BIOFUNCTIONAL MICROPATTERNED SURFACES TO STUDY NANO-SCALE ORGANIZATION AND DYNAMICS OF THE LFA-1 ADHESION RECEPTOR IN LIVING CELLS.

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Lymphocyte function-associated antigen-1 (LFA1;αLβ2) is a leukocyte transmembrane protein that functions as a intercellular adhesion receptor and as a co-stimulatory and signaling molecule. It mediates migration across the endothelium and within tissues and takes part of the immunological synapse by binding with high affinity to its ligand intermolecular adhesion molecule 1(ICAM-1). We have recently shown that on monocytes LFA-1 adhesion depends not only on receptor occupancy but also on its nano-cluster organization on the cell membrane (50-150 nm)[1]. However the signals and mechanisms which activate and dynamically reorganize LFA-1 into high avidity clusters are still a subject of many studies [2].

In order to get deeper insight on the mechanisms that control and regulate LFA-1 clustering, large ($1cm^2$) biofunctional micro-patterned surfaces containing ICAM-1 are fabricated to act as a second cell. Homogenous ICAM-1 regions (from 10μm-1μm) have been achieved over $1cm^2$ areas, with variations in ICAM-1 density below 9%. Immobilization of ICAM-1 via adsorption over Goat-anti-human antibodies, transferred onto glass substrates using micro contact printing technique[3], guarantees correct orientation of the binding site of the ligand to its receptor while retaining ligand functionality.

THP-1 cells (monocytic cell line) expressing LFA-1 have been stretched over the patterned surfaces and the diffusion of labeled LFA-1 in the ICAM-1 regions has been followed in time using single molecule sensitive total internal reflection (TIRF) microscopy. Single molecule fluorescence trajectories show an increase of intensity on individual fluorescent spots as well as reduction of its mobility consistent with selective recruitment of individual LFA-1 clusters on the ICAM-1 patterned areas. Fluorescent spots on the non-patterned areas (BSA coated) show on the other hand random diffusion. Furthermore, detailed quantitative analysis of LFA-1 trajectories on the BSA areas and in close neighborhood to the ICAM-1 patterns show no assisted diffusion of LFA-1 towards the adhesive regions, consistent with the notion of random ligand-encountering and binding. We are currently investigating the effect of cell membrane organizers, such as lipid rafts and tetraspanins, to regulate the spatio-temporal organization of LFA-1. These studies will shed light on the role of affinity vs. avidity of LFA-1 to its ligand ICAM-1 on a single molecule basis.

References:

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