O3: Endothelial Function and Shear Stress

O3-1 Arrangement and morphology of endothelial cells under the mechanical microenvironment changes after vascular stent implantation

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Vascular stent implantation will cause intravascular hemodynamic changes and Vascular endothelial cells (VECs) will be damaged during the process of stent expansion. The purpose of this study is to investigate changes in morphology and function of VECs after vascular stents implantation which can effect shear stress on the surface of stents. The stents were implanted to the coronary arteries of pigs and removed after 1 month, 3 months, 6 months, 12 months and 24 months. Then, the morphology of neointima with stents was observed by scanning electron microscopy. According to the results of the simulation, the VECs were cultured under different mechanical conditions to observe the morphology and arrangement of the cells. To verify the changes of intercellular junctions after stent implantation the expression of F-actin, VE-cadherin and Rac1 were investigated. We found 4 weeks after implantation, neonatal VECs had completely covered the surface of the scaffolds, and the cells were rounded. As the time prolonged, the cell morphology gradually changed to the spindle shape. Studies have shown that round-like VECs were unhealthy, and long spindle-shaped VECs could play a normal function to maintain the stability of the blood vessel environment. In the strut and V-shaped of the stent, the VECs were paving along the strut. The connecting rod ("S" position) was structurally complex, and the arrangement of the VECs in these parts was also complicated. The phenomena in animal experiments were basically consistent with the computer simulation. In vitro, cell experiments found the cytokines related to arrangement and intercellular connection, and obtained similar results.

O3-2 Blood Flow Regulates Zebrafish CVP Angiogenesis by Inducing ERK5 Signaling

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Vascular network formation induced by angiogenesis plays important roles in physiological and pathological processes. Much has been known about the role of blood flow in regulating angiogenesis for the straight or curving vessels in zebrafish. However, the contribution of blood flow and underlying mechanisms in vascular network formation such as caudal vein plexus (CVP) development is poorly understood.

Here, our data from tnnt2a-MO injection and treatment with chemical blood flow modulators showed that decreased blood flow disrupted CVP formation in zebrafish and the hemodynamic force was quantitatively analyzed. Furthermore, CVP angiogenesis in zebrafish embryos is inhibited by disruption of blood flow downstream effector ERK5, klf2a and nos2b by treatment with ERK5 specific inhibitor or injecting klf2a-MO, nos2b-MO. Meanwhile, overexpression of klf2a mRNA or nos2b mRNA could rescue vascular defects in tnnt2a or klf2a morphants. These data suggested that flow-induced ERK5-klf2a-nos2b signaling is involved in CVP angiogenesis in zebrafish embryos. Finally, we found the mechanical signal

transduction pathway in which mechano-sensitive F-actin polymerization induced cell contractility to activate klf2 and nos3 signaling.
Taken together, we have demonstrated that blood flow is essential for vascular network termed CVP angiogenesis in zebrafish. A novel genetic and mechanical mechanism was discovered in which the F-actin contractility facilitates integration of blood flow with the downstream ERK5-klf2a-nos2b signaling axis to guide CVP angiogenesis.

O3-3 The role of Id1 in oscillatory shear stress-mediated endothelial lipid uptake

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Inhibitor of DNA binding 1 (Id1) has been shown to be involved in lipid metabolism, which is pivot for atherosclerotic progression. However, it remains unclear that Id1 regulates endothelial cell functions and atherosclerosis in response to oscillatory shear stress. The current study aims to evaluate the effects of oscillatory shear stress on LDL uptake by endothelial cells and to delineate the roles of Id1 in this process. Using an in vivo ligation model of $ApoE^{-/-}$ mice and applying low and oscillatory shear stress (OSS) in vitro, we found that OSS can effectively promote lipid uptake. The results from in vivo en face staining showed that OSS exposure decreased Id1 expression. In vitro, OSS transiently promoted Id1 expression at early time, but eventually OSS resulted in a reduced expression of Id1 with the passage of time. Furthermore, we found that overexpression of Id1 can abolish OSS-mediated lipid uptake in ECs. Mechanically, we demonstrated that Id1 interacted with srebp1 to regulate LDLR expression, therefore influencing lipid uptake in endothelial cells. Together, our study shows a biomechanical role of endothelial Id1 in lipid uptake by down-regulating LDLR, which could help us understand oscillatory flow how to affect atherosclerotic development.

O3-4 Effect of DNA methyltransferase 1 in oscillatory shear stress-induced atherosclerotic vulnerable plaque formation

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Oscillatory shear stress (OSS) is one of the important hemodynamics factors contribute to atherosclerosis progression. Epigenetic mechanisms such as DNA methylation have been reported to play roles in regulating endothelial functions in this pathological process associated with flow characteristics. Here, we aim to explore the mechanism of OSS on vulnerable plaque formation. We established a carotid partial ligation model combining with high-fat diet, and found aggravated intima thickening and eventually vulnerable plaque formation accompany with abundant new immature vessels. Matrix metalloproteinase 9(MMP9) and vascular endothelial growth factor (VEGF) immunohistochemical staining also marked the neovascular locating in the vulnerable area. Furthermore, Masson and Sirius red staining confirmed the degradation of collagen which indicating the less stability of the

plaque. In vitro, we applied parallel flow chamber system to study how the OSS affects endothelial cell function. We found that OSS upregulated both DNA methyltransferase 1(DNMT1)and MMP9expressionPharmacological inhibition of DNMT1 by 5-Aza-2'-deosycytidine diminished the OSS induced DNMT1 and MMP9 up-regulation, and also inhibited the migration and angiogenic capacity of endothelial cells. In conclusion, our data demonstrated that OSS accelerated the vulnerable plaque formation may be through DNMT.
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O3-5 The influence of hemodynamic changes on proliferation and adhesion of endothelial progenitor cells

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It was well documented that vascular endothelium is directly responsive to hemodynamic changes. The purpose of this study is to investigate the effect of hemodynamic changes on proliferation and adhesion of endothelial progenitor cells (EPCs) so as to make use of the excellent characteristics of EPCs to provide theoretical basis for the prevention of in-stent restenosis.
In this study, we isolated endothelial progenitor cells from the rat bone marrow, and EPCs were exposed to different shear stress by flow chambers. We observed the dynamical change of the orientation and morphology of EPCs, which these cells adhered to various antibody substrates (either of CD133, CD31, VEGFR2) at the exposure of flow shear stress with different duration. We found that appropriate shear stress ($5 < \tau > 20 dynes/cm^2$) promote proliferation of EPCs and the EPCs adhered to CD133 substrate had the maximum proliferation ability. However, the proliferation rate of either too high or too low shear stress was decreased. In the test of retention rate determination, we found that EPCs rarely dropped off in central zone, but had a few in the edge, entrance and exit of flow chamber. The results indicate that the adhesion phenomenon of EPCs was taken off, which suggests that the effect of shear stress on physiological function still need to be further investigated.
In conclusion, our results proved that under flow shear, CD133 antibody substrate presented stronger absorption and retention ability to EPCs than the other two substrates, CD133 substrate is more specific for EPCs. The stent coating CD133 substrate could capture more EPCs in vivo, and promote the proliferation and adhesion of EPCs.
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O3-6 Short term effects of the Mediterranean Diet in human microvascular function - comparison between older and younger healthy, sedentary adults.

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Objective: To determine whether short-term adherence to the Mediterranean Diet (MD) is associated with improved microvascular function. Methods: We conducted a single-centre, cohort pilot study in Sheffield, UK. Twenty-four healthy, sedentary younger (18-35 years)

and older (55-75 years) adults were instructed and supported to adapt their current diet to meet the Mediterranean Diet adherence criteria for 4 weeks. We conducted baseline and post-intervention measurements of microvascular function using laser Doppler fluximetry, Transcutaneous oxygen pressure. Results: We identified statistically-significant improvements in axon-mediated microvascular vasodilation (2.24 (±0.56) to 3.14 (±0.84), P =0.03) and endothelial-mediated NO synthesis (2.59 (±0.67) to 3.32 (±0.87), P =0.022) in the younger group. No statistical significance was reached within the older participants' group, although Raw CVC increased following the intervention. Conclusion: Improvements in physiological function were observed following a short-term dietary intervention based on the MD in a younger population. These were not matched in an older group. Our findings suggest that different durations should be applied when designing dietary interventions in different age-groups, with expectations in physiological improvements differing between groups.