Rational Design of a Platelet-mimetic Hemostatically Active Nanoconstruct

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Statement of Purpose: Platelet transfusion is clinically important for managing bleeding complications in congenital or induced (e.g. myelosuppression) platelet disorders, and also in acute surgery or deep injury [1]. Current natural platelet-based clinical products (e.g. platelet concentrates) are expensive, have short storage life (3-7 days), and pose risks of contamination and immunoreaction [1,2]. Hence there is significant clinical interest in platelet-mimetic synthetic hemostats that are less expensive, have long storage life, and can avoid the biological risks. The two main hemostatic functions of natural platelets are to 'adhere' to the vascular injury site by binding to proteins like von Willebrand factor (vWf) and collagen, and to 'aggregate' via fibrinogen-mediated binding of the platelet surface integrin GPIIb-IIIa. Past research in synthetic platelet substitutes have mostly focused on accelerating only the 'aggregation' function by developing particle constructs surface-modified with pro-aggregatory proteins (e.g. fibrinogen) or peptides (e.g. RGD) [1-3]. These constructs do not have adhesion-specificity to vascular injury site and hence have minimal control over the location of 'aggregation'. This may pose the risk of thromboembolic complications from free-floating aggregates. We hypothesize that a nanoscale particle construct that bears platelet-mimetic pro-aggregatory and matrix-adhesive ligands simultaneously, can enable adhesion of these particles at site of vascular injury and subsequent recruitment of available platelets at that site for site-selective hemostatic function. To test this hypothesis, we have developed liposomal nanoconstructs, surface-modified simultaneously by three hemostatically active peptides, namely a vWf-binding peptide (VBP) and a collagen-mimetic peptide (CMP) to enable matrix adhesion under shear, and a GPIIb-IIIa-binding cyclic RGD peptide (cRGD) to enable platelet recruitment and aggregation specifically at the site of adhesion (**Figure 1**). We have studied the platelet-mimetic functionalities of these constructs at physiological shear on vWF/collagen surfaces using a parallel plate flow chamber (PPFC) and low platelet concentrations.

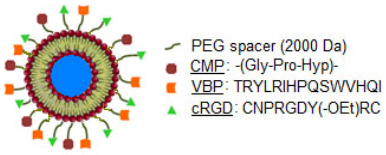

Figure 1. Platelet-mimetic construct design

Methods: The VBP sequence TRYLRIHPQSWVHQI, the CMP sequence $(Gly\text{-}Pro\text{-}Hyp)_7$, and the GPIIb-IIIa-binding cRGD sequence (cyclo-CNPRGDY(OEt)RC) were synthesized by solid phase Fmoc chemistry, characterized by mass spectrometry, and conjugated onto polyethylene glycol-modified carboxyl-terminated lipids (e.g. DSPE-PEG-COOH) via carbodiimide-mediated amidation. The adhesion of VBP-modified liposomes, CMP-modified liposomes, and liposomes modified by both CMP and VBP simultaneously, were studied on a collagen/vWF mixed surface under flow using PPFC and compared to adhesion of unmodified liposomes. The 'aggregation promotion' capability of cRGD liposomes was studied separately, by coating glass coverslips with unmodified or cRGD-modified liposomes, incubating platelet-rich plasma (PRP) on the coated surface, fluorescently staining the adhered aggregated platelets (with P-selectin-specific antibody), and measuring fluorescence intensity. After confirmation of successful 'adhesion' and 'aggregation' functionalities, green fluorescent liposomes simultaneously bearing all three peptides (VBP, CMP and cRGD), were introduced with red fluorescent platelets (at low concentration) in PPFC flow, over a vWF/collagen mixed surface, and ability of the liposomes to adhere to the surface and recruit platelets at the site of adhesion was monitored by measuring green and red fluorescence colocalization. Liposomes bearing only 'aggregatory' or only 'adhesive' peptides or no peptides were used as controls.

Results: VBP and CMP modified liposomes showed enhanced binding to the vWF/collagen mixed surface, compared to unmodified liposomes. Liposomes with both VBP and CMP showed enhanced binding to the vWF/collagen mixed surface, compared to single peptide modified liposomes. cRGD-modified liposomes showed statistically significant promotion of aggregation, compared to unmodified liposomes. Finally, liposomes simultaneously bearing both 'adhesive' and 'aggregatory' functionalities (all three peptides) showed significant ability to adhere onto vWF/collagen surface and promote platelet aggregation on the surface, under flow, compared to unmodified liposomes or those with only one functionality (**Figure 2**).

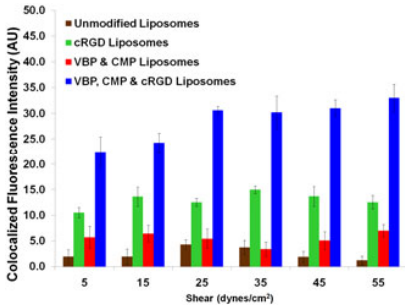

Figure 2. PPFC data from flow of liposomes and thrombocytopenic platelet concentration on vWF/collagen surface

Conclusions: We have successfully demonstrated the design and biomimetic function of a platelet-mimetic, peptide-modified nanoconstruct that holds promise as a hemostatically active synthetic platelet substitute.

References:

1. Mohanty et al, Asian J Transf Sci, 2009; 3: 18-21
2. Blajchman et al, Nature Medicine, 1999; 5: 17-18
3. Bertram et al, Science Trans Med, 2009, 1: 1-8.

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