A Platelet-Inspired Nanomedicine Approach for Targeted Thrombolytic Therapy

Clarissa Kos, Kavya Ravichandran, Wei Li, Christa Modery-Pawlowski, Dr. Anirban Sen Gupta
Case Western Reserve University, Cleveland OH 44106
The Cleveland Clinic Foundation, Cleveland OH 44106

Statement of Purpose: In occlusive vascular pathologies, rapid thrombolysis is necessary for restoring blood flow to critical organs. For this, it is advantageous to localize the delivery of thrombolytic drugs specifically at the clot site, so as to enhance site-selective drug action while minimizing systemic side-effects of coagulopathy and hemorrhage due to indiscriminate drug distribution. We hypothesize that clot-selective delivery of thrombolytic drugs can be achieved with nanovehicles that can actively anchor onto thrombus by binding to thrombus-associated *active* platelets. These vehicles can then allow locally triggered drug release via thrombus-relevant enzyme action. To test our hypothesis, we have engineered vehicles using liposomes as a model nanoparticle, where the liposome surface was heteromultivalently decorated with peptides that allow simultaneous binding to integrin GPIIb-IIIa via a fibrinogen-mimetic peptide (FMP) and P-selectin via a sialoprotein-mimetic peptide (SMP), on thrombus-associated active platelets [1]. The lipid components of liposomes were chosen to be amenable to enzyme-mediated degradation by thrombus-relevant enzyme, phospholipase A2 (PLA_2) [2, 3]. Here we present our studies on evaluating the ability of these liposomal constructs to bind to active platelets *in vitro* and thrombi *in vivo*. We also present our studies regarding PLA_2-triggered release of Streptokinase (SK) from these constructs. Lastly, we present studies from integrating the targeting and drug release components to evaluate 'targeted thrombolysis'.

Methods: *In Vitro and In Vivo Evaluation of Thrombus Targeting:* For *in vitro* studies, glass slides were coated with collagen and incubated with activated platelets. The resultant platelet-covered slides were assembled into a parallel plate flow chamber (PPFC) setup, and the fluorescently labeled surface-engineered liposomal constructs were allowed to flow over the slides for 30 min under wall shear stress range of 5-60 dyn/cm^2. The interaction of the constructs to the platelets was imaged using fluorescence microscopy and fluorescence intensity analysis was used to quantify the platelet-binding of the constructs. Particles containing no ligand decoration (unmodified) or particles bearing only one type of ligand (FMP-only or SMP-only) were used as comparison groups. Additionally, particle binding to a non-specific surface (albumin) was used as negative control. To test thrombus targeting *in vivo*, the ferric chloride-induced carotid artery thrombus model was used in mice [3]. Platelet-targeted and non-targeted (unmodified) fluorescently labeled liposomes were injected intravenously following thrombus formation, and intravital microscopy was used to monitor fluorescent particle localization at the thrombus site. Following the experiments, the animals were euthanized, the carotid artery was excised and imaged ex vivo with fluorescence microscopy.

Enzyme-Triggered Release of Payload: SK was loaded within the liposomes during the reverse phase evaporation process, unencapsulated SK was removed via ultracentrifugation, and the encapsulation efficiency was measured by UV-Vis spectrometry using an SK-specific chromogenic assay. Similarly SK-loaded liposomes were then suspended in buffer at 37°C in the presence or absence of PLA_2, and release kinetics was characterized using the same UV-Vis technique.

Evaluation of Targeted Thrombolysis: Platelet-rich thrombus was formed on collagen-coated glass slides using fluorescently labeled active platelets. The slide was assembled into the PPFC setup, and platelet-targeted, SK-loaded liposomes were allowed to flow through the system for 30 min in the presence of PLA_2. Clot lysis was evaluated by measuring a decrease in clot fluorescence over time using fluorescence microscopy. To evaluate thrombolysis in vivo, platelet-targeted SK-loaded liposomes were injected into mice following ferric chloride-induced thrombus formation, and thrombolysis was observed using intravital microscopy.

Results: Heteromultivalently-decorated platelet-targeted liposomes demonstrated enhanced thrombus binding compared to homomultivalently-decorated or unmodified constructs. Additionally, liposomes showed PLA2-triggered enhanced payload release. The platelet-targeted SK-loaded liposomal constructs demonstrated targeted thrombolytic capabilities.

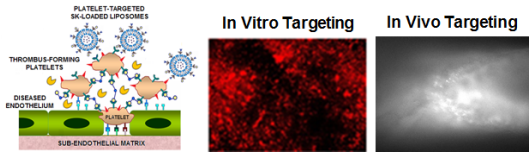

Figure 1. Design and representative results of platelet-targeted thrombolytic nanomedicine

Conclusions: Heteromultivalent targeting of active platelets enabled liposomal constructs to actively anchor onto platelet-rich thrombi and allow enzyme-triggered local drug release for targeted thrombolysis.

References: [1] Modery-Pawlowski et al. *Biomaterials* 2011; 32(35):9504**.** [2] Mueller et al. *Thromb Res* 1993; 72:519**.** [3] Li et al. *Redox Biology* 2013; 1(1):50