Summary

The human body contains various types of tissues, such as connective, nervous and muscle tissue. Each type of tissue consists of an ensemble of cells that, together, carry out a specific function. These cells are connected by intercellular contacts and through the extracellular matrix (ECM), which is constructed of different types of fibers that form a network. This network is not only valuable because it defines the surrounding of the cells, but also because it defines to a great extent the structure and integrity of the tissue as a whole. In this thesis I have employed a variety of techniques to look at the extracellular matrix of blood vessels, to find out how it defines the mechanical properties of the vascular wall, and to see how this is disrupted in a number of pathologies.

In this study I have mainly focused my attention on the aorta. This artery originates at the heart and extends down through the belly, and brings oxygen rich blood to a large portion of the body. The aorta of an adult human has a diameter of two to three centimeters and, at rest, has a blood flow of about five liters per minute.

The aortic wall is composed of three different layers. The inner layer, the intima, consists of a layer of endothelial cells which are in direct contact with the blood. The middle layer, the media, is constructed of a network of elastin fibers. These fibers are very elastic and can be stretched up to 150% without breaking. In this layer, smooth muscle cells are also present. They keep the tissue in the correct shape and produce the components of the extracellular matrix. The outer layer of the aorta, the adventitia, is composed of a loose network of collagen fibers, which, in contrast to the elastin fibers, are very stiff.

The outer two layers of the aortic wall give rise to its remarkable mechanical properties. The blood vessel is flexible at small deformations because the collagen fibers are not yet strained. The elastin fibers, however, will stretch a bit and ensure that the vessel maintains its shape. This flexibility is of great importance for the aorta because by expanding slightly it can respond to the temporal rise in blood pressure at every heart beat. At large deformations, however, the collagen fibers in the outer layer are put under tension, preventing the blood vessel to stretch too much and rupture. A similar principle is applied to the construction of a car tire, which is composed of elastic rubber and a metal framework. The rubber allows the tire to adsorb small bumps in the road, but the metal framework

prevents the tire from rupturing when it hits the kerb.

To comprehend the mechanical functioning of the extracellular matrix of the aorta, we mainly use two, complementary, techniques. By examining the tissue with an optical microscope, we can see how the different types of fibers are woven into a network. To this end we use the optical properties of the fibers themselves. Elastin, for example, lights up when it is illuminated with green light. We also use special labels to distinguish different types of fibers, for example an antibody that sticks to a specific type of collagen and which becomes visible in red light. In this study we have made much use of confocal microscopy. This type of microscopy allows for the generation of a three-dimensional representation of the tissue, making it possible to precisely determine how the different types of fibers are interwoven. In addition, we have used the optical microscope to image other components of the vascular wall, for example cells of the immune system, in order to see how they influence the extracellular matrix.

The other technique which we have used in this study is the atomic force microscope (AFM). The central element of this instrument is a small cantilever, about 40µm wide (the thickness of a hair) and 1µm thick, with at the end a sharp tip. With this cantilever we push on the individual fibers of the tissue to measure their stiffness and to determine how much force the network can carry before it breaks. By combining these measurements with information about the construction of the network, we try to come to a rational explanation for the mechanical properties of the aorta and to see how these properties have been affected in certain pathologies.

The first pathology for which we have used this approach, is an aneurysm of the abdominal aorta. In this disease, the lower part of the aorta locally swells up like a balloon, until it ruptures, which is almost always lethal. Despite the fact that this is a relatively common pathology — about 1 out of 20 adults above 60 years old have an aneurysm — and decades of research have been spent on this specific disease, the cause of this local weakening of the vessel wall was not known until a few years ago. There is, for example, no remarkable difference in the number of collagen fibers and the connections between these fibers between a healthy and an aneurysmatic aorta. Our three-dimensional representation of the collagen fibers in the healthy tissue showed how the individual collagen fibers together form thick bundles and that those bundles together form a dense network. For the aneurysmatic tissue, however, this arrangement appears to be completely lost and we only observed an unstructured aggregation of collagen fibers. When we indented the healthy tissue with the AFM, all the individual fibers in the tissue appeared to be connected in such a way that they shared the load, keeping the tissue intact. The network of the aneurysmatic tissue

was of such poor quality that all the fibers had to bear the load independently, causing the fibers to break, thereby damaging the network. This study shows the importance of the network structure of the collagen fibers in the vascular wall and demonstrates how a loss in the organization of the network can result in the local swelling and even rupturing of the aorta.

In a follow-up study, we used the same approach to examine the contribution of the individual components of the vascular wall to the mechanical properties of the whole tissue. This was done by selectively removing the individual building blocks of the tissue using enzymes and subsequently using optical imaging and AFM measurements to observe the effect of the treatment. This showed, for example, how after removing collagen from the adventitia, a coherent network remained, which was about 50 times less stiff than the original tissue. One of the remarkable results of our study was the effect of a neutrophil extract on the tissue. Neutrophils are cells of the immune system and it is known that this type of cells is present in elevated numbers in aneurysms. AFM measurements on the adventitita showed how the neutrophil extract changed, within 24 hours, the mechanical properties of the adventitia in such a way, that it showed large similarities with the aneurysmatic tissue. Further research has to clarify which specific elements of this extract cause this change and whether this carries a promise for the treatment of aneurysms.

After the successful measurements on aneurysms, we have extended the same approach of studying tissues towards other pathologies. Atherosclerosis is a slowly progressing disease, which starts with the accumulation of lipids in the vessel wall. The resulting inflammatory reaction produces a plethora of breakdown products of cells and the extracellular matrix. These products are covered by a layer of collagen to prevent them from entering the bloodstream. During the progression of the disease, however, this collagen layer becomes weaker and can eventually rupture. The breakdown products will then enter the blood, which will start clotting. This blood clot can ultimately block the blood supply to the heart or brain and cause a myocardial infarction or stroke. Recent studies in mouse models showed that lysyl oxidase, a protein which facilitates the connection between collagen fibers (LOX), reinforces the collagen cap of atherosclerotic plaques. We continued this study by first showing that human plaques with more LOX are more stable than plaques with less LOX. We did this both optically, for example by comparing the amount of collagen with the local LOX concentration, as well as with the AFM by showing that plaque regions with more LOX tend to be stiffer as compared to regions with less LOX. Subsequently, we showed that the amount of LOX is inversely correlated with adverse clinical symptoms, like, for example, the level of inflammation within the tissue. Lastly, we showed that patients who produce more LOX suffer less from atherosclerotic complications,

like a myocardial infarction, compared to patients who produce less LOX.

In the pathologies discussed above, the interactions between immune cells and the extracellular matrix play an important role in the progression of the disease. By means of a new animal model, in which human tumor cells are injected into zebrafish embryos, we studied how tumor cells infiltrate the extracellular matrix of, until then, healthy tissue. Using various optical techniques, we showed how neutrophils, the same immune cells which are present in aneurysms, alter the collagen network of the zebrafish and that this enables the tumor cells to invade new tissue. When we inactivated the neutrophils using various drugs, the tumor cells were no longer able to infiltrate the tissue. Although the results of this study are very fresh, they reveal hitherto unknown processes, that could possibly also play an important role in human tumor progression.

With this research we have seen that the extracellular matrix plays an important role in the integrity of tissues and that this is impaired in certain pathologies. This study opens a new way to analyze pathologies and processes in the human body. An example of a possible future study could be the ripening, or softening, of the cervix (the lowerpart of the uterus) during delivery. The cervix tissue consists mainly of collagen, but the mechanism in which the initial stiff tissue transforms into soft tissue, enabling the infant to be delivered, is still unknown. This process is even more interesting because of its reversibility: within a few weeks after birth the collagen network is back in its original state.