Platelet microparticle-inspired clot-responsive nanomedicine for targeted fibrinolysis

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**Abstract**

Intravascular administration of plasminogen activators is a clinically important thrombolytic strategy to treat occlusive vascular conditions. A major issue with this strategy is the systemic off-target drug action, which affects hemostatic capabilities and causes substantial hemorrhagic risks. This issue can be potentially resolved by designing technologies that allow thrombus-targeted delivery and site-specific action of thrombolytic drugs. To this end, leveraging a liposomal platform, we have developed platelet microparticle (PMP)-inspired nanovesicles (PMINs), that can protect encapsulated thrombolytic drugs in circulation to prevent off-target uptake and action, anchor actively onto thrombus via PMP-relevant molecular mechanisms and allow drug release via thrombus-relevant enzymatic trigger. Specifically, the PMINs can anchor onto thrombus via heteromultivalent ligand-mediated binding to active platelet integrin GPIIb-IIIa and P-selectin, and release the thrombolytic payload due to vesicle destabilization triggered by clot-relevant enzyme phospholipase-A2. Here we report on the evaluation of clot-targeting efficacy, lipase-triggered drug release and resultant thrombolytic capability of the PMINs in vitro, and subsequently demonstrate that intravenous delivery of thrombolytic-loaded PMINs can render targeted fibrinolysis without affecting systemic hemostasis, in vivo, in a carotid artery thrombosis model in mice. Our studies establish significant promise of the PMIN technology for safe and site-targeted nanomedicine therapies in the vascular compartment.

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**1. Introduction**

Vascular pathologies like myocardial infarction, stroke and peripheral arterial disease are major causes of morbidities and mortalities on a global scale [1]. A common clinical presentation in such disease pathologies is the formation of occlusive clots (thrombi) in blood vessels, that restrict blood flow to critical organs [2]. Therefore, rapid removal of occlusive thrombi to restore blood flow is a critical component of treating these conditions. One established clinical strategy for clot removal is the intravascular administration of thrombolytic (fibrinolytic) drugs [3]. These drugs, e.g. streptokinase (SK), urokinase-type plasminogen activators (uPA) and tissue plasminogen activators (tPA), act by facilitating conversion of plasminogen to plasmin, which in turn can break down the fibrin in the clot. While this fibrinolytic action is essential for therapeutic activity at the clot site, systemic off-target action of these drugs to convert circulating plasminogen to plasmin is harmful because this circulating plasmin can then break down circulating fibrinogen (systemic fibrinogenolysis), which affects normal hemostatic capabilities and leads to hemorrhagic side-effects [4,5]. According to the American Academy of Emergency Medicine (AAEM) and National Institute of Neurological Disorders and Stroke (NINDS), about 6% of stroke patients undergoing tPA-based thrombolytic therapy suffer from intracranial hemorrhage with about 45% fatality risk [6]. Another issue with the direct intravascular administration of thrombolytic agents is their rapid deactivation by plasma components (e.g. by plasminogen activator inhibitors) and resultant short circulation life, that in turn reduces their availability at the clot site [7]. All these issues can be potentially mitigated by designing technologies that can (i) encapsulate and protect the drug in...
circulation, (ii) anchor actively onto clot site under hemodynamic flow environment and (iii) allow triggered release of the drug specifically at the clot site to minimize off-target effects. In consideration of these design criteria, we have developed a vascular nanomedicine technology inspired by platelet-derived microparticles (PMPs). PMPs, originally reported by Wolf in the 1960s as ‘platelet dust’, are membrane fragments shed from activated platelets [8]. These PMPs are characteristically known to be lipid bilayer vesicles 100nm-1μm in diameter, with a high surface presentation of pro-coagulant anionic phosphatidylserine (PS) lipid, active integrin GPIb-lla, P-selectin, GPIIb-type receptors, thrombospondin, C-X-C type chemokine receptors and thrombin receptors [9,10]. Fig. 1A shows representative cartoon of a PMP, with (A1) showing fluorescence microscopy image of red fluorescent active platelets (shown with blue arrows) shedding PMPs (shown with yellow arrows) and (A2) showing representative scanning electron microscopy (SEM) images of the same at high resolution, demonstrating that the PMPs are sub-micron size vesicular structures. The PS-rich surface of PMPs facilitate intrinsic resolution, demonstrating that the PMPs are sub-micron size vesicular structures. The PS-rich surface of PMPs facilitate intrinsic resolution, demonstrating that the PMPs are sub-micron size vesicular structures. The PS-rich surface of PMPs facilitate intrinsic resolution, demonstrating that the PMPs are sub-micron size vesicular structures. The PS-rich surface of PMPs facilitate intrinsic resolution, demonstrating that the PMPs are sub-micron size vesicular structures. The PS-rich surface of PMPs facilitate intrinsic resolution, demonstrating that the PMPs are sub-micron size vesicular structures. The PS-rich surface of PMPs facilitate intrinsic resolution, demonstrating that the PMPs are sub-micron size vesicular structures.

For the PMIN design (Fig. 1B), the clot-specific active anchorage was rendered by heteromultivalently surface-decorating glycerophospholipid-based liposomal vesicles with peptide ligands that can specifically bind to thrombus-associated cellular phenotypes (e.g. platelets and leukocytes) via heterotopic ligand-receptor interactions, and (iii) secrete their payload locally to influence the thrombus pathology. Drawing inspiration from these structural and mechanistic aspects of PMPs (but not their pro-thrombotic functional aspect), we have chosen to construct (i) liposomal nanovesicles with non-coagulant lipid membrane, that can (ii) undergo active anchorage to platelet-rich thrombi via PMP-inspired heteromultivalent ligand-receptor interactions, and (iii) undergo active anchorage to thrombi, can release encapsulated thrombolytic drug to render site-specific thrombolytic action. We rationalized that these PMP-inspired nanovesicles (PMIN) will enhance therapeutic availability at the clot site, while protecting the encapsulated drug from plasma and minimizing systemic off-target side effects.

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