EXPERIMENTAL EVALUATION OF RECEPTOR-LIGAND INTERACTIONS OF DUAL-TARGETED PARTICLES TO INFLAMED ENDOTHELIUM

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Vascular-targeted carriers (VTCs) are often designed as leukocyte mimics, conjugated with ligands that target leukocyte adhesion molecules (LAMs) to facilitate specific adhesion to diseased endothelium. VTCs must adhere in regions with dynamic blood flow, frequently requiring multiple ligand-receptor (LR) pairs to provide particle adhesion and high disease specificity. To study LR kinetics under flow, multiple research groups have used protein-coated plates to study the adhesion and rolling of dual-targeted particles *in vitro*.[1-4] While important knowledge is contributed by these studies, they lack the complexity of a diseased physiologic endothelium, as spatiotemporal LAM expression varies widely. Despite decades of research with the ambition of mimicking leukocytes, the specificity of multiple LAM-targeted VTCs remains poorly understood, especially in physiological environments. More specifically, there is a lack of mechanistic understanding of how multiple ligands interact with biologically complex endothelial surfaces under dynamic *in vivo* environments.

We used cutting-edge intravital microscopy to investigate particle binding to an inflamed mesentery with a series of particles with well-controlled ligand properties. For all intravital experiments, we characterized the number of rolling and firmly bound particles, as well as the rolling velocity of each. We found that the total number of sites of a single ligand can drive particle adhesion to the endothelium, however, combining ligands that target multiple LR pairs provides a more than additive result. Combining sLe^A and anti-ICAM, LAM ligands targeting selectin and β_2 integrin mediated paths of adhesion respectively, resulted in ~3-7-fold increase of adherent particles at the mesentery endothelium over single-ligand particles. At a constant total ligand density, a particle with a ratio of 75% sLe^A: 25% anti-ICAM resulted in more than 3-fold increase over all over other ligand ratios tested in our *in vivo* model (Figure 1). *In vitro* parallel plate flow chamber experiments using human whole blood agree with these results. Particles with a ratio of 75% sLe^A: 25% anti-ICAM produced ~6-fold more bound particles over all other particle types at a time point of maximal ICAM expression on the endothelial cells (ECs). Taken together, we conclude the best dual-ligand design of a particle is heavily dependent on the surface expression of the ECs. Interestingly, particle adhesion is most efficient when more ligand is present for the lesser-expressed receptor. The novel use of intravital microscopy to investigate the effect of dual targeting ligands establishes the importance of LR-kinetics in ligand design for future therapeutics.

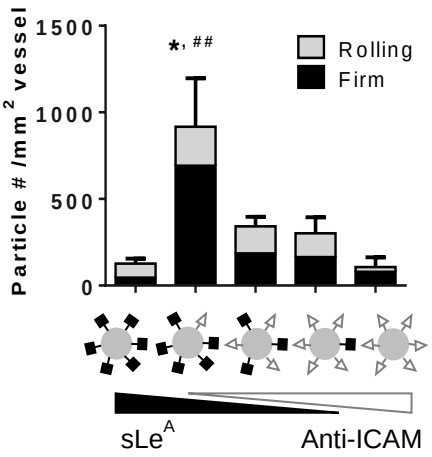

Figure 1 – Particle adhesion to inflamed mesentery endothelium as a function of varied ratios of sLe^A and aICAM. n=3 mice.

This work is specifically relevant to Nanotechnology in Medicine because it dives deeply into the mechanistic understanding of the molecular interactions between ligands on particles and receptors on cells. Our work propels the field forward by establishing the importance of particle ligand ratios based on EC receptor patterns.

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