Heteromultivalent Ligand Modification to Enhance Specific Bioactivity of Vascular Nanomedicine Platforms

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Statement of Purpose: In the area of targeted drug delivery, site-selective *active* targeting of nanomedicine constructs via surface-modification with specific ligands has been established as a way to enhance specificity and efficacy of the therapeutic cargo [1]. To this end, most research approaches have investigated the decoration of the nanovehicle surface with multiple copies of a single type of ligand that specifically binds with a target receptor epitope. Often, the target receptor or protein is not sufficiently over-expressed to ensure adequate binding of the nanovehicles. Hence, there is a growing interest in modifying the nanovehicle surface with multiple types of ligands (heteromultivalent modification) that simultaneously bind to multiple target epitopes at the disease site to compensate for possible insufficiencies of any one type of ligand-receptor interaction at the target site [2]. We postulate that such interactions may not only enhance the specific bioactivity of the nanovehicles towards the target, but they may be highly useful for active targeting in the vascular compartment where the carrier vehicles have to not only bind to the target site but also resist dislodgement under hemodynamic flow. Building on such rationale, we have investigated the heteromultivalent ligand modification approach in two different vascular applications: a thrombus-targeted model drug delivery carrier and a model particle-based synthetic platelet substitute (Figure 1). For the thrombus-targeted drug delivery platform, we have investigated simultaneous surface modification of liposomes with two different ligands that enable binding to activated platelets via two different mechanisms: a fibrinogen-mimetic peptide (FMP) which binds specifically to integrin GPIIb-IIIa and a sialoprotein-mimetic peptide (SMP) which binds specifically to P-selectin, both on activated platelets [3]. For the synthetic platelet substitute platform, we have investigated simultaneous modification of the liposome surface with two peptides that facilitate platelet-mimetic adhesion under shear flow: a vWF-binding peptide (VBP) and a collagen-binding peptide (CBP) [4]. With regards to heteromultivalent modification, we have further studied the effect of modulating the relative peptide ratios while maintaining fixed total peptide composition (liposome surface decoration density) on their binding activity.

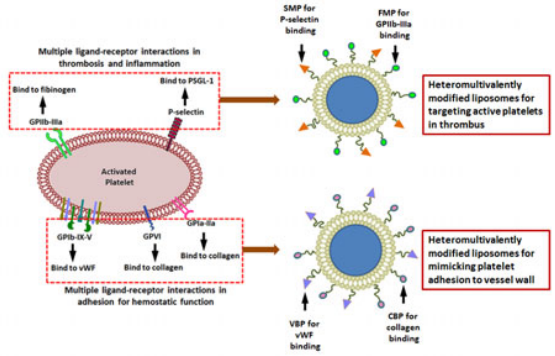

Figure 1: Platelet-associated biological mechanisms and design rationale for heteromultivalently modified nanomedicine constructs to enhance targeting specificity and binding stability under flow.

Materials and Methods: *Peptide-modified liposome fabrication:* Peptides were synthesized using Fmoc-based solid phase chemistry, characterized using MALDI-TOF mass spectroscopy, and subsequently conjugated via their N-termini to lipid-PEGCOOH via carbodiimide chemistry. Conjugation yield was determined using a Ninhydrin assay. Resultant lipid-peptide conjugates were incorporated in controlled compositions into Rhodamine-B-labeled liposomes (~150nm dia) using reverse-phase evaporation and extrusion. Liposome size was characterized using Dynamic Light Scattering (DLS).

Study of target cell (or protein) binding and shear-stable retention of peptide-decorated liposomes: Fluorescently-labeled, peptide-decorated liposomes were studied for their targeting specificity and binding stability under flow, *in vitro*, using a parallel plate flow chamber (PPFC). Glass microscope slides were coated with collagen-adhered activated platelets or with a vWF-collagen mixed surface. The slides were mounted within the PPFC, vacuum sealed and the various peptide-decorated liposomes were allowed to flow over the surface for 30 minutes under wall shear stress range of 0-60 dynes/cm^2. At various time points, the slides were imaged using fluorescence microscopy to quantify platelet-binding of liposomes. After 30 minutes, the liposome solution was replaced with PBS and flow was maintained for 15 minutes to remove loosely bound liposomes. Resultant liposome retention was again quantified by fluorescence imaging. As a negative control, the same experiments were conducted on slides coated with albumin. Liposomes without peptide modification or bearing only one type of peptide were used as negative control particles.

Results and Discussion: For both experimental models, results showed significantly enhanced binding and retention of the heteromultivalently modified constructs on their respective targets under low-to-high shear flow, compared to unmodified or homomultivalently modified liposomes. Only minimal non-specific binding was seen on albumin surface. Variation of ligand ratios allowed further modulation of binding capabilities.

Conclusions: The study demonstrated that heteromultivalent ligand modification of nanovehicles can enable enhancement of binding specificity, for potential applications in actively targeted vascular drug delivery. Furthermore, this approach can help optimize the binding strength for stable retention of the vehicles under flow.

References:

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