Platelet-targeted Nanoconstructs for Site-specific Drug Delivery in Vascular Disease

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Statement of Purpose: Vascular diseases are the leading cause of morbidity and mortality in America. The most prominent clinical events in vascular disease are arterial thrombo-occlusions that can lead to ischemia, unstable angina, myocardial infarction and stroke. Therefore, rapidly removing such occlusions and restoring blood flow is critical. Current interventional clinical strategies for removing vascular occlusions, like angioplasty/stenting and bypass grafting, often present post-procedural issues of restenosis and graft failure, respectively. Hence they are often coupled with systemic (oral or parenteral) pharmacotherapy, which suffer from issues of short plasma half-life of the drug, sub-optimal drug concentration at the thrombus site, and dangerous systemic non-specific side effects like hemorrhage. We hypothesize that a nanoscale construct that can encapsulate and protect vascular drugs in circulation and deliver them selectively and sustainably to the thrombus site under dynamic blood flow can significantly enhance therapeutic efficacy in vascular disease. To this end, we have developed a liposomal nanoconstruct that can specifically target activated platelets, which are the major components of an occlusive thrombus [1]. To achieve shear-stable activated platelet-selective binding under flow, we have decorated the liposomes with two small molecular weight peptides, one that specifically targets platelet surface integrin GPIIb-IIIa (fibrinogen-mimetic cyclic RGD peptide [2-4]) and one that specifically targets P-selectin (EWVDV peptide [5]) (Figure 1). We envision that these dual ligand-modified liposomes will provide parallel and synergistic pathways of activated platelet targeting and will enable stable binding to a thrombus site under a hemodynamic environment.

Methods: Peptides were synthesized using Fmoc based solid phase chemistry, characterized using MALDI-TOF mass spectroscopy, and then conjugated to lipids via carbodiimide chemistry-mediated amidation. Lipid-peptide conjugates were then incorporated into fluorescently-labeled (with Rhodamine-B) liposomal nanoconstructs (~ 150 nm dia) using the reverse-phase evaporation and extrusion technique. These peptide-modified nanoconstructs were studied for their platelet-targeting specificity and platelet-binding stability under flow, *in vitro*, using a parallel plate flow chamber (PPFC) and analyzed using epi-fluorescence microscopy. For targeting specificity analysis, the approach was to see whether pre-incubation of activated platelets with non-fluorescent peptide-modified liposomes blocked specific binding of fluorescent antibodies directed towards the same target receptors. For binding stability studies, fluorescently labeled untargeted or peptide-modified (single or dual-targeted) liposomes were incubated under physiological shear flow (in PPFC) over a monolayer of activated platelets for 30 min, followed by PBS flow for 15 min. Fluorescence from liposomes 'retained' on activated platelet monolayer was measured.

Results: *In vitro* blocking studies showed that our cRGD-modified liposomes specifically and significantly blocked binding of FITC-anti-CD41a (specific to GPIIb-IIIa), while our DAEWVDVS-modified liposomes specifically and significantly blocked binding of AlexaFluor647-anti-CD62P (specific to P-selectin). Hence, targeting specificity of peptides to their respective receptors was established. *In vitro* binding studies under dynamic flow, with shear between 5-60 dyn/cm^2, showed that nanoconstructs surface-decorated with both peptides (dual-targeting) stably bound activated platelets significantly more compared to those with any one peptide (single-targeting) under flow (Figure 1). Both single and dual-targeted nanoconstructs showed significantly enhanced platelet binding under flow compared to untargeted liposomes.

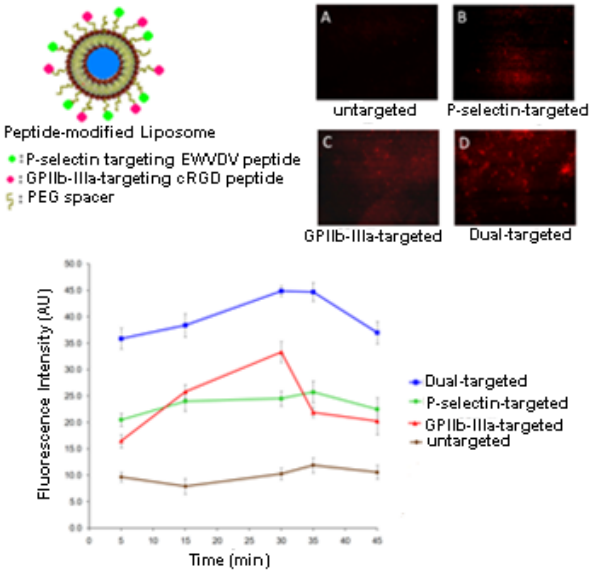

Figure 1. Bioengineering design of platelet-targeted liposomal nanoconstructs; representative fluorescent micrographs and quantitative platelet-binding data at 35 dyn/cm^2 in PPFC.

Conclusions: We have successfully demonstrated the activated platelet-targeting specificity and shear-stable platelet-binding ability of dual peptide-decorated liposomal nanoconstructs, under flow, *in vitro*. These results establish the potential of our nanoconstructs as platform vehicles for site-selective delivery in vascular disease.

References:

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