Oligosachharide modified biomimetic surfactant polymer for non-thrombogenic interface applications: Platelet Adhesion Studies

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Introduction The presence of proteoglycans, glycosaminoglycans and other glycosylated molecules is responsible for the inherent non-adhesive nature of the endothelial cell glycocalyx that prevents attachment of other cells and biomolecules to the intravascular endothelial cell lining [1,2]. Mimicking such a sugar-rich structure for vascular interfacial biomaterials provides a way to prevent the clinical problem of thrombus formation on blood-contacting biomaterials used for vascular grafts, cardiovascular assist devices, device housings etc. Shear-induced adhesion and aggregation of activated platelets on biomaterial surfaces is a major cause of subsequent thrombotic events. The presence of a non-adhesive glycocalyx-mimetic interface between the biomaterial surface and blood would potentially suppress platelet interactions and hence impart non-thrombogenicity to the biomaterial surface. Based on this rationale, dextran-modified poly(vinyl amine) surfactant polymers were developed that adsorb spontaneously on a variety of polymeric biomaterials. The polymer consists of a flexible poly(vinyl amine) backbone with pendant hexanoyl groups for binding to a hydrophobic biomaterial surface and pendant dextran (oligosaccharide) side-chain that orient into the aqueous environment to provide a glycocalyx-mimetic interface. The chemical structure of the polymer is shown in Figure 1.

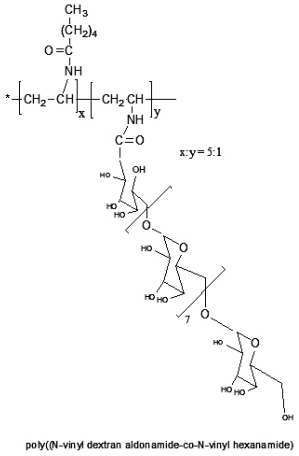

Figure 1. Chemical structure of dextran-modified surfactant polymer

The polymer shown in Figure 1 was adsorbed onto medical grade polycarbonate (PC) disks which were exposed to platelet rich plasma (PRP) or human whole blood (WB) in a rotating disk set-up such that a physiological shear environment could be simulated. Uncoated PC disks were used as controls. Platelet adhesion from PRP or WB onto uncoated and surfactant-coated disks was compared, in order to gain insight into the potential non-thrombogenic nature of the glycocalyx-mimetic coating on medical grade PC. Polycarbonate is clinically used in blood-contacting medical devices like blood pumps.

Experimental Low-molecular weight poly(vinyl amine) (PVAm) with well-defined structure and narrow polydispersity was synthesized from poly(N-vinylformamide) according to previously published procedures [2,5]. The PVAm thus obtained was derivatized simultaneously by hexanoyl groups via reaction with N-(hexanoyloxy)succinimide and by dextran aldonamide groups via reaction with dextran lactone. The ratio of pendant dextran to hexanoyl groups on the final polymer could be controlled stoichiometrically at 1:5. The chemical structure of the final polymer, *poly*(*(N-vinyl dextran aldonamide-co-N-vinyl hexanamide)*, was characterized by NMR and IR spectroscopic methods, as discussed elsewhere [2-5]. PC disks, 16 mm in diameter, were obtained with the help of a cork borer, from Lexan [TM] (grade 8040 MC) polycarbonate sheets (GE Structured Products, Mt. Vernon, IN). For surfactant coating, the disks were submerged in a polymer solution (aqueous) of concentration 2 mg/ml for 24 hrs, washed with PBS and dried in air.

Blood was drawn from healthy, aspirin-refraining adult donors into 3.8% sodium citrate anticoagulant (Sigma) in a 9:1 ratio (by volume). For WB based experiments human whole blood was used directly while for PRP based experiments, the blood was centrifuged at 950 rpm (~160 g) for 15 minutes at 25^{o}C to obtain supernatant PRP. The platelet count, as measured by an AcTDiff Coulter Counter system, was in the range of 2 x 10^5 - 3 x 10^5 platelets/ μl. The surfactant-coated and uncoated disks were exposed, for one-hour period, to PRP and whole blood under well-defined dynamic flow conditions and variable shear stress environments with the help of a rotaing disk system. Assuming PRP and WB to behave as a Newtonian fluid near the surface of the disk, the shear stress (τ_s, dynes/cm^2) at the surface of the exposed disk varies linearly with the radial distance, r, from the center of the disk, according to the equation:

$$\tau_s = 0.800\ \eta\ r\ (\omega^3 / \upsilon)^{1/2} \qquad [A]$$

where η is the absolute viscosity of the testing medium (poise, gm/cm.sec), υ is the kinematic viscosity (stokes, cm^2/sec)) and ω is the angular velocity (radians/ sec). The value of ω in [A] was manipulated in the RDS, such that the value of τ_s ranged from 0 dynes/cm^2 at the center of the disk, up to ~70 dynes/cm^2 along the radius. Following the RDS exposure, the adhered platelets on the disks were stained with FITC-anti-CD41a mAb (labels GPIIb-IIIa) and fixed in paraformaldehyde. The disks were finally mounted onto glass slides and observed in an epifluorescence microscope using a 450-490 nm excitation filter, a 510 nm dichroic mirror, and a 520-560 nm barrier filter. Images were captured in triplicate using MetaMorph software from the center of each disk to the circumference along the radius and the number of adhered platelets per mm^2 on the coated or uncoated disks was calculated from the images.

Results The number of adhered platelets per mm^2 was found to be significantly lower for surfactant-coated disks compared with uncoated disks in both WB and PRP media. For uncoated disks, the number of adhered platelets per mm^2 was found to be maximum near the center of the disk (close to zero shear conditions) and decrease towards the edge of the disk, especially above shear values of 50 dynes/cm^2. At higher shear conditions above that value, platelets get possibly detached by force, from the disk surface. For both WB and PRP-based experiments, the number of adhered platelets on surfactant-coated disks was found to be ~90% lower than uncoated disks within the shear range of 0-50 dynes/cm^2. Figures 2A and 2B show representative fluorescence microscopy images (at 1000 X magnification) of platelets adhered onto polycarbonate disks from PRP and WB, respectively, in the rotating disk experiments. Only images captured at zero shear and at ~50 dynes/cm^2 are shown for convenience of comparison. The figures also show the quantitative data for platelet adhesion over the entire shear range of 0-73 dynes/cm^2 for both WB and PRP experiments. The trend of the surfactant coating resisting platelet adhesion is evident from the quantitative data. The fluorescent photomicrographs from the WB studies for uncoated and surfactant-coated disks were further magnified and contrast-adjusted using Adobe Photoshop® to observe the presence of platelet-derived microparticles close to the edges of the spread platelet membrane. These particles, about 100-200 nm in size, have been found to play a potentially significant role in the maintenance of hemostasis and thereby might have an effect on amplification of coagulation and thrombosis at sites of intravascular injury or on blood-contacting biomaterial surfaces [6]. Microparticles on the surfactant-coated disk surface were found to be significantly less than on the uncoated one, commensurate with fewer adherent platelets, providing further evidence of the non-thrombogenic nature of the glycocalyx-mimetic

interfacial coating. Figure 2C shows magnified sections of representative images of the platelet adhesion studies from whole blood, where platelet-derived microparticles could be observed.

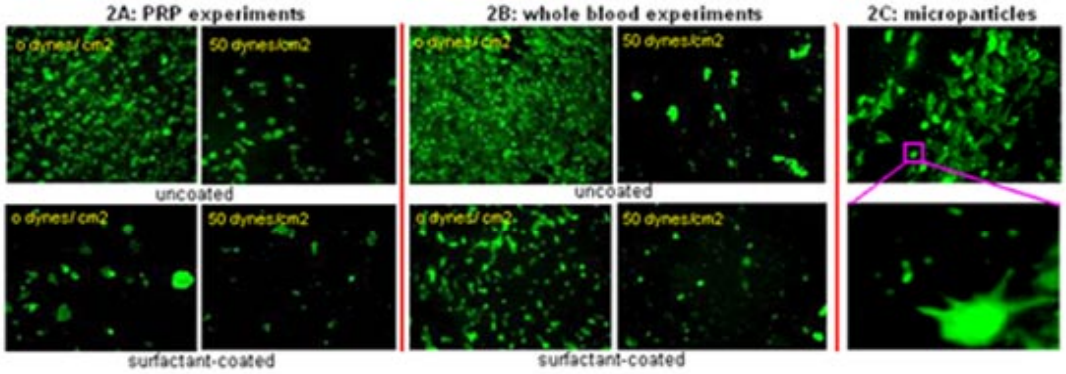

Figure 2. Representative fluorescent images for quantitative determination of platelets adhering to uncoated and surfactant-coated PC disks from PRP (2A) and WB (2B) media in the roating disk system; 2c shows

Discussion The hydrophobic derivatization of the PVAm polymer with pendant hexanoyl groups rendered the surfactant able to spontaneously adsorb on hydrophobic biomaterial surfaces like the polycarbonate used in these experiments. The hydrophilic derivatization with oligosaccharide moieties protruding in a comblike fashion into an aqueous environment, provided the mimic of the sugar-rich hydrated shell environment of the endothelial glycocalyx system. The sugar-rich environment potentially imparted a non-adhesive nature to the surfactant polymer as was evident from the reduction in number of adhered platelets on the polymer-coated disks in the RDS experiments. The coating itself was found to be considerably stable in low and high shear conditions in whole blood at 37^{o}C, the physiological body temperature. Such a coating can be envisaged to be utilized as a non-thrombogenic interfacial layer between blood and a variety of blood-contacting biomaterials.

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