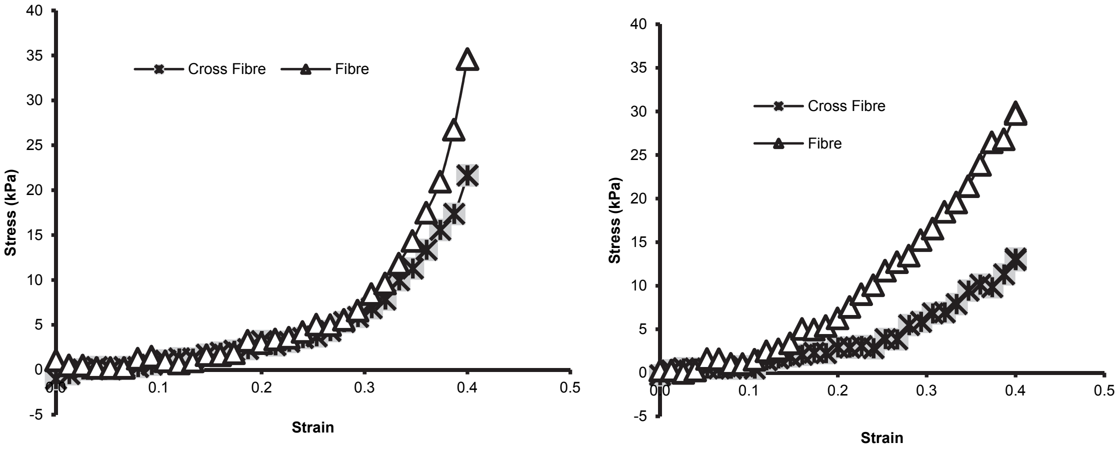
(a) (b)

(c) (d)

Fig. 8. Experimental results of stress-strain curve of septal wall (Septum) at 30% strain biaxial stretching (a) Sample 1, (b) Sample 2, (c) Sample 3 and (d) Sample 4



(a) (b)



(c) (d)

Fig. 9. Experimental results of stress-strain curve of right ventricle wall (RV) at 30% strain biaxial stretching (a) Sample 1, (b) Sample 2, (c) Sample 3 and (d) Sample 4

4. Discussion

The mechanics of anisotropic soft tissues and biomaterials are usually investigated using biaxial testing. During these tests, the specimens are generally clamped to the machine actuator using mechanical clamps (Perez et al., 2014, Hu et al., 2014, Nolan and McGarry, 2016). The interpretation of biaxial test results is complex and the direct use of material parameters in computer simulation may be complex (Tian et al., 2015, Delgadillo et al., 2015). In the present study, the stress–strain on the LV, septal and RV walls was presented to investigate the cardiac mechanics. Considerable differences were found when comparing the mechanical properties of the LV, septum and RV walls, as these were found to exhibit non-linear mechanics. Physiologically, the wall thickness of the RV was found to be much less than that of the septum and the LV. The RV wall was found to be non-linear, stiffer and significantly thinner than the LV and the septal walls. These differences will introduce differences when assigning material properties to bi-ventricular modelling of the heart. It is likely that the mechanical properties of the RV wall would provide more accurate results in the simulation of the heart. Therefore, a significant difference in the mechanical properties of the resting porcine heart in the LV, septum and RV tissue was observed when performing equibiaxial tensile tests. The LV and septal walls exhibit non-linear stress–strain behaviour of up to 40% strain and are both thicker when compared to the RV. On the other hand, the RV wall exhibits anisotropic and strain stiffening non-linear behaviour in both the fibre and the cross-fibre directions. These differences should be taken into consideration when modelling porcine myocardial tissue. In this study a porcine resting heart was used, which implies that these results may differ from those obtained from a human heart and a separate experiment should therefore be conducted to confirm similarities with the data presented here.

The development of accurate and representative heart models for the mechanical behaviour of healthy and infarcted myocardium is dependent on the formulation of suitable constitutive laws and precise determination of myocardial properties (Masithulela, 2016b). In most cases, the challenge is that experimental data may not be suitable for producing an accurate constitutive model. Therefore, data generated by this work could be further applied for the development of accurate and reliable constitutive models of the resting porcine heart. Accordingly, the novel data of biaxial extension in the passive porcine ventricular myocardium will assist in analysing the competence of current constitutive models.

Over the past decade, several investigations have been carried out to determine the stress–strain distribution in porcine hearts, as well as in other small and big animal hearts including the human heart (Kichula et al., 2014, Sommer et al., 2015). However, most of these studies have focused on one region only, namely, the LV (Nikou et al., 2016, Wise et al., 2016, Miller et al., 2013, Kortsmit et al., 2013). The stress–strain curve of the LV has then been used as being representative of the whole heart. This approach has resulted in the generation of several computational models which use the material properties of the LV as being representative of the whole heart. As proven by this study, the material properties in the RV, septal and RV walls differ. This means that, when developing computational models, each region of the heart should be treated differently and accurate material properties should be assigned. Accordingly, the accuracy of simulations may be affected if the simplified material properties are used. For example, if the Fung model (Zhang et al., 2015) is used as a constitutive model for simulating heart, each region should be assigned different material parameters in order to simulate the material behaviour accurately.

The mechanical behaviour of heart tissue has been discussed with a special focus on left ventricular functioning. Various finite element models have been developed based on the assumptions that the material behaviour of the whole heart is the same throughout. In this paper, it is clearly shown that the stress–strain in different regions of the heart differs. As a result, the stress–strain of the LV, septum and RV has been studied extensively. Overall, the comparison of rested LV, septum and RV has shown that these tissues have different mechanical properties. Understanding the mechanical behaviour in the various heart regions of the heart is a critical factor in facilitating the surgical implantation. Computational models of cardiac mechanics can be applied as effective tools in diagnosis and in planning patient-specific therapy. All computational models developed should seek to use accurate stress–strain data in the various regions. These have the potential to greatly increase the accuracy of these computational models when studying various mechanisms of heart disease, including myocardial infarction. These models may also assist in classifying myocardial pathologies, adopting therapeutic procedures, and even predicting therapy outcomes.

One of the limitations of the study is that because of the lack of more accurate modalities during tensile testing of the porcine heart, the fibre direction was determined by visual assessment. However, visual assessment is not accurate and could preclude the precise fibre alignment with specific and direct axis of the testing equipment. This limitation will be addressed in future work in which a reliable modality will be used to accurately align the fibre direction with the perfect axis of the testing equipment. Another limitation of this study was that a larger sample needs to be considered in future studies for greater statistical power. To reduce dehydration in the sample, samples were tested as soon as possible. This could also have introduced some errors.

5. Conclusions

In summary, biaxial testing techniques were applied to determine the elastic modulus of the LV, septum and RV in the fibre and cross-fibre directions. These moduli were compared by looking at different regions of the porcine heart, namely, the LV, septal wall and RV. A significant finding in this study is that the different regions of the heart differ in terms of stiffness in both the fibre and the cross-fibre direction. Therefore, a significant difference in mechanical properties of the resting porcine heart in the LV, septum and RV tissues was observed when performing equibiaxial tensile tests. The LV and septal walls exhibit non-linear stress–strain behaviour up to 40% strain and both are thicker when compared to the RV. On the other hand, the RV wall exhibits anisotropic and strain stiffening non-linear behaviour in both the fibre and cross-fibre directions. These differences should be taken into consideration when modelling porcine myocardial tissue. In this study a porcine resting heart was used. Accordingly, these results may differ from those obtained from a human heart and thus a separate experiment should be conducted to confirm similarities with the data presented here. Further work is necessary to study the effect of strain rate on the stiffness and elastic modulus of the porcine heart in different regions including the LV, mid-wall and RV.

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