O1: Cellular Rheology and Biophysics

O1-1 Albumin solder covalently bound to a biodegradable polymer membrane: New approach to improve binding strength in laser tissue soldering

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As the gold standard of small blood vessel anastomosis micro-suturing shows drawbacks such as increasing risks of hypoxia as well as tissue damage. Laser tissue soldering (LTS) might be a promising alternative. However the achieved sheer strength is too weak in many cases. It has been shown that the cohesive strength of the liquid solder can be enhanced by using carrier materials. In the present study a poly(ether imide) (PEI) membrane served as carrier material and indocyanine green (ICG)-supplemented albumin as solder substrate. In order to further strengthen the obtained solder weld albumin was covalently coupled to the carrier membrane. The coating was performed under physiological conditions to prevent structural protein changes. The albumin functionalized carrier membrane was placed onto the tunica media of explanted pig thoracic aortae forming an overlapping area. Using a diode continuous-wave laser an ICG-mediated heat-denaturation of the albumin could be achieved. The LTS could generate a membrane-blood vessel connection corresponding to 15% of the tensile strength of the native blood vessel. According to these results the sheer strength of a native blood vessel can be achieved by applying this method at an overlapping zone of appropriate size. Further studies in animal models should be conducted to confirm the beneficial effects of the obtained in vitro results.

O1-2 Circumferential alignment of smooth muscle cells in micro-tube environment

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Ordered arranged smooth muscle is an important component of tubular tissues（such as trachea and blood vessels）in vivo, which is responsible for maintaining morphology and mechanical properties. Growing evidences reveal that tubular envirronment could affect the movement and alignment of cells, but it is unknown whether this is sufficient to form a stable arrangement of cells. Thus, we fabricated micro-patterned cylindrical concave and convex surface with different curvature diameters（cell scale）with PDMS and inoculated with different types of cells in the tubular tissues. Results showed that the different degrees of morphology and biology differentiation occurred in all kinds of cells compared with the planar environment. Significantly, both of the two kinds of smooth muscle cells（ASMCs, VSMCs） could form a highly ordered pattern perpendicular to the tube axis in a given curvature environment. In our models, these behaviors of cells might be related to the architecture on curved surface and change with cell tension and adhesion ability. Altogether, our finding helps to better understand the tissue development and provide new idea for tissue engineering.

O1-3 Subhaemolytic mechanical trauma increases RBC aggregation by altering cell electrochemistry

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The ability of red blood cells (RBC) to aggregate, disaggregate, and deform greatly influences systemic blood fluidity and oxygen delivery. The major intrinsic disaggregating force of RBC is determined by their electronegative charge, created by sialic acids (SA) located within the glycocalyx. Given subhaemolytic shear environments within mechanical circulatory support (MCS) have been reported to alter cell morphology, we hypothesised that similar shear exposure would also cleave membrane bound SA, altering the electrochemical and physical properties of RBC.

RBC from 20 healthy donors were isolated and resuspended in an isotonic viscous suspending medium at 0.15 L/L. A Poiseuille shearing system was constructed and used to expose RBC suspensions to 125 Pa for 1.5 s. RBC were examined for: aggregation in autologous plasma, disaggregation shear rate threshold, ability to aggregate extrinsic to plasma factors (i.e. aggregability), SA concentration (utilising periodate-resorcinol method), and electrophoretic mobility.

Shear exposure increased RBC aggregation, disaggregation shear rate threshold, and RBC aggregability. The concentration of SA significantly increased in the shearing supernatant, and decreased in isolated RBC membrane ghosts. The electrophoretic mobility significantly decreased following shear exposure, confirming RBC had become less negatively charged.

Acute subhaemolytic shear exposure may remodel the RBC membrane, removing SA, thereby altering electrochemical and physical properties of RBC. As RBC aggregation/disaggregation is a primary determinant of blood fluidity (and oxygen delivery), the present observation may partly explain the increased incidence of microvascular dysfunction and ischaemic complications in patients receiving MCS.

O1-4 Subhaemolytic mechanical damage alters erythrocyte behaviour in subsequent low-shear flows

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Background: Shear environments within mechanical circulatory support (MCS) are associated with subhaemolytic trauma of red blood cells (RBC), that alters their physical properties, hindering their function. Given current complications of MCS use may be explained by microvascular impairment, we aimed to explore the behaviour of RBC in low-shear flows following exposure to subhaemolytic shear environments.

Methods: Healthy RBC were washed and isolated before being resuspended in a plasma-like substitute. RBC were exposed to 100 Pa of shear in a Couette shearing system for 60 s. RBC were then transferred to a slit-flow microfluidic chamber that allowed for visualisation and

concurrent laser-diffractometry. Flow rates of RBC suspensions were progressively increased to yield a shear range of 0.01-5 Pa. Laser-diffractometry was used examine RBC deformability, while visualisation provided assessment of cell orientation, altered morphology, and presence/size of aggregates.

Results: Shear exposure increased formation of aggregates that tumbled in low-shear flow, resulting in atypical trends of captured diffraction patterns. Increased shear was subsequently required to disaggregate rouleaux prior to cell orientation and deformation. Curiously, laser-diffractometry with concurrent visualisation revealed that laser-diffraction distortions were also observed with deforming subpopulations of rouleaux that had failed to disaggregate.

Conclusion: Exposure of RBC to subhaemolytic shears alters cell behaviour in subsequent low-shear flows. It is plausible that blood exposed to similar shears in MCS may demonstrate similar responses in vivo, and thus may disrupt low-shear blood behaviour in addition to the well-described changes in cell deformability.

O1-5 Ultrafast imaging of cell elasticity with optical microelastography

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Elasticity is a fundamental cellular property that is related to the anatomy, functionality and pathological state of cells and tissues. However, current techniques based on cell deformation, atomic force microscopy or Brillouin scattering are rather slow and do not always accurately represent cell elasticity. Here, we have developed an alternative technique by applying shear wave elastography to the micrometer scale. Elastic waves were mechanically induced in live mammalian oocytes using a vibrating micropipette. These audible frequency waves were observed optically at 205,000 frames per second and tracked with an optical flow algorithm. Whole cell elasticity was then mapped using an elastography method inspired by the seismology field. Using this approach, we showed that the elasticity of mouse oocyte is decreased when the oocyte cytoskeleton is disrupted with cytochalasin B. The technique is fast (less than 1 ms for data acquisition), precise (spatial resolution of a few micrometers), able to map internal cell structures, robust, and thus represents a tractable novel option for interrogating biomechanical properties of diverse cell types. This new technique is opening the possibility of studying dynamic cellular processes and elucidating new mechanocellular properties.
We call this technique “cell quake elastography.

O1-6 The Effects of Substrate Stiffness on HUVEC Adhesion with THP-1 Cellsand Molecules Associated with Adhesion

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Vascular stiffness makes the condition favorable for various cardiovascular disease. Apart

from collagen and elastin, adhesion between endothelial cells and immune cells mediated by molecules associated with adhesion and chemokines. Substrate stiffness can influence molecules associated with adhesion. The relationship between vascular stiffness and endothelial cell adhesion may provide a new target for the treatment of cardiovascular diseases. We sought to simulate the stiffness of normal and abnormal vessel, and assess the influence of substrate stiffness on molecules associated with adhesion in endothelial cell. Arcylamide and bis-arcylamide were reacted with various proportion for each polyacrylamide gel. The Young's modulus of gel were 11.15±0.1kPa, 32.51 ±1.91kPa and 80.64±2.11kPa. The influence of stiffness on adhesion molecules(ICAM-1,VCAM-1 and MCP-1) was tested by IF, WB and q-PCR technology. The relation of inhibitors of differentiation-1 (Id1) with adhesion molecules was studied by Id1 overexpression HUVECs. Protein and mRNA expression levels of adhesion molecules associated with HUVECs on physiological stiffness (30kPa) were lower than the pathological stiffness (80kPa and 10kPA). We found that both mRNA and protein levels of Id1 in HUVECs cultured on gel with different stiffness have same trend as adhesion molecules, namely the lowest on 30kPa. Likewise, Id1 is also highly expressed in pathological stiffness and molecules associated with adhesion will be highly expressed in transfected HUVECs, which may provide a new sophisticated way for prevention of cardiovascular disease by controlling vessel stiffness.
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