Endothelial cell forward migration in a disturbed wall shear stress environment is promoted by ROCK inhibition

S. Hsiao1; FJ. Tovar-Lopez2; J. Gunn1; T. Spencer3; I. Halliday3; C. Perrault; PC. Evans1
1University of Sheffield, Cardiovascular Science, Sheffield, United Kingdom; 2RMIT University, Melbourne, Australia; 3Sheffield Hallam University, Materials and Engineering Research Institute, Sheffield, United Kingdom

Purpose: Stent deployment to treat coronary artery disease causes damage and loss of endothelial cells (EC). Repair of injured arteries by EC migration is co-ordinated by Rho family GTPases. It is also regulated by wall shear stress (WSS), a mechanical force exerted by flowing blood on the vessel wall, via mechanisms that are poorly understood. It was hypothesised that stent struts may impede repair of injured endothelium by inducing localised disturbances in WSS.

Methods: To simulate a stented artery in vitro, chamber slides were fabricated with ridges (100 μm high) positioned perpendicularly to the flow direction. Confluent EC monolayers seeded on one side of either ridged or non-ridged (control) chambers were exposed to flowing culture medium (Ibidi system). Flow patterns were determined by computational fluid dynamic (CFD) modelling and particle velocimetry. Live cell imaging and analysis using ImageJ software enabled quantitation of EC migration velocity and directional persistence (DP).

Results: CFD modelling and live cell imaging indicated that EC on the non-ridged chamber slide were exposed to a uniform WSS of 13 dyn/cm² and migrated relatively uniformly in parallel with the flow direction (average velocity 1.13 ± 0.20 μm/min; DP 0.59 ± 0.14). By contrast, significant spatial differences in WSS were observed over the ridged slide in CFD, with significant spikes above physiological levels (>70 dyn/cm²) at the corners of the ridges and distinctive flow recirculation zone immediately upstream/downstream from the ridge (<4 dyn/cm²). These features were verified by particle velocimetry using fluorescently labelled polystyrene beads (2 μm diameter). Time-lapse imaging further revealed interrupted EC migration at the ridges. Specifically, although EC could migrate over the ridges, those that reached the recirculation zone downstream from the ridge migrated with non-uniform directionality (DP 0.25 ± 0.06) and displayed a reduction in velocity (0.78 ± 0.18 μm/min). Inhibition of the RhoA/ROCK signalling pathway with ROCK inhibitors (Y27632 or HA1077) promoted EC forward migration within the recirculation zone by significantly elevating DP (0.41 ± 0.04, p = 0.01) and velocity (1.16 ± 0.09 μm/min, p = 0.01).

Conclusions: Disturbed WSS downstream from stent strut-like ridges prevented the forward migration of EC. Inhibition of the RhoA/ROCK signalling pathway promoted EC migration and re-population of these sites. Our data suggest that treatment using a ROCK inhibitor may promote re-endothelialisation of stented arteries, a concept that is currently being tested using a porcine model.