S21-3 Adhesion of human hepatoma cells to vascular endothelial cells and the role of adhesive molecules

G. Song[a,b], J. Qin[a], T. Ohashi[b] and M. Sato[b]
[a]Department of Bioengineering, College of Bioengineering, Chongqing University, Chongqing 400044, P. R. China; [b]Department of Bioengineering and Robotics, Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan

Cancer is a malignant disease characterized by disorganization of the cell cycle, and the resulting unlimited and uncontrolled proliferation is very harmful to human health. Invasive growth and metastasis are the hallmarks of cancer which differentiates it from benign diseases. Metastasis through blood circulation is a complex multistage process involving several aspects of tumor-host interaction: adhesion, angiogenesis and proteolysis. Adhesion of tumor cells to vascular endothelial cells plays a key role in the early stages of metastasis, and expression of adhesive molecules performs an important function in regulating this adhesive process. However, the detailed mechanisms that control the adhesive behavior of tumor cells to endothelial cells and the roles of related adhesive molecules are not fully been understood. Using the micropipette aspiration technique, we studied the adhesive force of human hepatoma cells (HC) to human umbilical vein endothelial cells (HUVEC) and evaluated the contribution of adhesive molecules E-selection and integrin β1. We demonstrated that expression of integrin β1 reflected a cell cycle difference in HC: synchronized S phase HC gave rise to a significantly higher integrin β1 expression than G1 phase and unsynchronized control HC. The adhesive force of S phase HC to HUVEC was also markedly higher than that of G1 phase and unsynchronized control HC. Monoclonal anti-human integrin β1-pretreated HC yielded a pronounced decrease of adhesive force to HUVEC. We also found that the adhesive force of HC to recombinant human interleukin-1β- (rhIL-1β)-stimulated HUVEC was significantly higher than that of unstimulated control cells, and that immunofluorescence of E-selectin in rhIL-1β-stimulated HUVEC showed a higher fluorescent intensity compared to control cells. Moreover, addition of monoclonal anti-human E-selectin decreased the adhesive force of HC to stimulated HUVEC by approximately 50%. Collectively, these results suggest that adhesive molecules integrin β1 and endothelial E-selectin may be the main mediators of carcinoma metastasis of malignant tumors through blood circulation, possibly via increasing the adhesive force of human hepatoma cells to HUVEC in the early stage of metastases. (Supported by Research Grants NSFC-19972077, 30770530 and SRF for ROCS, SEM-20071108-9.)

S21-4 L-selectin shedding regulates two-dimensional binding to PSGL-1

M. Long[a] and X. Jia[a,b]
[a]National Microgravity Laboratory and Center of Biomechanics and Bioengineering, Institute of Mechanics, Chinese Academy of Sciences, Beijing 100190, P. R. China; [b]Bioengineering Department, Beihang University, Beijing 100083, P. R. China

L-selectin shedding is crucial to many biological processes such as inflammatory cascade, tumor metastasis and thrombosis formation. However, the underlying mechanisms of how L-selectin shedding contributes to the binding kinetics with its ligand are not well understood. We have thus developed *in vitro* models of L-selectin shedding by activating Jurkat cells using PMA or IL-8. The effects of L-selectin shedding on two-dimensional (2D) binding kinetics to P-selectin glycoprotein ligand 1 (PSGL-1) were quantified using an adhesion frequency assay together with a probabilistic kinetic model. Our data indicate that the 2D binding affinity of shed L-selectin was enhanced by reducing the reverse

rate or lowering both the reverse and forward rates, suggesting that L-selectin undergoes a conformational change induced by PMA or IL-8. Three-dimensional (3D) kinetic measurements using Scatchard analysis confirmed the enhancement of the binding affinity of shed L-selectin to its soluble antibodies. Morphological observations of Jurkat cells and membrane L-selectin distribution demonstrated that the enhancements of binding affinities were associated with surface microtopology as well as L-selectin clustering and co-localization with lipid rafts. These results provide new insight into understanding the physiological function of L-selectin shedding. (Supported by National Key Basic Research Foundation of China grant 2006CB910303, National Natural Science Foundation of China grants 30730032 and 10332060, Chinese Academy of Sciences grant 2005-1-16, and National High Technology Research and Development Program of China grant 2007AA02Z306 (M.L.).)

SYMPOSIUM 22: HEMORHEOLOGICAL ASPECTS OF MECHANICAL BLOOD TRAUMA

S22-1 Shear induced blood trauma and alteration of red blood cell deformability in rotary blood pumps

S. Takatani, M. Koso, D. Sakota, R. Sakamoto and T. Yasuda
Department of Artificial Organs, Tokyo Medical and Dental University, Tokyo, Japan

Red blood cells (RBC) in rotary blood pumps are exposed to shear stress resulting in alteration of their deformability and in hemolysis. Although the previous approach to evaluate RBC trauma in rotary blood pumps was to look at the level of hemolysis, in this study we paid attention to the deformability of the survived and damaged RBC. To achieve this goal, we have developed a cyclically reversing shear flow generator to observe the dynamic deformation process of RBC. The specially built cyclically reversing shear flow generator consisted of a microscope system under which RBC go through cyclically reversing shear stress. A high speed video camera, capable of 5000 frames per second, was used to capture the dynamic deformation process of individual RBC. In analyzing the deformation process, the ratio of the long, L, and short axes W, L/W, as obtained from the two-dimensional images was used. We used fresh, anti-coagulated porcine blood obtained from a local slaughterhouse. In the first part of the study, a cone and plate uniform shear stress generator was used to apply shear stress levels of 21, 43 and 64 Pa for the duration of 10, 20, 30, 40 and 60 min. In the second part of the study, whole blood was centrifuged to separate RBC according to their mass related to the ageing process. The older the cells become, the smaller become their volume and the higher the mass or the hemoglobin concentration. We found that for whole blood mixtures of old and young RBC, their deformability did not change even after exposure to a shear stress of 21 Pa for 60 min, while exposure to shear stresses of 43 and 64 Pa, changed their deformability after 40 and 20 min, respectively. With older cells, hemolysis occurred at lower shear stress than in younger blood cells. We speculate that shear stress speeds up the ageing process to alter their deformability together with a lower threshold level for hemolysis. (Supported by Japanese Promotion of Science Grant #18300149.)

S22-2 Computational indices for prediction of flow-induced blood trauma

J.F. Antaki
Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA

Computationally-based design of blood-wetted devices requires mathematical models that are amenable to numerical implementation in order to predict flow-induced blood trauma. Direct numer-