



Mechanical Properties of the Myocardium in the Ischemic Heart

Dinely Colon
Department of Biomedical Engineering,
The City College of New York

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Acknowledgements

This project was not possible without the support from both the Louis Stoke Alliance for Minority Participation (L-SAMP) and the Marshall Plan Foundation, thank you very much for this. Very special thank goes to my supervisor in this project Dr. Gerhard Sommer for all his support and guidance. Also, I would like thank all the members of the Biomechanics laboratory at the Graz University of Technology (TUGraz), but in special at to Stefan Lind for his contribution helping me with the Matlab code. In addition, I gratefully acknowledge Prof. Gerhard A. Holzapfel for the wonderful opportunity to work in his laboratory at TUGraz.

Abstract

The biomechanical “machine” that keeps the human body going is the heart; this organ is responsible for blood and oxygen supply to the rest of the body. Unhealthy diet and heart diseases can cause damage to the heart. This damage may be irreversible even with all the advances in science and technology. One of the most common medical conditions that affect the heart is a myocardial infarction, which results into a permanent defect to the myocardial tissue and often leads to a dysfunctional heart. In the present study we are investigating how the area of the heart that is most affected by a myocardial infarction behaves mechanically in comparison to the healthy heart tissue in the same subject. The samples for this experiment were obtained from the Division of Transplantation Surgery of the Medical University of Graz. There, we performed a left anterior descending artery blockage for 45 minutes in order to induce ischemia/reperfusion damage to the heart; a procedure, which induces a heart attack resulting into damage at the lower part of the left ventricle including some portion of the apex of the heart. The pigs were differently treated for two months afterwards. Three groups of porcine hearts were investigated in the mechanical tests: *control*, *myocardium infarction with treatment* and *myocardium infarction without treatment*. We performed triaxial shear tests to identify the mechanical differences between healthy and necrotic tissues. Therefore, three cubic samples with the dimension of ~4x4x4 mm were prepared from both the healthy and the necrotic side of the left ventricular myocardium. We applied shear deformations in six different directions in order to investigate all three main directions of the orthotropic myocardium (i.e. the fiber direction, sheet direction and sheet-normal direction). The results show a decrease in stiffness in the samples that were treated with a cardio-protective solution in comparison with the other groups. Unfortunately, at this point we don't have enough samples to perform any statistical analysis. In

future, we are planning to perform more experiments and use multi-photon microscopic structure investigations of the samples in order to be able to correlate the mechanical properties with its underlying microstructure of both the healthy and the necrotic myocardial tissues. Moreover, we will analyze possible residual stresses in healthy and necrotic tissues on circular-shaped (in a transverse cut) and elliptical-shaped (in a longitudinal cut) samples.

Introduction

The heart has the ability to contract and expand during the process of blood injection/ejection to both the lungs and the remaining body. The tissue composition and architecture is what provides the heart the unique ability to serve as a biological pump in order to supply blood and oxygen to the entire body. The lack of oxygen in the tissue can lead to irreversible damages. Medical conditions such as cardiomyopathy can restrict the oxygen supply to the heart, resulting in permanent damage to the heart tissue. Even though the heart has the ability to generate its own bypass, in case of a blockage in the artery, this systematic response does not always fulfill the demand of blood and oxygen supply that the tissue requires resulting in irreversible damage in the affected area [4]. The heart is divided into 4 parts: two atria and two ventricles (Fig. 1). Each of this part has a specific function in the process of blood circulation in the body. The heart has been categorized as the left and right heart. The reason for this lies in the fact that the heart has two compartments, the left and the right ventricle, which are divided by the septum. Each of the heart side has its function in the process of supplying organs and tissues with blood. The left side receives oxygenated blood from the lungs and sends it to the organs and supplying them with oxygen, while the right part of the heart collect the deoxygenated blood from the body and sends it to the lungs for re-oxygenation. This process repeats itself while there is life or heart function in the body. The ventricle moves based on a combination of contraction, extension and torsion [6, 13]. The beating movement is due to a network of muscle fibers, which wrapped around both ventricles [6, 12, 13]. During the deformation, the collagen prevents the muscle fiber from sliding or rupture and protects it from overstretching and assist in re-lengthening [6, 15]. The heart consists of muscular fibers that are composed of myocytes, which are muscle cell that form fibers [4]. This unique organ is composed of three layers of tissue:

endocardium (inner layer), epicardium (outer layer) and myocardium (middle layer). Both the inner and the outer layers are mainly composed of collagen, which allows the heart to deform during contraction/expansion motion without changing the physiology of the tissue. The types of collagen in the heart tissue are mainly I and III, and they assist in the expansion process by providing protection to the muscle fibers from over stretching [4, 6]. Myocardium fibers are surrounded by endomysial and perimysial collagen that form sheets [1]. These sheets form laminar structures that are responsible for the mechanical properties of the cardiac tissue [6, 10, 11]. The sheet surface orientation varies spatially showing a complex structure inside the heart [6, 16] (Fig. 1). The mechanical properties of the myocardium are characterized along three orthogonal axes [6]. The three axes represent the 6 possible shear modes. These directions have been identified as FS, FN, SF, SN, NS, NF (see Fig. 4(b)). This behavior of each direction is caused by bundles of myocytes, which change their fiber directions [1, 4, 6]. During infarction, the wall muscle of the heart is permanently damaged because of the massive cell loss and the inability of the heart to regenerate itself [1, 5]. To what extent the damage is, in terms of mechanics, is still unknown. The few studies conducted after myocardial infarction show that there is a remodeling of the fiber orientation and laminar structure [6, 17, 18]. From a mechanical point of view, this results in a less optimized architecture that lead to poor prognosis [19, 20, 21].

In order to investigate the mechanical properties of the heart, scientist had applied simple shear deformation to the passive myocardium in the left ventricle. Simple shear imposes tensile strain in the rotated plane in addition to shear, making it a more complex mode of deformation than for pure shear [1]. The complexity of the heart and its function makes it impossible to test this organ *in vivo* in order to obtain an accurate determination of its mechanical properties. We

are limited to investigate the *post mortem* state of the cardiac tissue as well as its composition due to the biological changes in the tissue after death. In an attempt to better understand the tissue composition of the heart, Eriksson et al. [2] investigated the myocytes orientations. In their investigation, they found that myocytes are bundled and form layers of up to six cells, which are referred to as sheets. These sheets contribute to the orthotropic material properties of the myocardium. These properties allow the left ventricle the ability to spread electrical activation, which leads to re-polarization and the mechanical response to pressure load and active contraction [2].

Passive myocardium has nonlinear viscoelastic shear properties that depends on the amount of strain that it is applied to the sample. Shear deformation or relative sliding of the myocardial layers may play an important role in the function of the heart [1]. Previously reported data suggest that this facilitates the left ventricle ejection by thickening the subendocardial wall during systole [1, 8]. This thickening is associated with the reorientation or shearing of myocardial layers [1, 9].

Experimental investigation of the myocardium has been limited to biaxial testing. Also, it has been focused in healthy myocardium. To the best of our knowledge, Dokos et al. 2002 was the first one to use the cubic samples of healthy myocardium tested in the six different modes. He found no significant difference between simple shear deformation in the NS and NF across all heart at any magnitude of maximum shear displacement. NF and NS modes are controlled by tensile response to extension of the material axis that is normal to the muscle layers, resulting in a very small contribution from shear. He concluded that the response to left ventricle myocardium to simple shear deformation is dominated by the tensile material properties of the

tissue along the local microstructural axes and that the contribution of shear stiffness to this response was relatively small [1].

In this study we investigated how the area of the heart, most affected by a heart attack, behaves mechanically in comparison to the healthy heart tissue in the same subject. Therefore, the mechanical properties of the necrotic region, due to the blocked artery, and a healthy region were determined by the aid of triaxial shear tests. In particular, six modes of simple shear were applied to the orthotropic myocardium. These modes can be evaluated by using cubic samples of the tissue. For the purpose of this particular study we took into account the arrangement of the fibers and sheets in the myocardium, in order to represent each of the above-mentioned modes of simple shear.

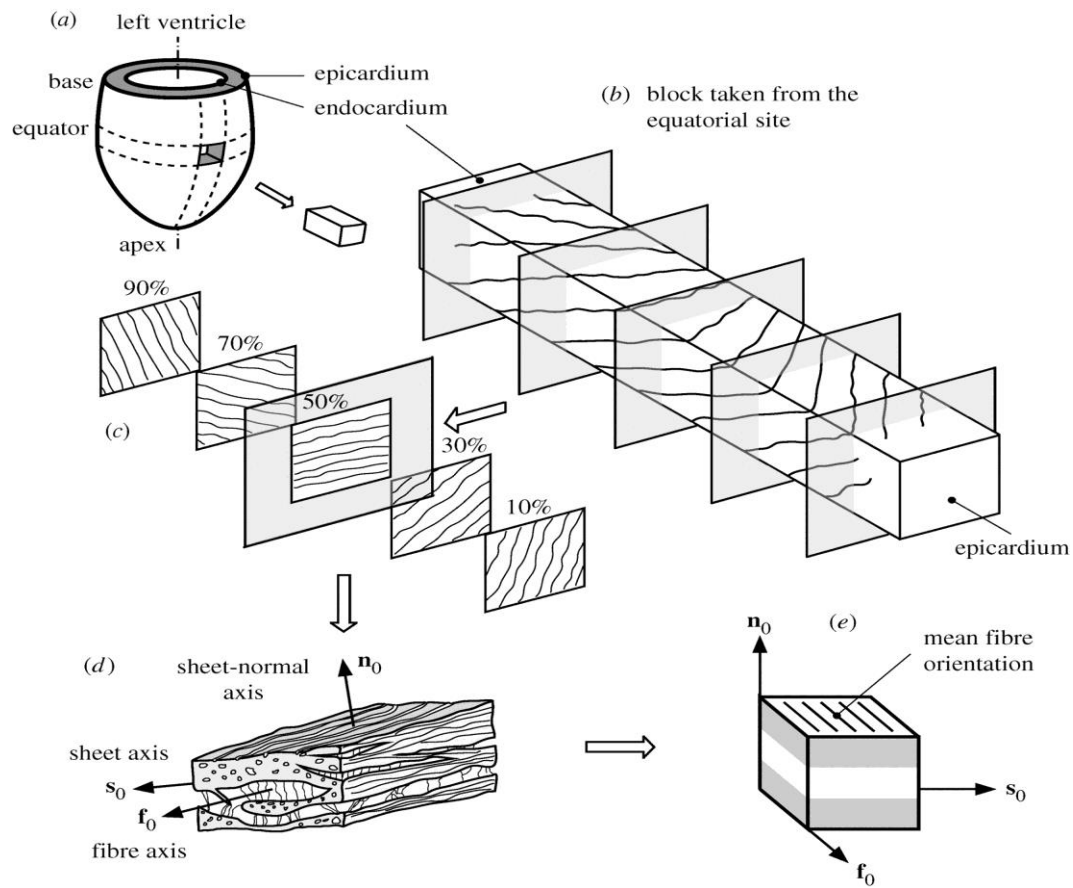


Figure 1. Graphical representation of the heart tissue along with fiber orientation a) left ventricle, b) representation obtain from the ecuatorial region

Image borrow from Holzapfel and Ogden, 2009

Materials and Methods

The ischemic porcine hearts were prepared at the Division of Transplantation Surgery of the Medical University of Graz. The left anterior descending coronary artery was gradually blocked during a 45 minutes period to mimic ischemic cardiomyopathy in humans. This procedure induces a heart attack resulting in a damaged myocardium by formation of necrotic tissue at the lower part of the left ventricle of the heart. Three groups of porcine hearts were investigated by mechanical tests: control (n=5), myocardium infarction without treatment (n=3) and myocardium infarction with treatment (n=12). The subjects in the treatment group were treated for two months after ischemia was induced. In particular, the acute and the chronic impacts of the cardio-protective antioxidant solutions (Angiotensin-(1-7) and Karal (α -Ketoglutarate and 5-Hydroxymethyl-furfural) either alone or in combination) were tested during regional ischemia/reperfusion on cardiac performance and reperfusion arrhythmias.

For mechanical testing, we performed triaxial shear tests using the custom-built experimental triaxial shear device at the Institute of Biomechanics, Graz University of Technology, Austria, in combination with the testing software package TestXpert II (Zwick/Roell, Germany) (Fig. 2).

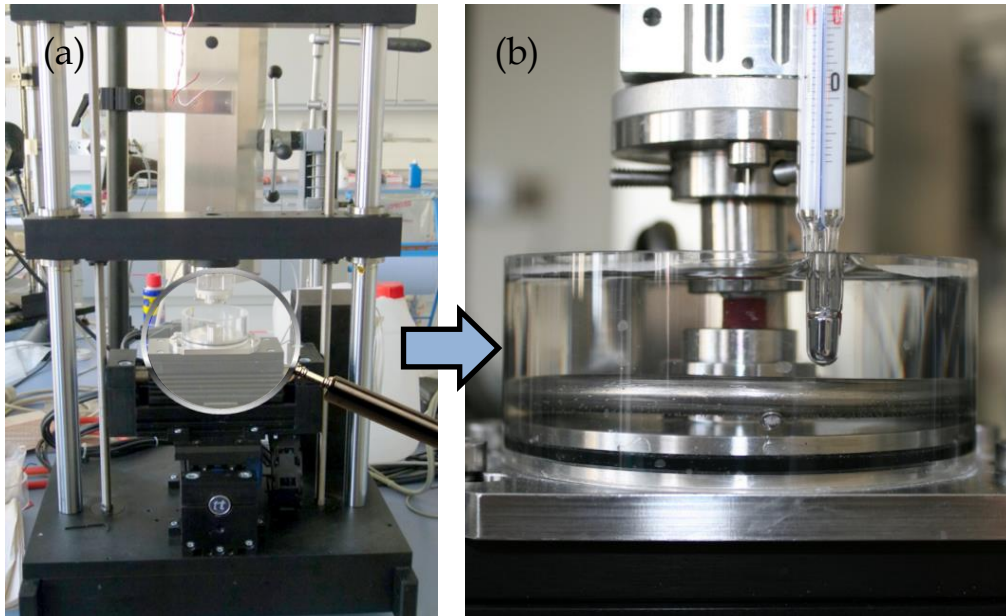


Figure 2: (a) Photograph of the triaxial shear testing setup; (b) a cubic porcine myocardial tissue specimen inserted in the triaxial machine and ready for testing.

Figure 3(a) and 3(b) show the anterior part and posterior part of an ischemic porcine heart, respectively. From each part three cubic specimens were prepared for mechanical testing.



Figure 3: (a) Anterior and (b) posterior view of a porcine heart obtained from the Medical University of Graz.

The tests were performed on cubic myocardial samples ($\sim 4 \times 4 \times 4$ mm) obtained from both the necrotic and the healthy region at similar latitude regarding the porcine heart (Fig. 4(a)). Cubes were prepared with their sides aligned with the fiber, sheet and sheet-normal axes (Fig. 4(b)). After the proper orientation was set, the cubes were glued to the shear testing holders, using super adhesive gel glue from Loctite. After the samples were properly glued to the top holder, glue was added to the bottom holder and the gluing protocol was activated to insure the application of a proper distributed force throughout the whole sample and to guarantee the same gluing

process for all the samples. During this process the machine applies a constant force of approximately 0.1 N for 5 minutes. During testing the samples were submerged in cardioplegic solution maintained at constant temperature of 37°C by a heater circulation unit. Three cycles (two for preconditioning and one for further data analysis) of sinusoidal simple shear (0.1–0.5 in 0.1 steps of specimen thickness) were applied to the specimens in two orthogonal directions. The samples were tested in different orientations in order to cover the simple shear responses along the six directions. After the tests, the samples were marked and stored in 4% formaldehyde for further microstructural investigations.

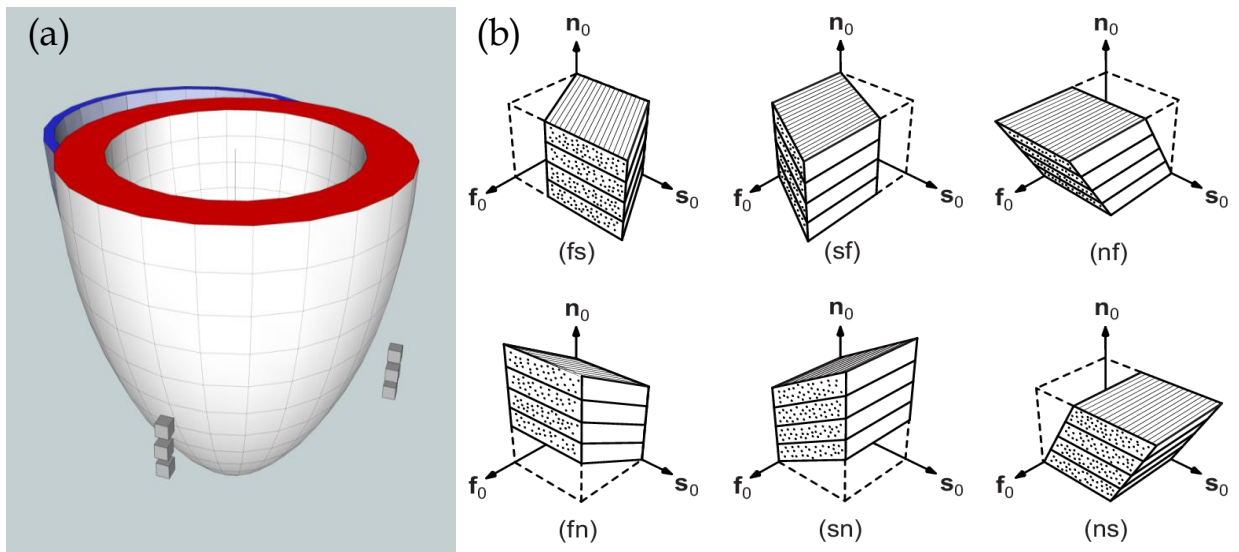
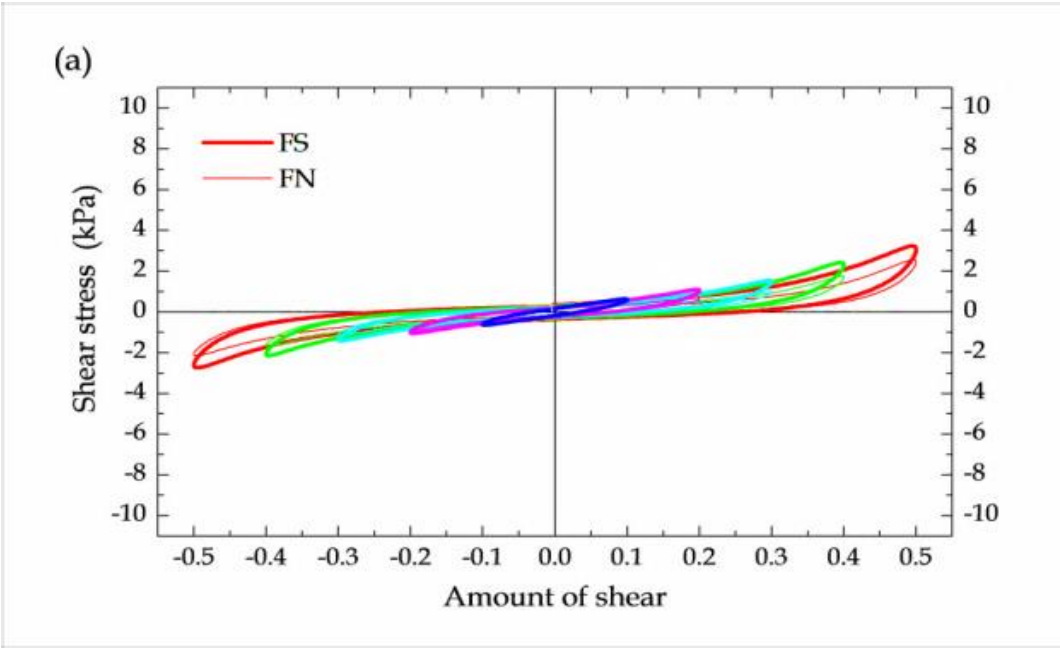


Figure 4: (a) Schematic representation of the ventricles, and six cubic samples extracted from the lower region of the lateral left ventricle; (b) six possible modes of simple shear (FS, FN, SF, SN, NF, NS) represented by the fiber f_0 , sheet s_0 and sheet-normal n_0 vectors.

Results

Representative shear stress vs. amount of shear behavior for all six shear modes is displayed in Fig. 5. Depending on the type of tissue, healthy or necrotic, we saw a difference in the sinusoidal behavior of the data. Distinct differences are apparent in both the healthy tissue (Fig. 5(a)) and the necrotic tissue (Fig. 5(b)) for the simple shear modes of FS and FN, i.e. the amount of shear stress present in the necrotic tissue was bigger than the healthy tissue.



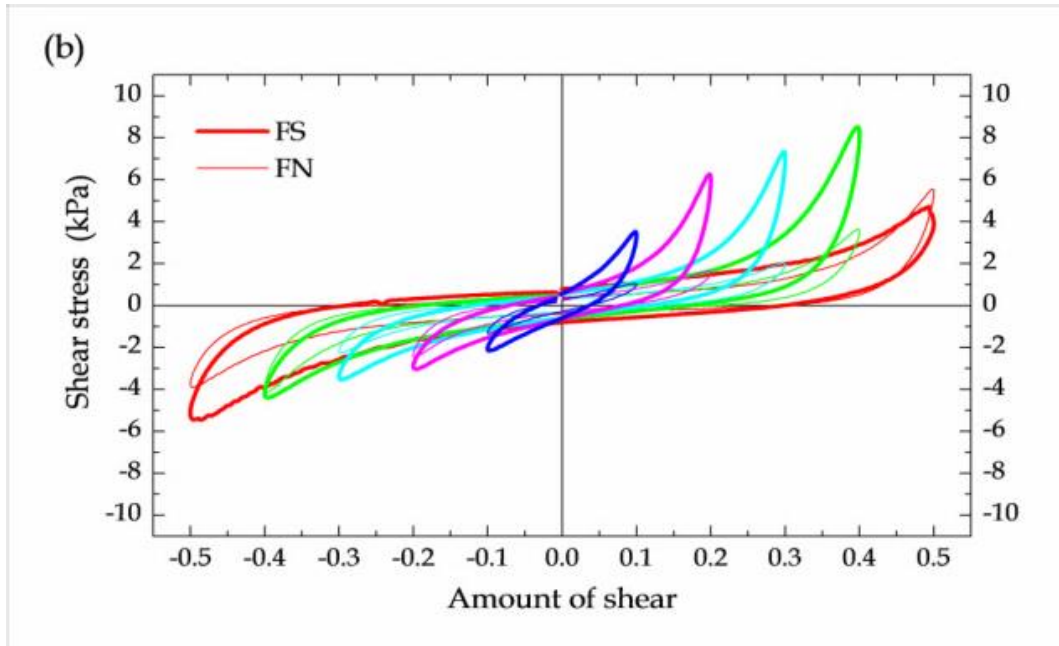
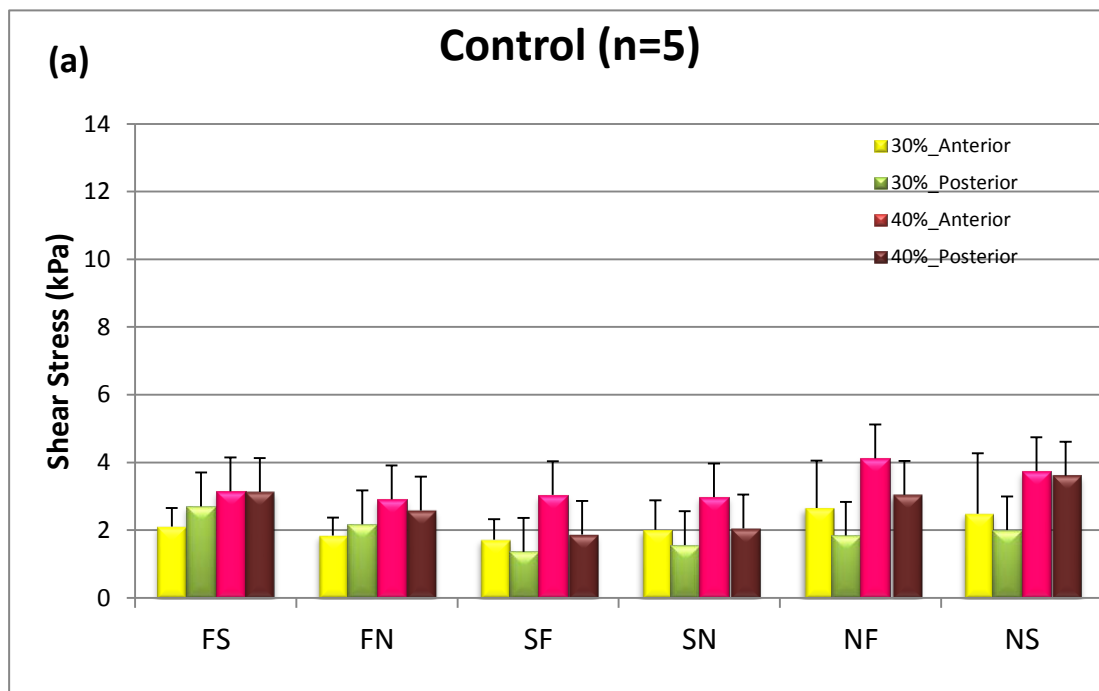


Figure 5: Representation of the sinusoidal shear stress vs. amount of shear of porcine myocardial tissue in both FS and FN shear mode for healthy (a) and necrotic tissue (b).



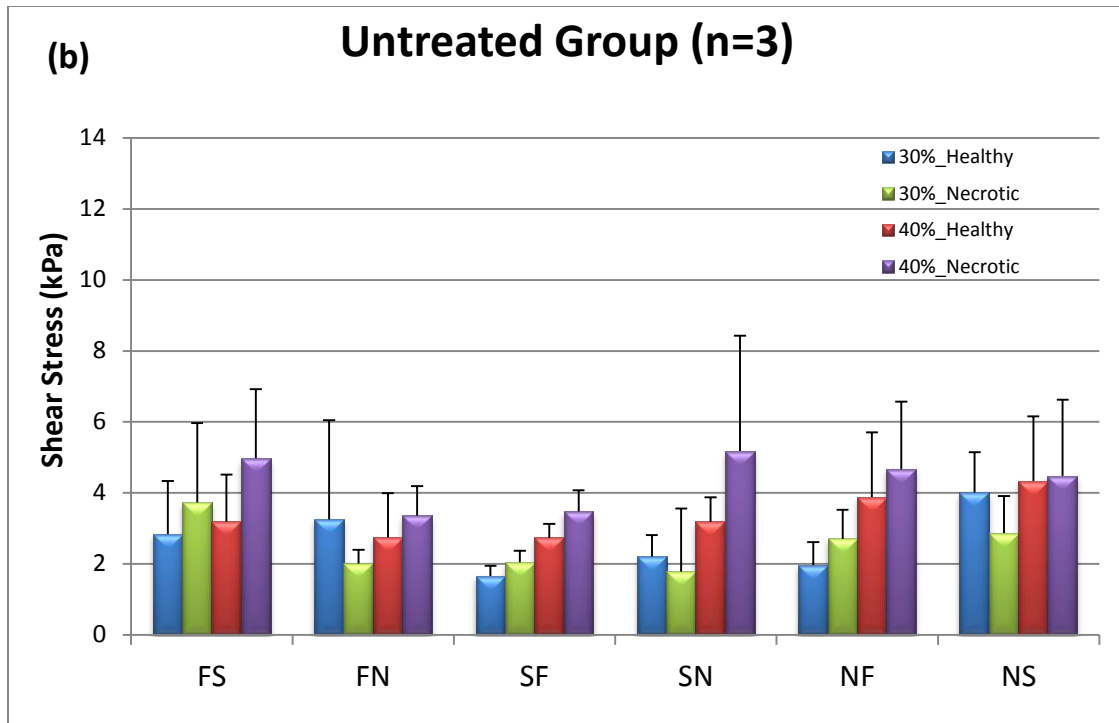
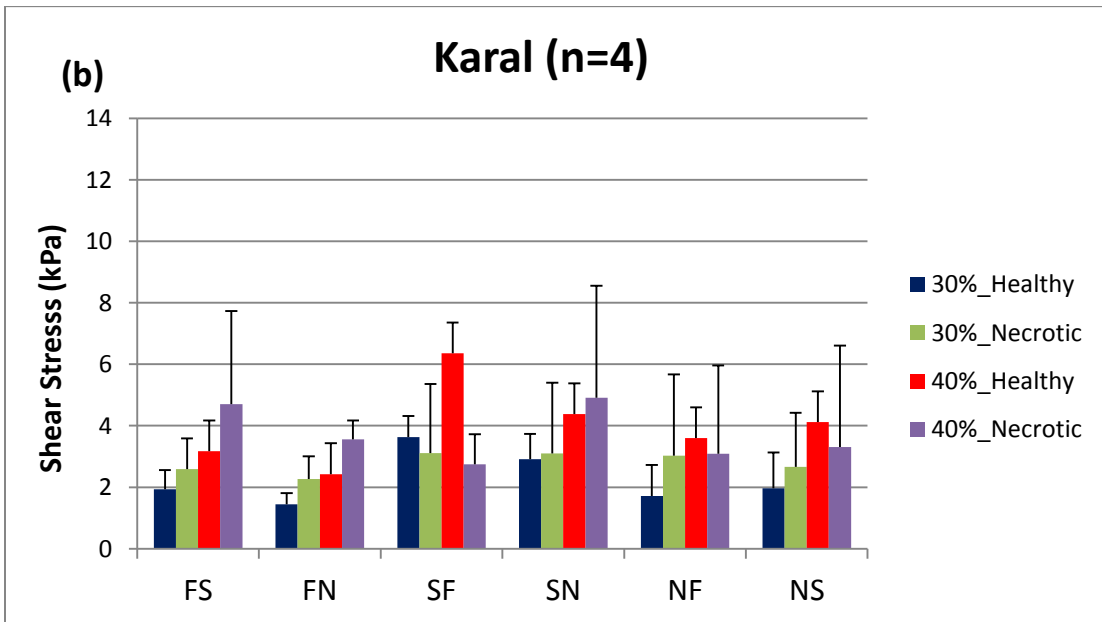
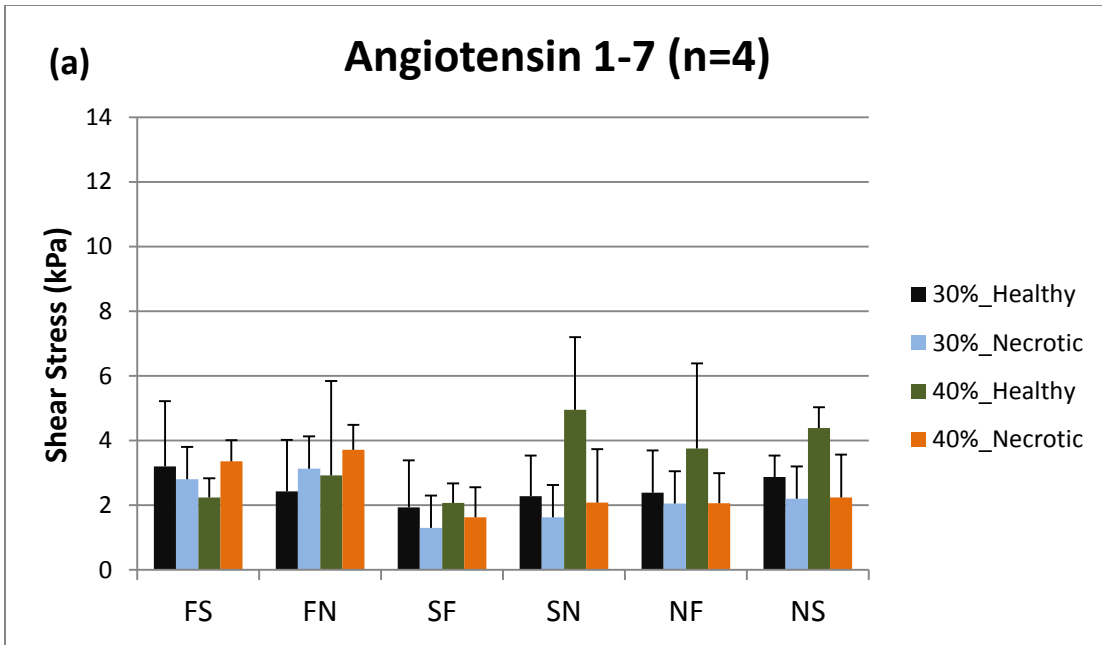


Figure 6: Graphical representation of the maximum shear stresses obtained from the control group (a) and the untreated group (b) at 30% and 40% amount of shear.

Since the control group has no necrotic region, both the anterior and the posterior wall of the heart were tested. Figures 6(a) and 6(b) show a bar chart obtained for each mode tested of the control and the untreated group. The maximum shear stress values in the control group lie between 1.5 and 4.1 kPa, while in the case of the untreated group these values lay between 1.8 and 5.8 kPa. Overall, for the 30% and 40% amount of shear the necrotic region of the untreated group was stiffer than in the healthy region (Fig. 6). Unfortunately, at this point we are not able to perform meaningful statistical analysis due to the low number of tested samples, but a trend can be observed.



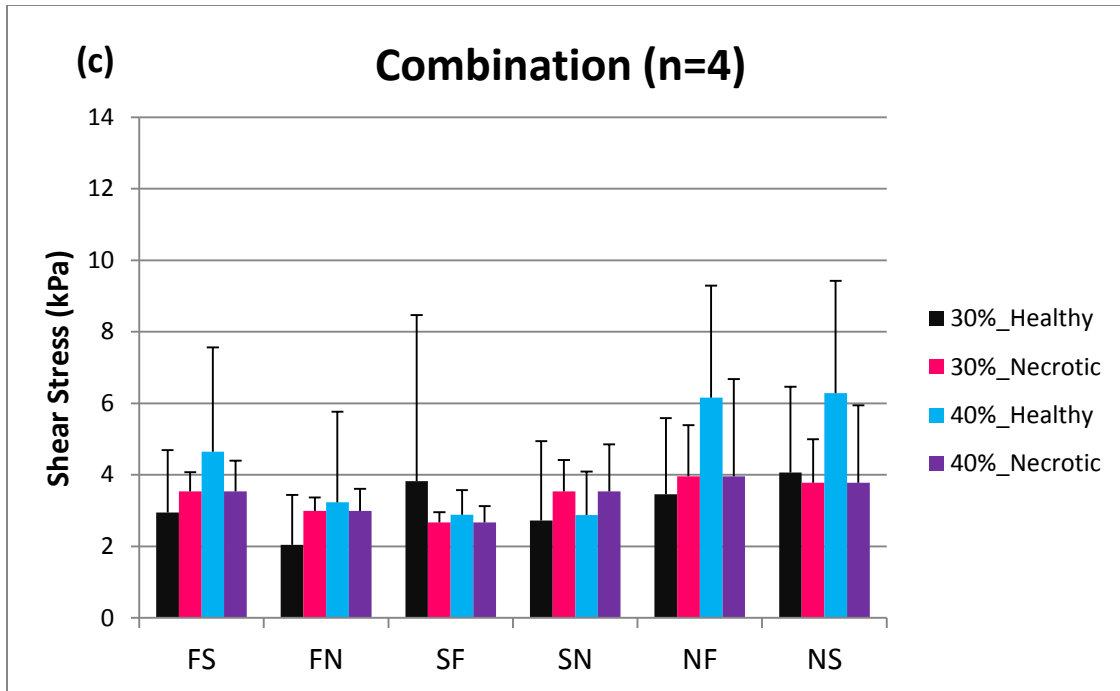


Figure 7: Graphical representation of the maximum shear stresses obtained from the treated groups. (a) Angiotensin, (b) Karal and (c) a combination of Karal and Angiotensin for 30% and 40% amount of shear.

When comparing the three different groups that were tested (Figs. 7(a), (b) and (c)), one can see that all of them have different maximum shear stress values. Angiotensin shows the lowest maximum shear stress values per mode in comparison to the other treated groups, the Karal and the combination group (Fig. 7).

Discussion

Depending on the position of the cardiac cycle, the mechanical behavior of the myocardium may vary. Previous data show that the thickness of the myocardium is greater in the end systole in comparison to the beginning of systole and diastole [7]. This change in wall thickness can contribute to the rearrangement of the fibers, changing the tensor direction in the myocardium [2]. Unfortunately, the physiological state of our sample was not monitored during sacrifice. During the two months after the procedure, echography was performed to monitor the recovery process and it shows a great improvement in the healing process for the treated group in comparison to the control group. The data to support this finding are not shown in this paper because it is still a work in progress.

Dokos and colleagues [1] reported that the stiffness of the myocardium varies in the following order $F > S > N$ mode of shear, this indicates a progression of shear stiffness [1]. Our results showed a different behavior from that reported by Dokos et al. [1]. The reason for this may lie in the fact that the samples of the study by Dokos et al. [1] were obtained from the equatorial line, while the samples for the present study were collected from the apex. Also, Dokos et al. only tested healthy heart tissue while our samples were both necrotic and healthy in the same tissue. In the constitutive modelling study by Holzapfel and Ogden [3], they considered the mechanical behavior of the myocardium tissue as orthotropic. Furthermore, they treated the myocardium as a non-homogeneous thick material with nonlinear elastic and incompressible properties. Based on these assumptions they developed a theoretical framework based on invariants associated with the three orthogonal directions: f , s , and n . With their theoretical considerations they affirmed the results obtained by Dokos et al. [1].

A limitation of this study is the limited number of subjects per group. We definitely need to perform more experiments in order to establish a viable trend in the data. Another source of error is the fact that the specimen alignment based on the fiber and sheet orientations were done by the naked eye. To circumvent this problem we will use a visual aid, e.g. a microscope. We also suggest conducting multi-photon microscopic investigations of the tested specimens in order to determine the orientation and distribution of the fibers and sheets in the tested samples. Moreover, we would like to also perform residual stress analysis to this investigation. Therefore, we will perform both a transverse cut and a longitudinal cut in both the healthy and the necrotic tissues.

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