Ai miei genitori e a mio fratello.
Vi porto sempre nel cuore.

Abstract

Aorta is the large arterial trunk carrying oxygenated blood from the heart to all the parts of the body through the systemic circulation. Aorta is usually divided in several anatomical districts with different specific arterial functions; in fact the composition of the aortic wall layers and the corresponding arrangement of elastin and collagen fibers vary along the vascular districts, leading to a different mechanical response.

A number of cardiovascular diseases can impair the aortic wall structure leading to aneurysm or dissection. The assessment of the mechanical response of the aortic wall with respect to its anatomical districts, both in healthy and diseased conditions, could provided useful information for clinical research or vascular prosthesis design.

The present study is collocated within this scenario since it aims at investigating the mechanical response of the aortic wall through uniaxial tensile tests. In particular, we developed a testing protocol based on literature, evaluating two main mechanical parameters: i) the maximum elastic modulus (MEM) and ii) the physiological elastic modulus (PM). MEM corresponds to the mechanical behavior for ultimate conditions before failure, while PM resembles the elastic response of the aortic tissue within physiological ranges of pressure (i.e., 130/80 mmHg).

In the first part of the study we have set-up a testing protocol to perform uniaxial tensile test on soft tissue samples exploiting a MTS Insight 10 kN machine. A preliminary performance of such tests and the related tuning have been performed on aortic samples derived from freshly slaughtered pigs.

Subsequently we have characterized the mechanical response of human aortic samples derived from several districs and related to different pathologies; in particular we analyzed the following cases: i) aneurysmal ascending aorta, ii) aneurysmal ascending aorta with Bicuspid Aortic Valve (BAV), iii) dissected descending aorta, iv) dissected descending aorta with Loeys-Dietz Syndrome (LDS)

Wide knowledge about the ascending aorta is available in literature, while articles related to the other segments are rare, especially referring to pathological states. For

this reason, the experimental data obtained from the ascending aorta are compared with previous works found in literature, while the analysis of descending aorta is reported as new data feeding.

The results obtained from pigs, on one hand, support the reliability of the adopted testing protocol, on the other do not confirm the indications available in literature, since we have not found the anisotropic response of the aortic wall. The experiments achieved on human samples excised from ascending aorta, globally agree with previous studies, proving the anisotropy of the tissue. The circumferential direction, in fact, is stiffer than the longitudinal in the last part of the curves (17.13 MPa versus 10.87 MPa in the aneurysmal ascending aorta; 6.1 MPa versus 2.4 MPa for aneurysmal ascending aorta with BAV). Even the results on descending aorta are in line with this trend, with the circumferential orientation stiffer than the longitudinal.

All the stress-strain relationships obtained in this work can provide new data in the assessment of the mechanism in which diseases effect the mechanical behavior. Such results can, finally, be exploited to calibrate ad-hoc constitutive models of aortic tissue for computational analysis, dedicated to wall failure investigation of aneurysm or dissection.

Sommario

L'aorta è il lungo tronco arterioso responsabile del trasporto di sangue ossigenato a partire dal cuore fino a tutte le parti dell'organismo attraverso la circolazione sistemica. Tipicamente l'aorta si distingue in diversi distretti anatomici con specifiche funzioni; infatti, la composizione strutturale degli strati della parete aortica ed il corrispondente arrangiamento delle fibre di elastina e collagene variano lungo i segmenti vascolari, comportando una risposta meccanica diversa.

Esistono delle patologie cardiovascolari in grado di alterare la struttura della parete del vaso, portando ad aneurisma e dissezione. La definizione della risposta meccanica del tessuto rispetto ai distretti anatomici, sia in condizioni fisiologiche che patologiche, potrebbe fornire informazioni utili per la ricerca clinica e la progettazione di protesi vascolari.

La presente attività si inserisce all'interno di questo scenario, dal momento che mira allo studio della risposta meccanica della parete aortica attraverso prove di trazione monoassiali. In particolare abbiamo sviluppato un protocollo di test basato sulla letteratura, valutando due parametri principali: i)il massimo modulo elastico (MEM) e ii) il modulo elastico fisiologico (PM). Il MEM corrisponde al comportamento meccanico in condizioni di massima trazione prima della rottura, mentre il PM rappresenta la risposta elastica del tessuto aortico per range fisiologici di pressione (i.e., 80-130 mmHg). Nella prima parte del lavoro abbiamo settato un protocollo per effettuare le prove di trazione monoassiale su campioni di tessuti soft, utilizzando la macchina di prova MTS Insight 10 kN. Una verifica preliminare su questo tipo di test ed il relativo perfezionamento sono stati fatti su campioni di aorta provenienti da maiali macellati in giornata.

In seguito abbiamo caratterizzato la risposta meccanicaaorte umane in diverse condizioni patologiche e diversi segmenti. In particolare abbiamo concentrato l'attenzione sui seguenti casi: i) aneursima dell'aorta ascendente, ii)aneursima dell'aorta ascendente affetta da Valvola Aortica Bicuspide, iii) dissezione dell'aorta discendente, iv) dissezione

dell'aorta discendente affetta da sindrome di Loeys-Dietz.

I risultati ottenuti dai maiali confermano, da un lato, la validità del protocollo di test adottato, mentre dall'altro, non coincidono con le indicazioni riportate in letteratura, infatti non si evidenzia la risposta anisotropa della parete aortica.

Gli esperimenti condotti su campioni umani estratti da aorta ascendente, concordano globalmente con studi pecedenti, dimostrando l'anisotropia del tessuto. La direzione circonferenziale, infatti, risulta più rigida di quella longitudinale nell' ultima parte delle curve (17.13 MPa versus 10.87 MPa nell'aorta ascendente aneurismatica; 6.1 MPa versus 2.4 MPa nell'aorta ascendente aneurismatica con valvola bicuspide). Anche gli esperimenti sull'aorta discendente sono in linea con questa tendenza, con la direzione circonferenziale più rigida di quella longitudinale.

Tutte le relazioni di stress-strain ricavate possono fornire nuovi dati per la definizione del meccanismo in cui le patologie influenzano le proprietà meccaniche. Questi risultati possono, infine, essere impiegati nella calibrazione di modelli costitutivi ad-hoc del tessuto aortico, utili per le analisi computazionali, improntati all' indagine della rottura della parete per aneurisma e dissezione.

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Table of Contents

Chapter 1

Introduction

Aorta, which is the largest artery of the cardiovascular system, arises from the left ventricle of the heart and, forming an arch, it extends down to the abdomen where it branches off into two smaller arteries. The main function of the aorta is to carry and distribute oxygenated blood to all arteries. Aorta experiences a complex mechanical loading due to the pulsatile blood flow ejected by the left ventriculum, thus, an appropriate mechanical response is required to accomplish the physiological task of this important vascular district.

The interest for the aortic mechanics has fascinated researchers since 1800 (Roy, 1880) as it may be confirmed by the large number of publications available in the literature (Carew et al., 1968; Halloran et al., 1995; Holzapfel, 2006; Duprey, 2008; Choudhury et al., 2009; Guinea et al., 2010; Khanafer et al., 2011).

The knowledge of aortic mechanical properties is of the outermost importance to better understand the mechanical behavior in healthy condition as well as to predict the mechanical behavior in pathological states.

In fact, severe pathologies connected to genetics and cardiovascular diseases can affect the aortic wall leading to a micro-structural degeneration of its layers and, consequently, impairing its mechanical response. It appears evident that dysfunction of the vessel can induce negative effect on all the system.

The underlying aortic composition, mechanical properties as well as the mechanisms responsible for age-related changes and vascular disease are however largely unknown. In order to identify mechanically the aortic wall components of healthy and diseased aorta, *in vivo* and *ex vivo* experimental tests may be carried out.

In vivo tests through imaging techniques, such as computed tomography, magnetic resonance imaging and echocardiography, give the possibility to evaluate the relative change in vessel cross-sectional area occurring during the cardiac cycle as well as to detect aneurysm formation. Together with simultaneous pressure registrations, stress-diameter curves can be obtained by inflation tests (Koullias et al., 2005). Nevertheless, in vivo examinations do not contribute in tissue analysis and, more importantly, in assessing potential rupture of the aortic wall.

With respect to *ex vivo* experiments, typical tests are the uniaxial and biaxial tensile tests. In particular, uniaxial tensile tests are conducted to determine one dimensional elastic properties such as the elastic modulus (Duprey, 2008), tensile strength (Guinea et al., 2010) and failure point (Mohan and Melvin, 1982).

Moreover, uniaxial tensile tests can highlight the differences between healthy and pathological vessels, as it has been presented in the recent study of Khanafer et al. (2011). However, although unidirectional tests are easier to perform and control, they are not able to evidence the anisotropic properties of soft tissues. In order to overcome this limitation, it is common practice to test strips excised at different orientations, circumferential and longitudinal in relation to the vessel, or to consider biaxial tests. If, on the one hand, biaxial tests are more appropriate for anisotropic tissues, on the other hand, they may be difficult to perform and to control due to the small size of aortic wall specimens (Nielsen et al., 1991).

The aim of this study is to analyze the mechanical response of the human aorta specimens undergoing external excitement and, thus, to investigate its mechanical properties. Particular emphasis is given to the changes of the elastic properties in relation to pathological conditions as well as to the aortic district from which the sample is extracted.

For this purpose, uniaxial tensile tests have been performed on pathological samples of patients underwent to surgical repair at Policlinico of San Donato, Milan, for aneurysm or dissection. The pathologies evidenced are: i) Bicuspid Aortic Valve (BAV) and ii) Loeys-Dietz Syndrome.

Each tensile tests is carried out following standard protocols as described in the literature by using the uniaxial testing device of the Department of Structural Mechanics, University of Pavia. The procedure has been validated on porcine samples. It is worth noting that testing soft tissue is not a trivial task since it is necessary to maintain the original mechanical properties before the test.

The experimental data have been post-processed in order to investigate the elasticity of the aortic wall in relation to two main conditions: (i) ultimate failure condition and (ii) normal situation.

In particular, the elastic modulus has been evaluated: i) in the region of strain preceding the failure, highlighting the behavior in critical conditions, and ii) in the the physiological range of blood pressure (130/80 mmHg), underlying the importance of understanding how aorta acts in normal conditions.

First of all, it is necessary to own a deep knowledge of the arterial tissue, in particular of the aortic wall, since it slightly differs from the common arterial structure. For this reason, in the *Chapter 2*, the material and mechanical properties of the aorta will be presented, taking into account the variation of its typical behavior along the vessel and for connective tissue disorders.

The *Chapter 3*, indeed, will focus on the materials and methods adopted to accomplish a uniaxial tensile test. Specifically, it is attempted to define a new protocol, as long as no standards on mechanical tests for the biological tissues are assessed in literature. Many challenges will be there faced, due to the nature of the samples above all, involving preservation, sizing and the eventual damaging of the aortic tissue. On that account peculiar tools and methods in data acquisition will be described.

Finally, the *Chapter 4* will report the results of the unaxial experiments performed, at first, on porcine samples, so that the procedure assessed could be validated, and then on human pathological tissues. This part of the work work will provide new data about aneurysmal ascending aorta and dissected descending aorta, increasing the understanding of the mechanisms involving the degeneration of these districts.

Experimental tests performed on the other segments, instead, are briefly described in *Appendix* since no effective data are extracted. The results here achieved on ascending aorta are compared to other works, while the ones related to descending aorta are just commented since no study, to our knowledge, concerned that district dissected . However taking new experimental data into this domain has a great value, as not many samples are available to improve the knowledge about the changes in the mechanical properties of the aortic wall in relation to the location and the pathologies correlated.

Chapter 2

Aorta biomechanics

Biomechanics is broadly defined as mechanics applied to biology and provides the physical and analytical tools to understand problems in physiology with mathematical accuracy (Kassab, 2006).

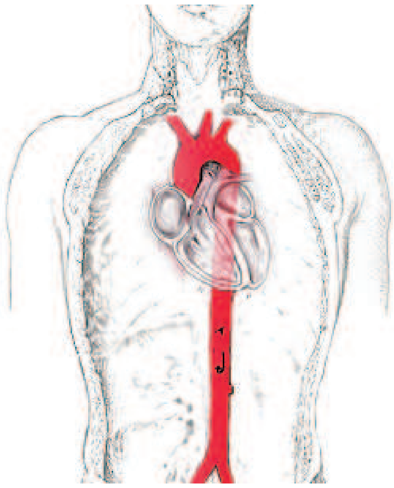
In the present work, we focus on the biomechanics of the aorta. Initially the anatomy of the aortic wall is described, taking into account the structure and composition of the three different layers in which the vessel is subdivided. Afterward, aneurysm and dissection, are described since they are the main diseases that affect the aortic wall. The mechanical behavior of aorta, strictly related to the wall microstructure, is then presented, paying attention to the variations of material and mechanical properties with regard to the location along the trunk and pathological states.

2.1 Aortic wall anatomy

The arterial system consists of a branching network of elastic conduits and high resistance terminals, which transforms the intermittent discrete cardiac output into capillary flow with moderate pulse (Sokolis, 2007).

According to dimensions and location, arteries can be subdivided into two groups: *elastic* arteries and *muscular* arteries. Elastic arteries, which include the aorta, pulmonary artery, common carotids, and common iliacs, are closer to the heart and have relatively large diameters (25-20 mm) (Redaelli and Montevecchi, 2007).

On the contrary, muscular arteries, which include coronaries, cerebrals, femorals, and renals, have smaller dimensions and in general are located at the periphery (except



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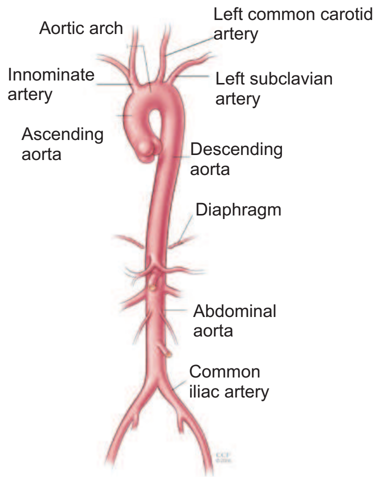


(b)

Figure 2.1: (a) Trunk of aorta located into the human body and (b) aortic districts

for the coronary arteries).

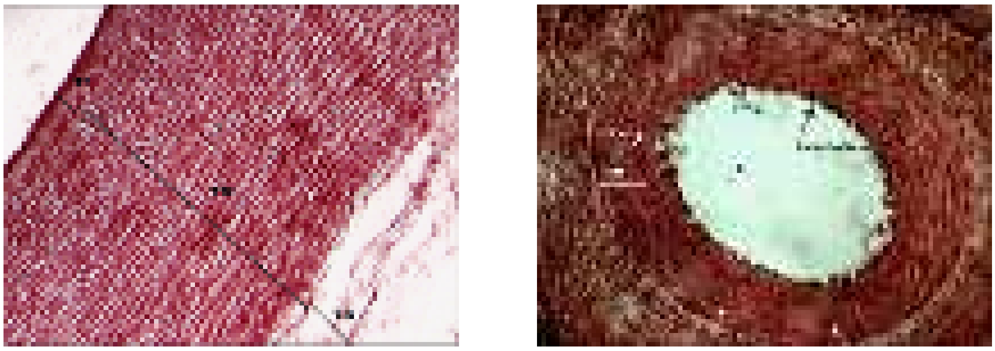
In this work, we focus on the *aorta* which is the largest artery of the human body. Aorta has not only to conduct the blood to the different organs, but to act, also, as the major player of the circulatory biomechanics.

Aorta originates from the left ventricle of the heart and extends through the abdomen where it splits in the common iliacs (see Fig. 2.1(a)).

With respect to its position on the human body, four segments may be identified along the aorta: i) ascending aorta – first 5-7 cm out of the heart, ii) aortic arch – from the second sterno costal joint to the 4th thoracic vertebrae, iii) descending aorta – till the diaphragm, iv) abdominal aorta – below the diaphragm till the common iliac arteries (see Fig. 2.1(b)).

At microscopic level the arterial wall appears as a layered structure, composed of three concentric zones (tunica intima, tunica media, and tunica adventitia) separated by elastic membranes and containing primarily elastin, collagen fibers, fibroblast, endothelial and smooth muscle cells, embedded in an amorphous, gel-like, ground substance consisting mostly of water.

It is worth noting that the histology of each arterial segment changes with location according to the own physiological function in the circulatory system. However, no abrupt variations are evidenced between large and small vessels and some arteries can exhibit both types of morphological structures. In the following, we describe briefly



(a) (b)

Figure 2.2: Photomicrographs at low magnification showing the layers of (a) elastic arteries; (b) muscular arteries

each arterial layer (tunica intima, media, and adventitia) from the histological point of view (see Fig. 2.2).

Tunica intima is the innermost layer of the artery made of a single layer of endothelial cells embedded in extracellular matrix and of an underlying thin basal lamina. In the aorta, the tunica intima may also contain a subendothelial layer of connective tissue and axially oriented smooth muscle cells. The intima is responsible for the transition of nutritional substances and chemical signals through the conduct, as it is in direct contact with the blood flow. Despite the great functional importance of aorta, its mechanical contribution in healthy young human arteries is neglected due to its small thickness. With aging and atherosclerosis –the most common disease of arterial wall- the intima becomes thicker and stiffer developing a more complex and heterogeneous structure (Holzapfel, 2006).

Tunica media is the middle and thickest layer of the artery, made of smooth muscle cells, elastin and collagen immersed into an aqueous ground substance (matrix) containing proteoglycans. In elastic arteries as the aorta, these constituents are arranged in repetitive lamellar units, forming concentric medial layers. The lamellae, in average 5-15 μm thick, are separated by thin (3 μm) fenestrated sheets of elastin.

The number of such units decrease as the distance from the heart increases. In fact, the thoracic aorta contains 50-60 lamellar units divided into vascular and avascular zones, while the entirely avascular abdominal aortic media typically contains 28-32 units (Ruddy et al., 2008). This laminated structure confers high strength to the media and explains how the medial layer determines the mechanical properties of the whole vessel. Unfortunately, this organization is prone to split, creating a cleavage between lamellae (dissection). In the media, collagen fibers are aligned along the the circumferential direction with a very little dispersion. This structural arrangement gives the media the ability to carry loads in the circumferential direction (Gasser et al., 2006).

Tunica adventitia is the outermost layer of the artery, mainly composed of fibroblast and collagen bundles undulated at physiological pressures. Histological evidence proves that collagen fibers tend to maintain an axial orientation with dispersion. Collagen fibers remain slack at low pressures but, as the pressure increases, they straighten, reinforcing the arterial wall and preventing the over-stretching and the rupture of the artery (Holzapfel, 2008). Adventita is involved in mechanics only for extreme pressure values, when collagen straighten, reinforcing the arterial wall and preventing the over-stretching and the rupture of the artery. Collagen fibers, which tend to maintain an axial orientation, remain slack at low pressures (Gasser et al., 2006).

2.2 Aortic diseases

Cardiovascular disease is the leading cause of death in developed countries and it is projected to become the leading cause of death worldwide in the near future. Several genetic and cardiovascular diseases affect the aorta wall, leading to a microstructural degeneration of its layers and, consequently, impairing its mechanical response. The most common types of aortic diseases are the dissection and the aneurysm (see Fig 2.3) where generally the former arises as a consequence of the latter (Hebballi, 2009).

Aortic dissection is an intramedial splitting caused by a radial tear of the intima and of a portion of the underlying media. The blood entering in the media through the tear creates a false lumen with variable extension. Moreover, blood can even re-enter the true lumen at any point, thus making a communicating dissection. The primary biological mechanisms in aortic dissection are decreased strength of the inner layer, increased blood pressure and augmented diameter coupled to thickness reduction. Intima tears when the inner layer is no more able to bear the stress (Okamoto et al., 2002).

Dissection may occur spontaneously during accident or as a complication of balloon angioplasty. The risk of dissection increase in patients with hypertension, many connective tissue disorders such as Marfan, Loeys-Dietz, Ehlers-Danlos syndromes and congenital heart diseases as Bicuspid Aortic Valve (BAV).

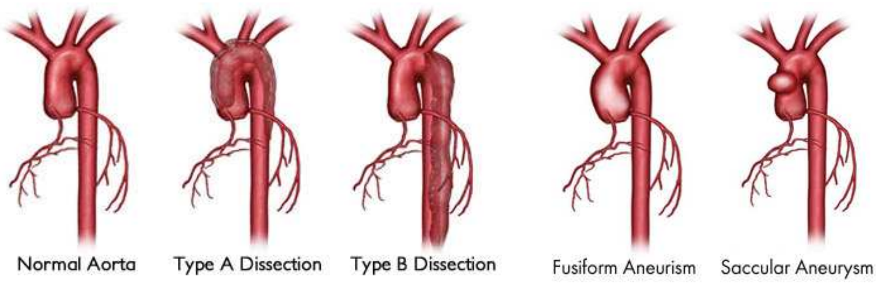


Figure 2.3: Different types of dissection and aneurysm

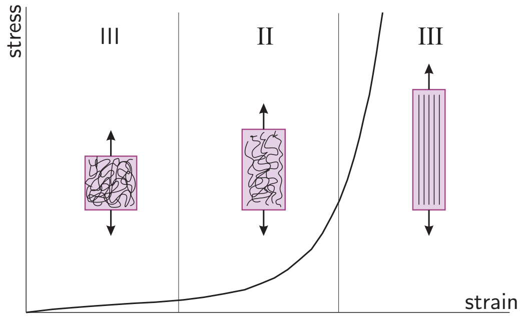
Aneurysm is the progressive balloon-like dilation of the artery in correspondence to a weaker portion of the vessel wall, that in worst cases leads to rupture of the tissue and death of the patient. Aneurysm rupture occurs when the mechanical forces acting on the aneurysm exceed the strength of the degenerated wall (Vorp, 2007). Such forces are directly related to the local diameter of the aorta.

Despite the increase in diagnose of aortic aneurysm over these last 30 years, thanks to advances in imaging techniques and screening programs, aortic aneurysm represents the 13th largest cause of death in USA. For the aorta, the most common forms are the abdominal aortic aneurysm and the thoracic aortic aneurysm . Abdominal aortic aneurysm affects approximately 3-5% of the population beyond 50 years of age in Western countries (Ruddy et al., 2008) . Approximately 150,000 new cases are diagnosed each year, and the incidence is increasing. Thoracic aortic aneurysm is relatively rare; each year, only 0.0059% of a given population is diagnosed with this condition. Thoracic aortic aneurysms are often caused by some form of genetic syndrome, involving connective tissue disorder and by the congenital deformation of the bicuspid aortic valve.

2.3 Aortic biomechanical behavior

The mechanical behavior of each single arterial layer and, therefore, the overall artery behavior, is strongly influenced by the concentration and the structural arrangement of its constituents (Holzapfel, 2000).

In experimental and theoretical studies, under physiological conditions, arteries are regarded as nearly incompressible solids (Carew et al. (1968), Chuong and Fung (1984)). The physiological range of the stresses in arterial tissue is assumed to be in the order of $50 - 100$ kPa under the mean blood pressure (100 mmHg) and the in situ axial



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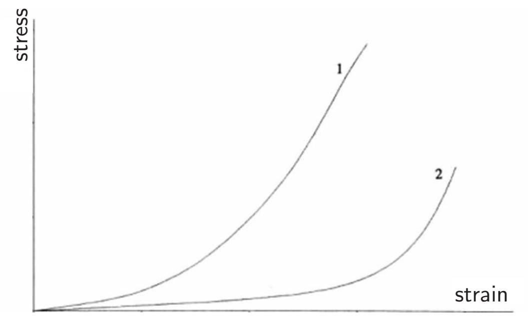


(b)

Figure 2.4: (a) Typical non linear elastic behavior with the three phases indicated; (b) different stress-strain response in the longitudinal (1) and circumferential (2) highlight an anisotropic behavior

stretch (Holzapfel et al., 2002). The structural arrangement of collagen fibers, which have preferred direction, leads the arterial tissue to be anisotropic. This means that if the same force is applied in different directions, the resulting strain will differ between the directions.

Moreover, it is well established that healthy arteries behave as highly deformable composite structures and exhibit a nonlinear stress-strain response with a typical J-shape with stiffening at the physiological strain level (see Fig. 2.4).

This particular form, representative for many soft tissues, depends mainly to the elastin and collagen components. In fact, the first part of the stress-strain curve, related to low loads, involves the elastin, whereas at higher stress the influence of collagen increases. In the low-pressure region, collagen bundles have a wavy form and the fibers are not straight until the waviness of the bundles have unfolded.

It is worth noting that, elastin can experience uniaxial extension of 150% when being straight, without breaking and return to its original configuration when unloaded. On the contrary, collagen can extend less than 10% when straight and have an elastic modulus 250 times greater than elastin. Thus, collagen seems to have a protecting effect to prevent overextension of the vessel.

Arterial walls can also exhibit several types of inelastic phenomena within and above the physiological loading domain. For example, arteries show viscoelastic effects under constant load (creep and relaxation) and exhibit hysteresis under cyclic loading. Another important point to be considered is the presence of a residual stress in the artery. It has been known for some years that the load-free configuration of an artery is not stress free. This phenomenon can be observed by cutting an arterial ring, which assumes the shape of an open sector by a springing effect. In general the cut open sector is also not stress free, since the opening angles of the separate layers are different (Okamoto et al., 2003).

2.3.1 Regional variations of material and mechanical properties

It is well appreciated today that the aorta not only serves a conduit function but it has an important role in modulating the left ventricular function, the myocardial perfusion and the arterial system (Sokolis et al., 2008).

Microstructural changes of the tissue and consequently variations of the elastic properties along the trunk, are the main responsible for the management of the whole cardiovascular system through all the body. Recent studies reported that aortic biomechanical and microstructure are affected by location.

Haskett et al. (2010), used the SALS technique, combined with biaxial planar tests, to approach the precise mechanism for these changes and progression along the axial length of the aorta as well as with age. Fiber directionality through the thickness of the aorta was found to be primarily in the circumferential direction, which was also the direction of highest compliance. Afterward, the degree of fiber alignment was found to decrease along the length of the aorta.

Moreover, circumferential and axial stiffening occurs with age and increases from proximal to distal aorta. In particular, abdominal region displays the highest stiffness. Inherent differences in the organization and content of the major vascular structural proteins contribute, furthermore, to the regional variation in mechanical properties. The content of collagen and elastin relative to lumenal surface area, progressively diminishes with increasing distance from the heart (Halloran et al., 1995).

The elastin decrease is associated to the reduction of the amount of elastic lamellae, balanced by the commensurate collagen content till the suprarenal aorta, level below which the proportion is altered for a major percentage of collagen. Since the arterial pressure is essentially constant through all the length of aorta and the elastin component varies along the trunk, the stiffness of the segments increases in the distal part, in the abdominal region above all (Ruddy et al., 2008). Finally, it is reasonable to agree with the tensile tests data of Sommer (2008), where the failure properties obtained suggest that the tensile strength of aorta decreases with increasing distance from the heart.

2.3.2 Mechanical properties in pathological state

Changes in biomechanical properties of aortic wall are strictly related to pathologies formation. Connective tissue disorders, involving the rearrangement or degradation of the proteins structures, deeply influence the elastic properties of the aorta.

Genetic diseases, such as Loeys-Dietz and Marfan Syndromes, raised from the mutation of genes responsible for synthesis of tissue constituents, weaken the aortic wall, while cardiovascular malformations, like Bicuspid Aortic Valve (BAV), changes the hemodinamics on the tissue, both remodeling the intrinsic structure of the wall.

Aneurysm and dissection, are generally considered side effects of the diseases mentioned above, but they can even occur in apparently healthy, elder people, till the occurrence of a critical event.

Thubrikar et al. (2001), found the global stiffening of the tissue, with higher value in the circumferential direction than in the longitudinal. They focused even on the variation of mechanical properties in relation to the regions of the aneurysm, feature taken into account even by Ravaghan et al. (1996) in their work. They agreed that the change in characteristic is strictly related to the variation in wall thickness.

Experimental data obtained by Vorp et al. (2003), showed that aneurysmal ascending aorta was significantly weaker than control, having tensile strength lower for both circumferential and longitudinal orientations. The same trend was found for stiffness values, greater in pathological tissues than in healthy patients for both directions (4.67 MPa versus 3.25 MPa in the circumferential direction; 4.48 MPa versus 2.61 MPa in the longitudinal direction). No significant mechanical differences were displayed considering the two orientations.

Iliopoulos et al. (2009) sustains that the reason why aneurysmal and dissected, from ascending to abdominal, aorta stiffens, is related to reduction in tissue extensibility and especially in elastin content. In particular, as medial thickness decreases, the intimal layer increases, keeping constant the wall dimensions; in this way a no more elastic tissue hardly withstands the physiologic hemodinamic forces.

A recent work of Khanafer et al. (2011), reveals that the circumferential orientation is significantly higher (9.19 MPa) than the longitudinal (3.13 MPa). Analog results were obtained in the previous work (Duprey, 2008), for the same region and disease, underlying the significant difference in stiffness for circumferential and longitudinal orientation, that suggests that aortic wall with aneurysm is anisotropic.

Chapter 3

Materials and methods

In this chapter, we present the standard protocol used for testing specimens of porcine and human aortic tissue. In particular, we describe the tensile testing system, the method for preserving living tissue and the data analysis. Emphasis will be reserved to the definition of parameters to investigate the elasticity of the wall.

3.1 Testing protocol

3.1.1 Testing system

The uniaxial tensile tests are executed by using the *MTS Insight 10 kN* (MTS System Corporation) machine recently acquired by the Structural Mechanics Department of the University of Pavia. The MTS Insight material testing system consists of: i) a load frame, ii) an electronic frame controller, and iii) a *TestWorks*® software. In the following, a brief description of each testing system component is presented.

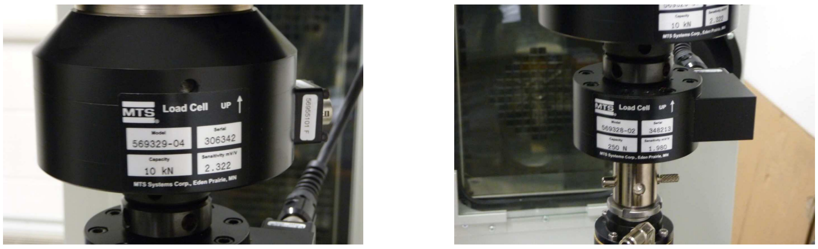
The *load frame* includes a base unit and two vertical columns with an upper transverse member (see Fig. 3.1). The moving crosshead is driven by precision ball screws with high-strength, precision ball nuts and rides on the ball bearings. This configuration is very efficient in minimizing friction and wear. The ball screws are anti-backlash. This feature removes the backlash so that position can be measured with increased accuracy over non preloaded ball screws. The screws are driven by a series of pulleys and belts which in turn are driven by a precision dc servo motor. The ball screw is connected to an optical encoder for precise position and velocity control.

Figure 3.1: MTS Insight 10 kN testing machine

The forces applied during the test are detected by a *load cell* (see Fig. 3.2) plugged to the moving crosshead. By using a specific algorithm, the load cell transforms resistance variations measured by a Wheatstone bridge into force values. The maximum load supported by the system is 10 kN. However, the forces involved in the biological domain are significantly lower, so that a load cell of 250 N with an accuracy of 0.01 N may be adequate for our tests.

Other important components of the testing system are the *grips* which allow the fixation and the consequent extension of the specimen. Wedge jaws are furnished with the *MTS Insight 10 kN*, but they are usually used for hard materials. On the contrary, pneumatic grips are suitable for soft tissues since they are able to keep constant the pressure during the testing execution (see Fig 3.3). In order to measure correctly tissue deformations, a *video extensometer* has been used which can read the original as well as the variation of the distance between two targets.

Finally, the software program *TestWorks4 (TWS4)* is responsible of the full machine control, data acquisition, management, and advanced data analysis. The program,



(a) (b)

Figure 3.2: Load cells furnished with the *MTS Insight 10 kN* device: (a) load cell of 10 kN and (b) load cell of 250 N

(a) (b)

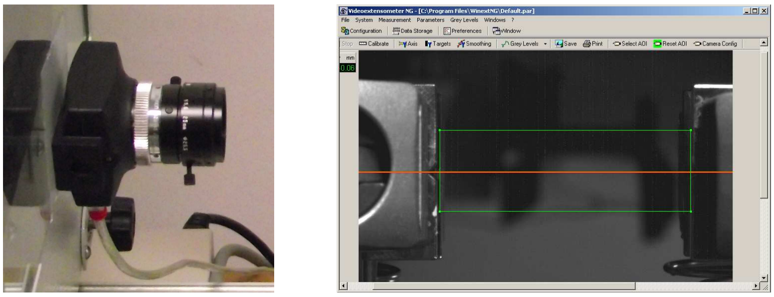
Figure 3.3: Grips furnished with the *MTS Insight 10 kN* device: (a) wedge grip and (a) pneumatic grip

offering a host of features, makes the material testing process fast and easy to use. The software has various method templates available, providing a starting point in the configuration of specific test methods. The user can define new variables, formulas and routines, depending on the needs, and follow the test easily during execution (see Fig. 3.4).

3.1.2 Samples

The samples investigated during the experiments belong to porcine and human species. Pig aortas which are harvested from a local slaughter (Rovescala, Pavia) are used only to verify the reliability of the testing method. The human aortic samples are obtained from patients undergoing to surgical repair at Policlinico San Donato, Milano.

It is worth noting that samples of soft biological tissues are very difficult to analyze through mechanical devices since experiments are done in a completely different envi-



(a) (b)

Figure 3.4: (a) Video extensometer; (b) software window

ronment from which the specimens belong. In order to perform successfully mechanical testing, it is necessary to keep an appropriate sample preservation.

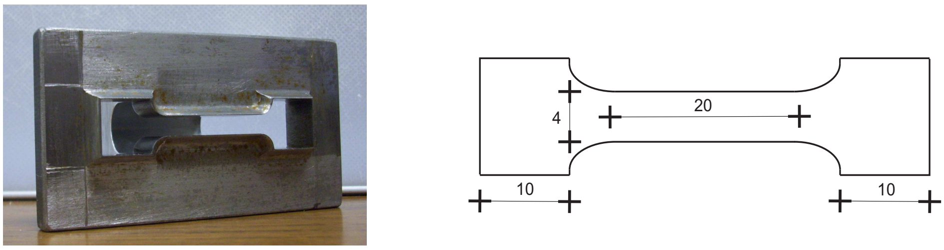
For this purpose, the tissues are preserved in normal solution at 4°C for reproducing environment conditions similar to the original (Chow and Zhang, 2010) and tested within 48 hours of extraction in order to maintain unchanged the mechanical properties (Haskett et al., 2010).

After equilibrium at room temperature, the samples are cut with a metal dyne (see Fig. 3.5(a)) providing a dogbone shape of 50 mm of total length, 20 of gage length, 10 mm of endings and 4 mm of width (Adham et al., 1996). Considering the fact that the human samples are extracted from alive patients, generally the amount of tissue cut is not large enough to obtain a dogbone shape, so that 3-4 mm wide almost rectangular strips of variable length are obtained (Sommer, 2008). The thickness is measured with a digital caliper at three different points: at the center and at the two extremes of the gage length, so that the average value of the three thickness measurements is assumed as thickness of the specimen (Adham et al., 1996).

In order to evaluate the anisotropy of the aortic wall tissue, the specimens are excised from each samples at two orthogonal directions, i.e., circumferential and axial.

It is worth noting that soft tissues contain water for the 60-70% of their volume, so that aorta specimens can easily slide out of the grips. Consequently, the experiments on soft tissues require some specific designed tools in order to face the problem of slippage. For example, a common method consists in gluing the extremes of the specimen to low-grit sandpaper.

Finally, black markers are applied on the central zone of the specimen tissue in order to permit video extensometer measurements (see Fig. 3.6). In fact, the abrupt lighting contrast between markers and tissue allows the video extensometer to follow



(a) (b)

Figure 3.5: (a) Metal dyne which provides the dogbone shape; (b) schematic representation of the dogbone specimen with dimensions;

Figure 3.6: Rectangular specimen ready to be tested with markers applied on the central zone and grit sandpaper at extremes

the black markers during the test.

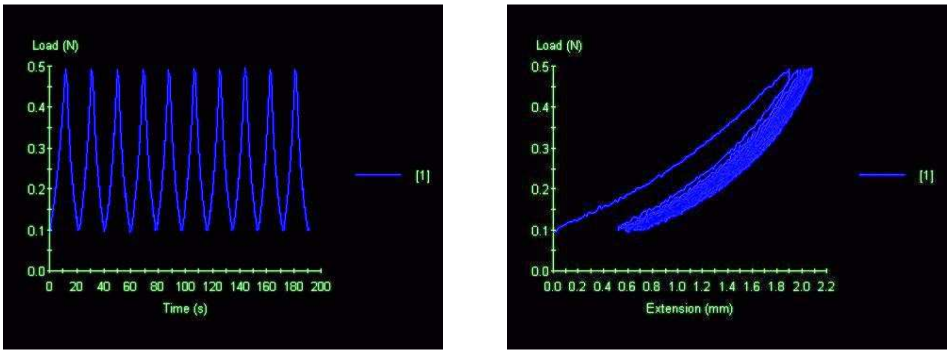
3.1.3 Uniaxial Tensile Testing

The uniaxial tensile procedure is performed into two steps: i) preconditioning and ii) tensile testing. The former step is applied to make repeatable the test procedure.

Preconditioning. Aortic tissue as each soft tissue shows a viscoelastic behavior under particular loading condition. For example, difference in the stress-strain response are evidenced during cycles of loading and unloading, i.e., the *hysteresis phenomenon*. The area delimited by the two loading-unloading curves corresponds to energy dissipated as heat during the load cycle.

However, it has been noted that after a proper number of subsequent cycles, the loading-unloading response becomes repeatable with less energy dissipation. In the literature, this technique is called *preconditioning* (Sokolis et al., 2006).

Moreover, the preconditioning is also used to reproduce the *homeostasis* of the living tissue. In this work, each specimen is preconditioned for 10 cycles with a triangular wave ranging from 0.1 to 0.49 N at a constant speed of 10 mm/s of the crosshead (see. Fig. 3.7).



(a) (b)

Figure 3.7: (a) Hysteresis cycles observed as the triangular function applied; (b) stress-strain relationship on the software

Tensile test. After the preliminary phase of preconditioning, the uniaxial tensile test takes place. During the tensile test, the applied force and the extension between the two markers are continuously recorded (10Hz) at the same speed of 10 mm/s (see. Fig. 3.8). The test runs until the failure of the tissue specimen, detected by the software as a drop of 0.1 N between two following data points.

By using the directly measured values of loads F and extensions ΔL, the program can calculate the indirect measures of engineering stress, σ_E, and engineering strain, ε_E, rispectively:

$$\sigma_E = \frac{F}{A_0}, \qquad \varepsilon_E = \frac{\Delta L}{L_0}, \tag{3.1}$$

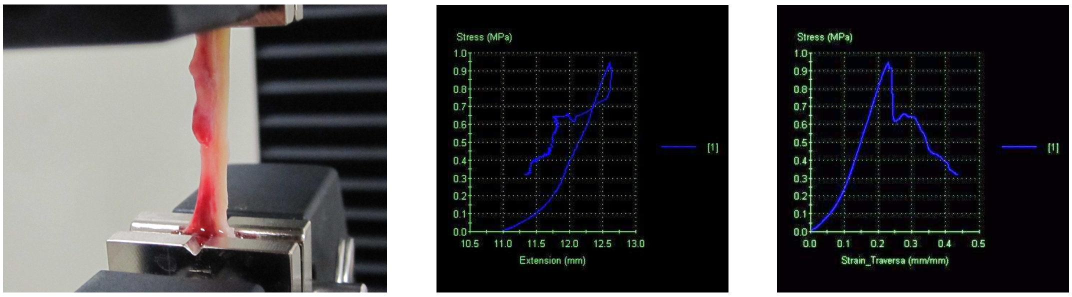
with L_0 and A_0 the initial length and initial cross-sectional area of the specimen, respectively.

Fig. 3.8(b) shows the plot of the quantities directly measured by the testing machine, i.e., loads and displacement, whereas Fig. 3.8(b) shows the stress and strain quantities computed by the software using the relations (3.1).

According to the size of the variations in the cross-sectional area, this choice may be questionable, so that true strain definitions for studying the mechanics of aortas have been introduced (Duprey, 2008; Khanafer et al., 2011).

3.2 Data analysis

The stress and strain data provided by MTS software have been post processed by Matlab software (The Mathworks Inc., Natick, MA, USA) in order to compute the



(a) (b) (c)

Figure 3.8: (a)Tensile test in execution; (b) output in terms of loads and extensions directly measured by the device; (c) output in terms of stress and strain calculated by the software

true stress and the true strain (Khanafer et al., 2011). Then, the true-stress versus true-strain curves are derived.

The passive mechanical behavior of the aorta has been investigated through the analysis of curve slopes in the physiological and supra physiological ranges of blood pressure. The physiological blood pressure in adults should be 130/80 mmHg. The top number (130 mmHg) is the *systolic* pressure whereas the bottom number (80 mmHg) is the *diastolic* pressure. Over the range 130/80 mmHg, we have considered the *failure* zone wherein the specimen breaks.

3.2.1 Stress-strain response

The stress and strain values obtained from the cross-head have been transformed into the corresponding true stress and true strain by using the relations:

$$\sigma_T = \frac{F}{A}, \qquad \delta\varepsilon_T = \frac{\delta L}{L}, \tag{3.2}$$

with A the current cross-sectional area, L the current length of the specimen and δL the instantaneous extension.

In order to correlate true with engineering quantities, we assume the arterial tissue to be incompressibility, i.e., no volume changing. The relationship between the current and the initial cross-sectional area is $A = A_0 L_0 / L$.

Consequently, the true stress is given by:

$$\sigma_T = \frac{F}{A} = F\frac{L}{A_0 L_0} = \sigma_E \frac{L}{L_0} = \sigma_E(1 + \varepsilon_E), \tag{3.3}$$

where the relations (3.1) and the position $L = L_0 + \Delta L$ have been used.

On the contrary, the true strain is obtained as as the sum of all current engineering strains:

$$\varepsilon_T = \int \delta\varepsilon = \int_{L_0}^{L} \frac{\delta L}{L} = \ln\frac{L}{L_0} = \ln\frac{L_0 + \Delta L}{L_0} = \ln(1 + \varepsilon_E). \tag{3.4}$$

3.2.2 Material elastic parameters

With the aim to describe more efficiently the elastic properties of an artery, some material parameters are considered: i) the *ultimate strain* and *ultimate stress* at the breaking point, and ii) *elastic modulus*.

According to classical elastic theories, an elastic and isotropic material shows a constant proportionality (within their elastic limits) between stress and strain. This proportionality is expressed by the familiar *Young modulus* (E):

$$E = \frac{force\ per\ unit\ area}{force\ per\ unit\ length}. \tag{3.5}$$

However, since aortic tissue is largely extensible and show a nonlinear stress-strain response, a single value of elastic modulus does not represent the continuously varying response of the tissue.

In order to take into account the variation of the elastic modulus, the *incremental elastic modulus*, which is defined as the differentiation of the stress-strain relationship ($\delta\sigma/\delta\varepsilon$) in generally used in literature (Sokolis et al., 2008).

With respect to the two investigated range of blood pressure, i.e., physiological and failure regions , two elastic moduli, i.e., *physiological elastic modulus* (PM), and *maximum elastic modulus* (MEM), are defined in a different manner.

Since the stress–strain curve is nearly linear within each investigated range, the elastic moduli are computed as the slope of the fitted line in the corresponding range.

In order to apply this method, we define the failure region and the physiological region as follows: (i) *failure region*: part of the stress-strain curve between the two points related to the ultimate strain, ε_{max}, and the 70% of ε_{max} (see, Fig. 3.9(a)); (ii) *physiological region:* part of the stress-strain curve between the two points related to the stress values σ_{80} and σ_{120} corresponding to a blood pressure of 80 and 130 mmHg, respectively (see, Fig. 3.9(b)).

Following Duprey (2008), the stress values σ_{80} and σ_{130} are computed by using the

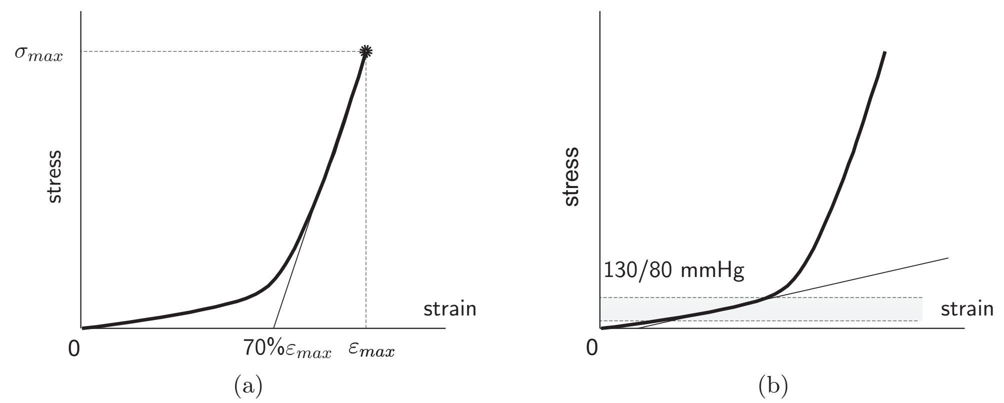


Figure 3.9: Graphical represantation of elastic modulus on an experimental curve: (a) maximum elastic modulus (MEM); (b) phisiological elastic modulus (PM)

Laplace law for thin-thickness tube:

$$\sigma_P = \frac{Pd}{2t}, \qquad \text{with} \qquad t/d < 0.10, \tag{3.6}$$

with p the inner pressure, d the inner diameter and t the thickness of the thin vessel.

The adoption of the *Laplace law* is widely spread in the mechanics assessment of aorta (Vorp, 2007), nevertheless the assumption of thin-thickness should be always validated. The samples under investigation in this study had an average thickness of 3 mm and a related diameter of 30 mm. For some samples this assumption would not be valid, especially in dissection occurrence, where the blood coagulation in the false lumen leads to a large increase in the wall thickness.

Chapter 4

Experimental results

The testing protocol described in the previous chapter has been adopted on samples of porcine and human aorta.

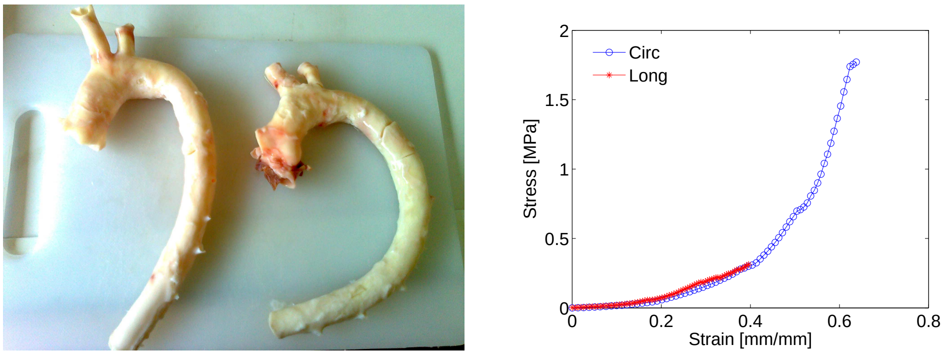
Although in the literature porcine data have been widely assumed as a good approximation of human, in this work porcine data are only used to validate the testing procedure. However, some recent studies as the work of Martin et al. (2011) demonstrated that significant differences exist between pig and human aortas, and this motivates our assumption.

The human aortic samples are excised from patient undergoing to surgical repair (aneurysm or dissection) at Policlinico of San Donato, Milano. The samples provided belongs to different aortic districts, such as ascending aorta, aortic arch, descending aorta and abdominal aorta. Based on information from pre- and post-operative exams, the aortic samples are classified according to the aortic district and the cardiovascular risk factor.

4.1 Results on porcine aorta

Two aortas freshly harvested are taken from a local slaughter (see Fig. 4.1(a)). Six specimens, three in both circumferential and longitudinal direction, are cut from ascending and descending aorta with the metal dyne, obtaining the typical dog-bone shape. Tensile tests are carried out without video extensometer strain acquisition, since it was not yet available.

The Fig. 4.1(b) shows the typical stress-strain response for circumferential and



(a) (b)

Figure 4.1: (a) Picture of the two pig aortas investigated; (b) two representative stress-strain curves obtained from the tensile tests

longitudinal directions. The curves display the common non linear elastic behavior of the aortic wall. All the longitudinal specimens fail at lower tensile strength than the circumferential. The responses in both tensile directions do not evidence anisotropy, in contrast with the work of Gundiah et al. (2008). The curve in that article, however, do not strongly display this kind of behavior that, probably, as Sokolis (2007) found, increases in the aorta distally, where circumferential direction becomes progressively stiffer.

In order to assess the validity of the method through which the highest stiffness is calculated, the MEM defined in this work is applied to another curve described in literature (Sokolis et al., 2008) and the value obtained is then compared to the corresponding result of the authors. A typical stress-strain relationship of a strip from a porcine aortic arch, undergone to uniaxial tensile test, is analyzed. Sokolis et al. (2008) defined the maximal stiffness as the peak of the incremental elastic modulus, leading to 0.78 MPa for this curve. The linear regression approach provides the same result, showing the legitimacy of the method.

4.2 Results on human aorta

The samples of human aortic are excised from five patients still alive undergoing to surgical repair at Policlinico San Donato. From the donors, eight samples are freshly obtained.

As shown in Tab. 4.1, the samples are correspond to the different aortic districts, i.e., ascending, descending and abdominal as well as the arch. With regard to the

Table 4.1: Donor informations

	Sex	Age	Reason of surgery	Correlated pathologies	Aortic district
CT-1945	M	66	Aneurysm	–	Ascending aorta Aortic Arch
TA-1963	M	48	Aneurysm	BAV	Ascending aorta
ZA-1956	M	65	Aneurysm	–	Suprarenal aorta Subrenal aorta
MA-1938	M	73	Dissection	–	Descending aorta
TC-1975	F	36	Dissection	LDS	Ascending aorta Descending aorta

reason of surgery it is possible to distinguish the aneurysm and the dissection.

Unfortunately, we made to investigate the elastic mechanical properties only in the ascending and descending aorta districts, so that the results obtained testing only the ascending and descending aorta samples are reported in this chapter.

In fact, the abdominal aorta samples and the ascending aorta tissue with Loeys-Dietz syndrome were heavily damaged, so that poor data have been obtained. On the contrary, the samples of aortic arch have been disregarded due to the little interest in literature and then to the paucity of experimental data for comparison. However, our experimental data concerning aortic arch , LDS ascending aorta and the abdominal aorta are summarized in Appendix.

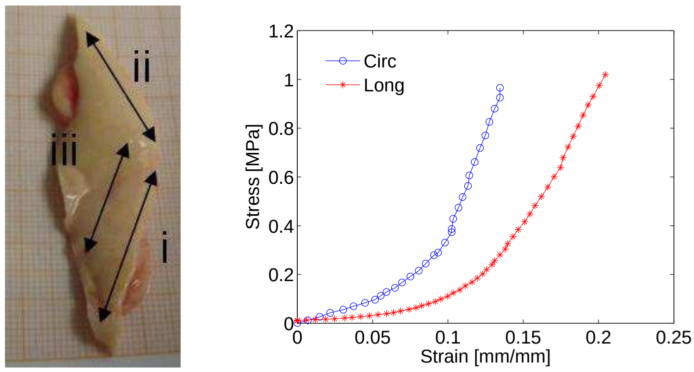
Finally, our results are compared with experimental data available in the literature. It is worth noting that ascending aorta aneurysm is a wide spread topic of the literature domain, so that many works may be found. On the contrary, experimental data on descending aorta are few since, only recently, the brand-new medical breakthrough (endografts) demands an increasing knowledge about this segment of the aorta.

4.2.1 Results on ascending aorta

Ascending aorta, CT-1945

With respect to ascending aorta, here, we refer to the sample labeled CT-1945, see Tab. 4.1. From this sample, three specimens are obtained: one in the circumferential direction and two in the longitudinal one, as indicated in Fig.4.2(a).

The obtained elastic moduli, MEM and PM, are reported in Tab.4.2, whereas the stress-strain curves are plotted in Fig.4.2(b). As shown in Tab.4.2, the values of elastic



(a) (b)

Figure 4.2: (a) Visual inspection of ascending aorta with specimens indication; (b) representative curves for circumferential and longitudinal direction

modulus are different in the circumferential and longitudinal direction. This evidences an anisotropy in the tissue. Moreover, we have also found that both MEM and PM moduli are higher in the circumferential direction than the longitudinal one.

Table 4.2: Elastic moduli in the circumferential and longitudinal direction for CT–1945 sample

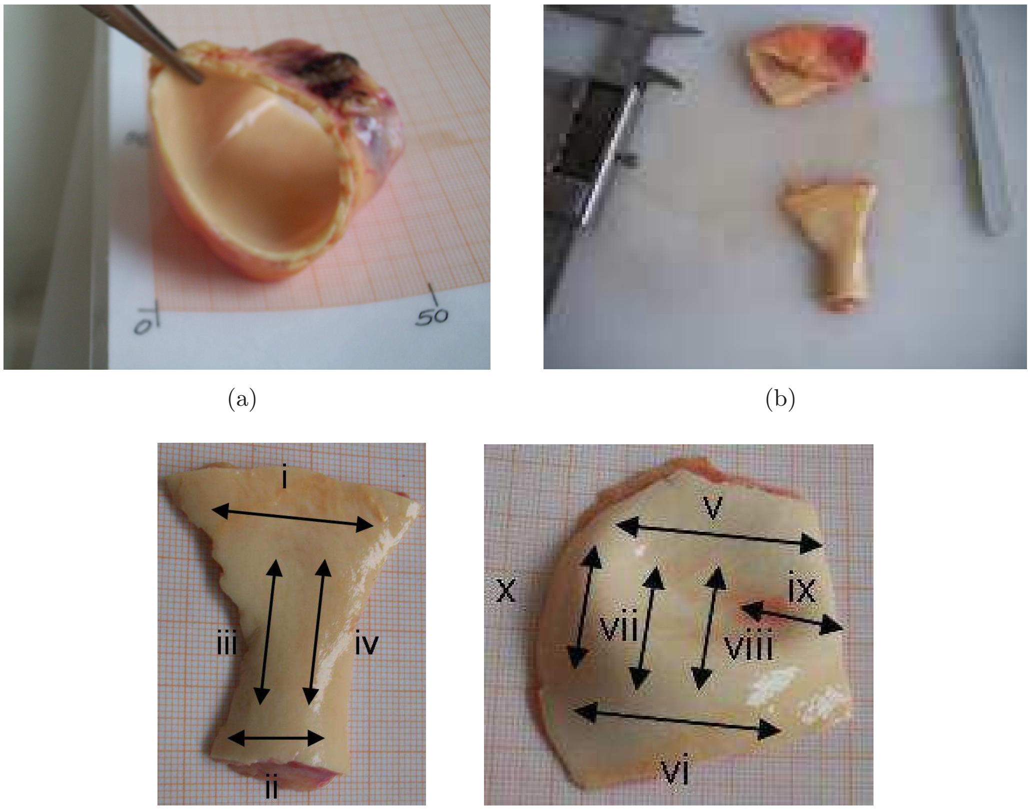
	Direction	MEM [MPa]	PM [MPa]
CT–1945	Circumferential	17.13	3.14
	Longitudinal	10.87	2.35

In the literature, Vorp et al. (2003), reported no significant difference in MEM between circumferential and longitudinal specimens (4.46 MPa versus 4.48 MPa). However Iliopoulos et al. (2009), and Khanafer et al. (2011) more recently, reported significant differences in MEM between circumferential and longitudinal orientations for ascending aorta aneurysms (7.15 MPa versus 4.6 MPa).

Ascending aorta with BAV, TA-1963

The sample TA-1963, resembles the patient with BAV (see Tab.4.1). This cardiovascular deformation is regarded as a risk factor for aneurysm occurrence, since it impairs the hemodiamic forces acting on the wall.

As long as the sample is a complete ring (see Fig.4.3(a)), we decide to cut it sagitally (see Fig.4.3(b)), in order to investigate the greater and lesser curvature of the ascending aorta. Four strips are cut from the greater curvature (GC), two for both directions (see Fig.4.3(c)); six specimens instead are obtained from the lesser curvature (LC), three in



(a) (b)

(c) (d)

Figure 4.3: (a) Visual inspection of the whole ring of the ascending aorta sample; (b) sagittal cut of the ascending aorta; (c) greater curvature with specimens indication; (d) lesser curvature with specimens indication.

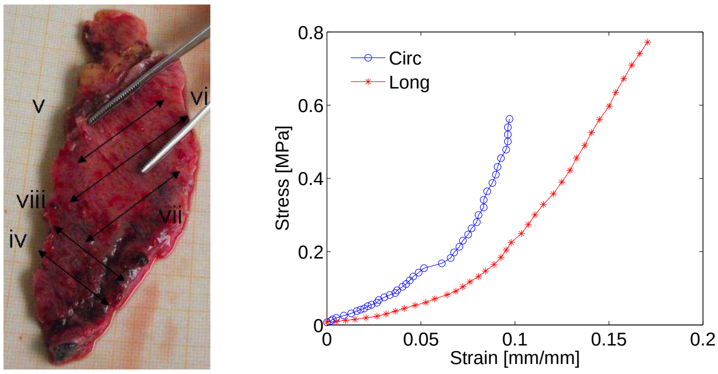
the circumferential orientation and three in the longitudinal one (see Fig.4.3(d)).

The elastic moduli, shown in Tab.4.3, display a different trend among curvature and direction. If on one hand the MEM is stiffer in the circumferential region for both the regions with higher value for the GC, the longitudinal direction appears as stiffer in the LC. The PM values instead show no large differences.

In a previous work, Choudhury et al. (2009) studied the mechanical properties of GC and LC in BAV ascending aortic aneurysm with equibiaxial tests. The stiffness of the greater curvature was underlined but no significant differences proved the anisotropy of the tissue. On the contrary, Duprey et al. (2010), performing uniaxial tensile tests, reported values close to ours: 9.37 MPa versus 4.39 MPa in GC and 9.96 MPa versus 2.78 MPa, confirming the anisotropic behavior.

Table 4.3: Elastic moduli circumferential and longitudinal orientation in relation to GC and LC, for TA-1963

	Curvature	Direction	MEM [MPa]	PM [MPa]
TA-1963	Greater	Circumferential	8.57	0.60
		Longitudinal	2.70	0.65
	Lesser	Circumferential	6.51	1.05
		Longitudinal	4.73	0.68



(a) (b)

Figure 4.4: (a) Visual inspection of a fragment of descending aorta with specimens indication; (b) representative curves for circumferential and longitudinal direction

Finally, the same work (Duprey et al., 2010) analyzed the elastic modulus in the physiological range (120/80 mmHg), sustaining that the circumferential orientation is stiffer than the longitudinal one.

4.2.2 Results on descending aorta

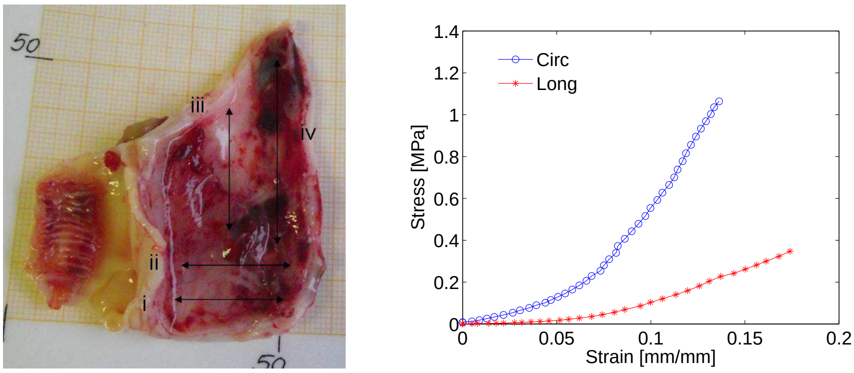
The experiments following described have no reference in previous studies, hence all data extracted will just be commented on the knowledge acquired in this work of thesis.

Dissected descending aorta, MA-1938

In relation to the dissected descending aorta, the first sample investigated is MA-1938 (see Tab.4.1). Three natural regions were obtained, differentially damaged. Eight strips were cut, but, since during preconditioning some failed, only five were actually tested: two in the circumferential direction and three in the longitudinal one (see Fig.4.4(a)).

Table 4.4: Elastic moduli in the circumferential and longitudinal direction for MA-1938

	Direction	MEM [MPa]	PM [MPa]
MA-1938	Circumferential	8.4	3.27
	Longitudinal	5.58	2.27



(a) (b)

Figure 4.5: (a) Visual inspection of descending aorta (LDS) with specimens indication; (b) representative curves for circumferential and longitudinal direction

The value of the elastic parameters calculated are reported in Tab.4.4, whereas two representative stress-strain relationship are depicted in Fig.4.4(b). The longitudinal stiffness is lower than the circumferential one for both maximum and physiological modulus.

Dissected descending aorta with Loeys-Dietz Syndrome

The last sample under our investigation, belongs to the young patient labeled with TC-1975. The LDS is an autosomal dominant syndrome related to the mutation of the genes encoding the transforming growth factors *TGBR1* and *TGBR2*. Such a mutation impair the microstructural interactions of the cardiovascular vessels. From the sample four strips were obtained: two in the circumferential and two in the longitudinal direction (see Fig.4.5(a)).

Table 4.5: Elastic moduli in the circumferential and longitudinal direction for TC-1975 sample

	Direction	MEM [MPa]	PM [MPa]
TC–1975	Circumferential	24.62	2.80
	Longitudinal	4.45	1.91

The Tab.4.5 reports the the elastic values obtained from the tensile test. The high stiffness calculated for MEM in the circumferential direction, is even appreciable in the curves depicted in Fig.4.5(b), where the longitudinal direction reaches higher deformations but fails at lower loads.

Chapter 5

Conclusions

The study carried out in this work of thesis consists in the investigation of the mechanical properties of the aortic tissue wall, in relation to the regional variation and pathologies involved, through uniaxial tensile tests. The biomechanics of main artery of the body, in fact, is deeply influenced by the microstructural changes of the components of the aortic wall and it is widely interesting to understand how the behavior is thus altered.

Actually, it was firstly assessed a new protocol for testing soft tissues, since no standars are defined, digging the knowledge out of previous works available in literature. Many challenges, such as the brittleness, the preservation and the manual management of the living tissues, made the task not trivial. Specific techniques and tools were adopted, indeed. The uniaxial tests, were achieved by the tensile test machine *MTS Insight*, controlled with the *TWS4* software that let the user to acquire data according to his needs. Owing a video extensometer for evaluating the samples deformation, it was possible to reduce the errors in data acquisition with respect to the traditional way of measurement (i.e., crosshead).

The elastic non linear behavior of the aortic wall, was analyzed, then, through the stress-strain relationship provided by the tensile system. In particular two elastic parameters of main interest were defined: i) maximum elastic modulus (MEM) and ii) physiological modulus (PM). As long as the changes in the modulus of elasticity let understand how the tissue deforms under stress application, the investigation of it, in the failure and physiological region of pressures, would help in assessing the mechanical response of pathological tissues.

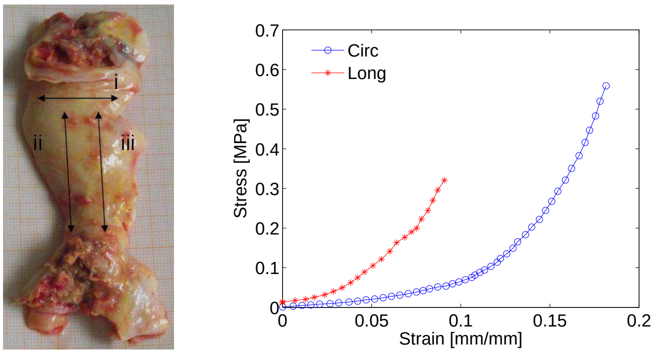
The first experiments were performed on porcine samples, in order to check the validity of the protocol in relation to the results of the literature. The anisotropy of the tissue was not confirmed, but the shape of the curves and the method applied for calculating the elastic parameters found good correspondences.

The last part of the study involved human samples testing. Several tissues were provided by the hospital of San Donato, Milano, but the unsteady nature of surgical repair, not always allowed meaningful tensile tests. With regard to the feasibility of the tests, two districts are analyzed in this work: i) ascending aorta and ii) descending aorta though the MEM and PM. If on one hand the results are globally in line with the indications found in literature, such as the stiffening of the circumferential orientation, on the other the small amount of specimens available do not allow to define a significant trend.

The new experimental data here obtained, however, encourage the understanding of the effects of rare diseases on the mechanics of aorta, for which little knowledge is still available. Moreover, specific constitutive models can be assessed in order to foresee the evolution of aneurysmal and dissected aortic tissue. Recently the clinical practice strongly requires such tools, with the aim of developing patient-specific treatment and endograft design.

Appendix

This appendix enclose some data with regard to the samples just mentioned during the discussion for the poor informations extracted.



(a) (b)

Figure 5.1: (a) Visual inspection of the sample with specimens indication; (b) two representative stress-strain curves obtained from the tensile tests.

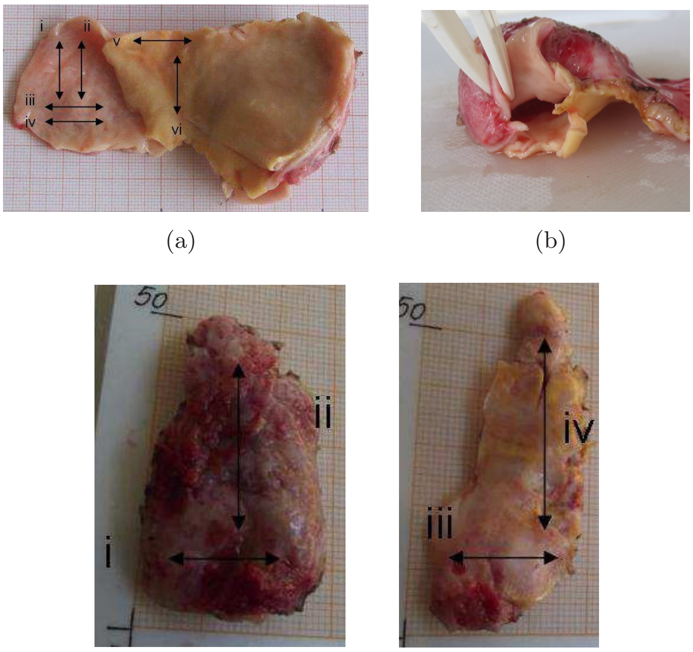
Aortic arch, CT-1945 In literature no studies, to our knowledge, are available on aneurysmal aortic arch. Moreover, the restricted number of specimens that were feasible to cut, lead us to insert the analysis in this part of the thesis. It is worth noting that samples availability is bare, hence these values need to be recorded.

The tissue provided three specimen: two in the circumferential orientation and one in the longitudinal (see Fig.5.1(a)). The stress-strain relationship of the main direction are reported in Fig.5.1(b).

Table 5.1: Elastic moduli in the circumferential and longitudinal direction for aortic arch CT–1945 sample

	Direction	MEM [MPa]	PM [MPa]
CT–1945	Circumferential	18.29	1.21
	Longitudinal	6.09	3.65

The sample under investigation showed the circumferential orientation stiffer than the longitudinal one with regard to the MEM, whereas the PM displays a higher value in the longitudinal direction.



(a) (b)

(c) (d)

Figure 5.2: (a) Visual inspection of the ascending aorta sample with specimens indication; (b) false lumen; (c) suprarenal abdominal aorta; (d) subrenal abdominal aorta.

Ascending aorta, TC-1975, and abdominal aorta, ZA-1965

The tissue damage of the two samples belonging to the patient indicated with TC-1975 and ZA-1965, respectively, compromised the success of the tensile tests. The ascending aorta was characterized by a blood coagulation (thrombus, see Fig.5.2(b)), that limited the extraction to two strips of small dimension on the whole tissue (see Fig.5.2(a)) Instead the suprarenal (see Fig.5.2(c)) and subrenal (see Fig.5.2(d)) aortic samples showed the delamination of the layers, after the excision with the metal dyne. The curves obtained had no typical J-shape on which it is possible to make the analysis.

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