Determination of induced vascular damage by in vitro stenting

Gerhard Sommer*[1], Melanie Pranger[2], Lisa Wallinger[1], Dagmar Kolb[2,3], Gerd Leitinger[2], Christian Viertler[4], and Gerhard A. Holzapfel[1,5]

[1]Institute of Biomechanics, Graz University of Technology, Austria – Austria
[2]Gottfried Schatz Research Center, Medical University of Graz, Austria – Austria
[3]Core Facility Ultrastructure Analysis, Medical University of Graz, Austria – Austria
[4]Diagnostic and Research Institute of Pathology, Medical University of Graz, Austria – Austria
[5]Department of Structural Engineering, Norwegian University of Science and Technology, Norway – Norway

Abstract

Restenosis remains the most challenging complication after coronary stent implantation (CSI) (1), where the degree of restenosis largely correlates with the severity of vascular injuries (2). To reduce the failure rate of such coronary artery interventions, scientists and manufacturers must develop 'safer' stents by significantly reducing the risk of vascular injury. In this study, we investigated the damage to coronary arteries caused by *in vitro* stenting, where the vascular damage is based on a biomechanical experiment that simulates the loading conditions of stenting *in vitro*.
Damage to porcine coronary arteries caused by *in vitro* CSI was examined at different structural levels using histological investigation, electron microscopy modalities, and collagen hybridizing peptides (CHPs). In a custom-built testing chamber, the *in vitro* loading condition during CSI was simulated by biaxially stretching squared porcine coronary artery samples while a stent-mimicking punch was pressed into the sample with defined loads and orientations. Electron microscopy investigations were used to investigate the structural alterations under different loading conditions of the coronary artery walls (unloaded vs biaxially loaded and punched). Histological stains applied to cross-sections of arterial tissue specimens were utilized to identify mechanical damage at the microstructural level. Moreover, the surface and cross section of the vessel were examined using scanning electron microscopy (SEM). Mechanically important components such as collagen, smooth muscle cells (SMCs) and proteoglycans (PGs) in the arterial layers were visualized using 3D-TEM (electron tomography). Finally, fluorescently labeled CHPs shed light on the molecular unfolding of collagen and provide insights into damage mechanisms that occur at the molecular level.

Light microscopy images of the stained histological sections revealed focal, prominent, longitudinally oriented vascular SMC bundles in the intima, accompanied by a duplicated internal elastic membrane. In the indentation-treated specimens, significant compression below the indentation mark, stretched-out nuclei on the lateral walls of the lesion, bulging at the lesion edges, and structural weaknesses in the intima and media within the lesion were observed. The SEM results showed that the surface at the punch ends was more damaged when the

*Speaker

punch was oriented axially rather than circumferentially. Moreover, the endothelial layer beneath and near the indented punch was completely removed. The results further revealed that not only the intima but also the media were severely damaged. The functionality of SMCs around the punch is questionable due to the damage. Interestingly, collagen fibrils appear to be able to withstand the enormous mechanical stresses caused by the applied punch loads. The 2D TEM and electron tomography studies showed rearrangement, reorientation and clustering of PGs between collagen fibrils due to the high loadings of the punch. The reorientation and accumulation of PGs was more pronounced below the punch mark than to the side of the punch edges. Analysis of the CHP fluorescence data revealed mechanically induced damage at the molecular level, particularly in the intimal region lateral to the punch mark, which was aligned with elongated nuclei, instances of intimal tearing, and other observed structural weaknesses. Overall, these findings suggest that the functionality of arterial tissue is significantly affected by the applied indentation force. Functional alterations in stented arteries may ultimately lead to the development of restenosis.

References

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Acknowledgements
This work was supported by grant no. P32713 from the Austrian Science Fund (FWF).