ULTRASTRUCTURAL STUDY OF INDUCED VASCULAR DAMAGE CAUSED BY IN VITRO STENTING

Gerhard Sommer (1), Melanie Pranger (2), Dagmar Kolb (2,3), Gerd Leitinger (2), Gerhard A. Holzapfel (1,4)

1. Institute of Biomechanics, Graz University of Technology, Austria; 2. Gottfried Schatz Research Center, Medical University Graz, Austria; 3. Core Facility Ultrastructure Analysis, Medical University Graz, Austria; 4. Department of Structural Engineering, Norwegian University of Science and Technology, Norway

Introduction

The number of coronary stent implantations (CSI) is rapidly increasing worldwide, with the most challenging complication after CSI being restenosis [1]. In addition to inflammatory processes, the degree of restenosis primarily correlates with the severity of vascular injuries [2], which are unavoidable during and after CSI interventions. To reduce the fatality rate of stents in CSI, scientists and manufacturers must develop safer stents by significantly reducing the risk of vascular injury. Therefore, in this study, we aimed to investigate coronary artery damage induced by *in vitro* stenting. This study of vascular damage is based on a biomechanical experiment simulating the loading conditions of stenting *in vitro*. After mechanical testing, damage to a coronary artery by *in vitro* CSI was investigated at the ultrastructural level using electron microscopy.

Methods

To simulate the *in vitro* loading condition during CSI, inside a custom-built test chamber, a square sample of a porcine coronary artery was stretched in two orthogonal directions while a stamp in shape of a stent-strut was indented into the sample with defined loads and orientations. To demonstrate the structural alterations under loading, coronary artery walls (unloaded vs. biaxially loaded and stamped) were chemically fixed with glutaraldehyde and prepared for electron microscopy investigations. On the one hand, the surface and the cross section of the vessel were examined using scanning electron microscopy (SEM), on the other hand, mechanically important constituents such as collagen, smooth muscle cells (SMCs), and proteoglycans (PGs) in the artery layers were made visible using electron tomography (3D-TEM).

Results and Discussion

The SEM results obtained in this study revealed that damage to the surface of the coronary artery after stenting was more pronounced when the stamp was oriented longitudinally than circumferentially. This could be explained by the structural composition of the media, where collagen fibers are preferentially oriented in the circumferential direction. Accordingly, it would be reasonable to design the stent network in such a way that the stent struts are primarily oriented in the circumferential direction. However, this is not the solution to avoid in-stent restenosis. The endothelial layer of the coronary artery was completely removed/injured under and around the area of the indented stent strut. Therefore, the development of restenosis will occur regardless of the orientation of the stent strut.

Regardless, the results showed that not only the intima of the coronary artery was damaged, but also the tunica media. The SMCs in the area of the stent strut were severely damaged, so that their functionality was questionable. It appears that the collagen fibrils were able to withstand the enormous mechanical stresses from the applied stent loads. Interestingly, PGs rearranged between the collagen fibrils. This behavior was detected by 2D TEM and could be confirmed with the help of 3D TEM and subsequent 3D reconstructions. Those revealed that PGs under the stented area were displaced by the dense structure of collagen fibrils, where PGs accumulated (see Fig. 1). This reorientation and clustering of PGs was more pronounced below the stamp mark than lateral to the stamp edges.

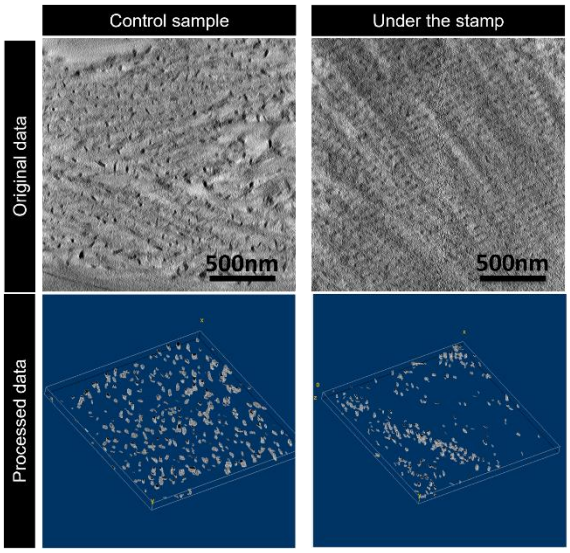

Figure 1: Representative reconstructed electron tomography images showing the arrangements of PGs in an uninjured control sample (left) and an injured sample under the stent strut (right).

References

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2. Mitra and Agrawal, J Clin Pathol; 59:232–9, 2006.

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