Unravelling the collagen network of the arterial wall

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8 Epilogue

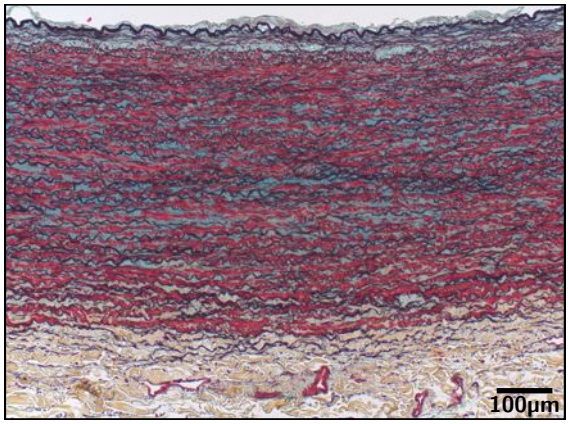

Figure 8.1: Movat stained coupe of the arterial wall.

In this thesis we have shown how the atomic force microscope, in combination with various other techniques, can be used to study the physics of the collagen network of several tissues: the aortic wall, the fibrous cap of atherosclerotic plaques in humans and the tail of zebrafish; We have also studied how the aortic tissue responds to various digestions. These studies have gained us new insights in the mechanical properties of the ECM of these tissues and how they are impaired in various pathologies.

Our experiments on aneurysms showed how the failure of the vessel wall is caused by the disruption of the spatial organization of the collagen fibers, rather than by a lower concentration of collagen.

In atherosclerosis a complex interplay between different ECM degrading enzymes on the one hand and ECM stabilizing proteins like LOX on the other, determine the mechanical stability of the fibrous cap. The AFM proved to be a valuable tool to measure local stiffness variations. This can give new clues on cap weakening by linking local stiffness variations to various inflammatory markers or ECM stabilizing proteins.

The examination of tumor development in zebrafish showed how cells from the immune system remodeled the collagen matrix, enabling the tumor cells to invade new tissue. This study revealed a new type of cell/ECM interaction in tumors which could also play a role in human cancer development

Finally we have shown how the arterial wall can be used as model system to study the effects of various proteolytic enzymes on the ECM. The measurements with the neutrophil extract showed, for example, how enzymes, produced by neutrophils, alter the tissue in such a way that it gives the same response upon indentation as aneurysmatic tissue.

This new way of studying diseases of the ECM could result in new insights in many other pathologies as well. Here we discuss possible future experiments on myocardial infarction and cervix ripening.

After a myocardial infarction the ECM of the heart has been locally disrupted. The newly formed collagen network, which has much resemblance with ordinary scar tissue, has different mechanical properties from the surrounding muscle tissue. The AFM could probe these differences, which will help to understand how the functioning of the heart is hindered by this stiffer scar tissue.

During pregnancy and delivery, the collagen network of the cervix, the lower part of the uterus, changes in such a way that the initial stiff tissue becomes soft, making it possible for the infant to be delivered. During cervix ripening, neutrophils are known to be active in the cervix, but the precise mechanism which softens the tissue is unknown. It has long been assumed that matrix metalloproteases play a major role in cervix ripening. Recent studies, however, have show that this assumption is not valid.[1] Moreover, the alteration of the ECM of the cervix is particularly interesting because of its reversibility: the ECM of the cervix is repaired within a few weeks after delivery. A combined AFM and histological study of the cervix at different stages during ripening, could link specific changes in ECM organization to the stiffness of the tissue. The AFM could furthermore be used to measure the effect of the individual proteolytic enzymes, present during cervix ripening, on the effective Young's modulus of the tissue.

Most of the biophysical research on collagen has largely focused on networks of purified collagen I, the most abundant type of collagen. In vivo, however, collagen I usually forms heterotypic fibrils together with other collagen types. The ratio between collagen V and I is known to change during tissue development and is different for various diseases, like atherosclerotic plaques and scar tissue. Piechocka et al. showed, using micro-rheology experiments, how collagen gels get softer when the ratio of collagen V to collagen I is increased.[2] It is still unknown, however, whether this effect is caused by a change in stiffness of the individual collagen fibrils or whether it is the result of a change in the connections between the fibrils. An AFM study, similar to the one we have performed on the aneurysmatic tissue, in which indenters of different sizes were used, could be used to discriminate on which length scale the network changes.

During the studies performed for this thesis, contacts have been made with a number of medical groups of different disciplines, which broadened the horizon

[1] R. A. Word, X. H. Li, M. Hnat and K. Carrick. Dynamics of cervical remodeling during pregnancy and parturition: mechanisms and current concepts. *Semin. Reprod. Med.*, 25:69, 2007

[2] I. K. Piechocka, A. S. G. van Oosten, R. G. M. Breuls, and G. H. Koenderink. Rheology of heterotypic collagen networks. *Biomacromolecules*, 12:2797, 2011

of all groups involved. The work described in this thesis fits into the growing sense among medical researchers to appreciate the ECM not only as the environment of cells, but also as an important part of tissues with its own dynamics. There is a growing awareness that for understanding the functioning of an organ the organization and characteristics of the surroundings of the cells is equally important as the cells themselves. For the physicists, these studies have been one of the first crossings of the gap between a well controlled in vitro experiment or a theoretical study to an in vivo system that involves many more components and uncertainties.

I hope that this study has contributed to the development of a new branch of multidisciplinary research, which, in the near future, will unveil exciting new physics as well as new clinically relevant insights.