Protein Interaction with Glycocalyx-mimetic Surfaces: A Candidate for Blood-compatible Materials

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Introduction

Materials for cardiovascular applications represent an enormous market (estimated to be $20 billion annually). This includes short term (e.g. sample tubes, and sensor), intermediate term (e.g. catheters, cardiopulmonary assist devices, blood storage, membranes for blood oxygenation and hemodialysis), and long-term (e.g. stents, heart valves) applications. One way to develop blood-compatible surfaces is to design a material which mimics the inside of the normal blood vessel where blood does not coagulate. Blood vessels are lined with cells that present a dense, polysaccharide-rich brush-like layer, called the endothelial glycocalyx. The polysaccharides in the glycocalyx are strong polyanions called glycosaminoglycans (GAGs). These GAGs regulate blood-surface interactions that contribute to the unique blood compatibility of the inside surfaces of blood vessels. This layer inspires the present work, which is focused on developing methods for preparing dense glycosaminoglycan brushes. In this work we report new glycocalyx-mimetic GAG brushes by first preparing chitosan-terminated, chitosan-hyaluronan polyelectrolyte multilayers (PEMs). These PEMs are subsequently modified by adsorption of negatively charged polyelectrolyte complex nanoparticles (PCNs), containing the GAGs heparin and chondroitin sulfate. These PEM+PCN surfaces provide access to heparin-rich or chondroitin sulfate-rich brush like layers on surfaces, with sub-micron surface heterogeneity.

Overall Goal

Develop model surfaces that can be used for studying blood and blood protein interactions with polymer brushes that mimic the endothelial glycocalyx.

Motivation

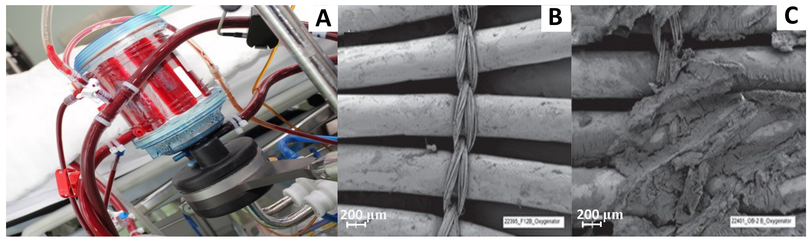
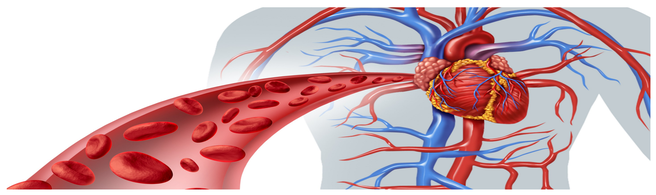


Figure 1. (A) An ECMO bypass system. SEM images of (B) Oxygenator capillaries of heparin-treated rabbits. (C) Thrombosis at oxygenator capillaries of saline-treated rabbits after 6 hours.
(Magnus Larsson, et al. Science Translational Medicine 2014).

Biological Background



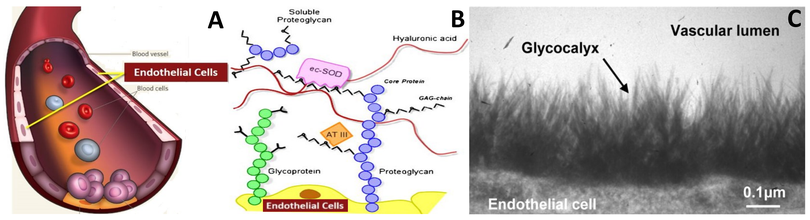


Figure 2. (A) Schematic diagram showing the location of endothelial cells. (B) Schematic representation of the endothelial glycocalyx, showing its main components (Reitsma S, et al. Eur J Physiol 2007).
(C) Electron microscopic views of glycocalyx (Daniel Chappell, et al. Cardiovascular Research 2009).

Methodology

PCN

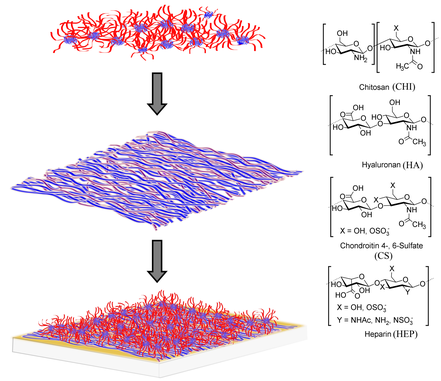
PCN formed from CHI and GAGs (CS & HEP)

PEM

PEM was produced by alternating solutions of the CHI and HA

PEM+PCN

PEM surface with the final layer of CHI was exposed to the PCN (CS-CHI or HEP-CHI)



Results

Size & Zeta Potential of PCN by DLS

Table 1. Average size, PDI, and zeta potential of PCNs

PCN	pH= 5
CS-CHI	
Size (nm)	235±2
PDI	0.11±0.04
Zeta Potential (mV)	-36±3
HEP-CHI	
Size (nm)	105±3
PDI	0.21±0.05
Zeta Potential (mV)	-23±2

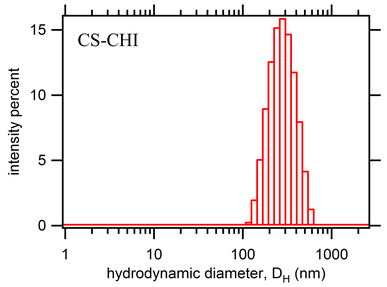


Figure 3. Size distribution by intensity at pH 5.

In Situ Surface Preparation by SPR

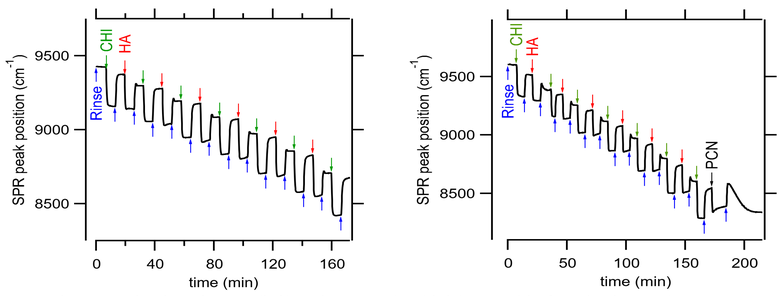


Figure 4. Kinetics of PEM (left) and PEM+PCN (right) assembly from in situ FT-SPR at pH 5.

Surface Chemistry by XPS

Table 2. S/N ratios of PCN on PEM

Sample	pH= 5
PEM+PCN (CS-CHI)	0.27±0.02
PEM+PCN (HEP-CHI)	0.38±0.02

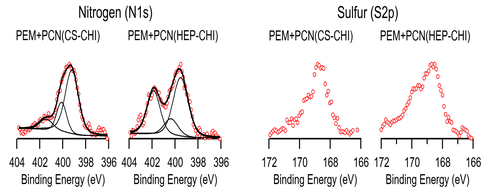


Figure 5. N1s envelopes (left) and S2p envelopes (right) for PEM+PCN at pH 5.

Surface Evaluation by AFM

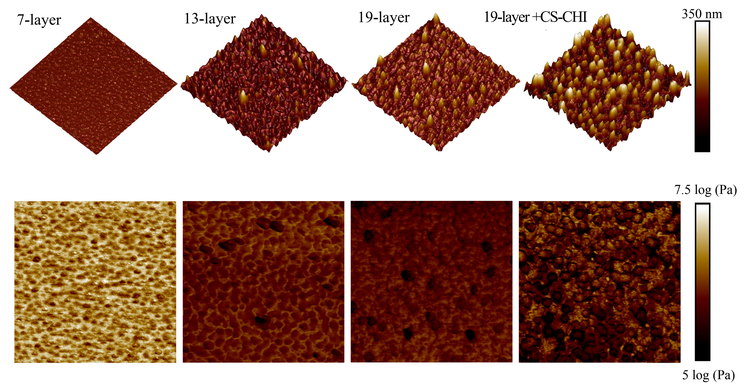
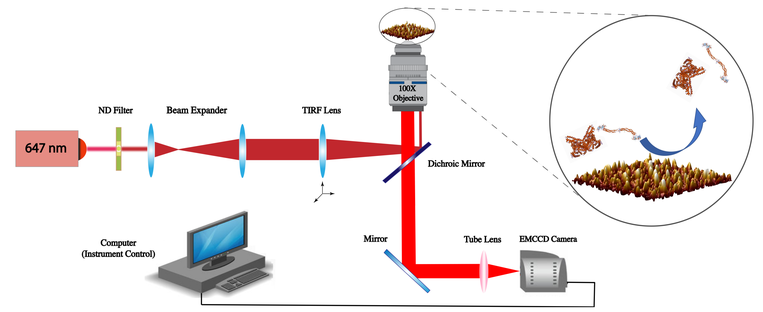


Figure 6. 5 µm × 5 µm AFM images showing the height channel (top) and log-modulus channel (bottom)

Blood Protein Adsorption Study by TIRF Microscopy



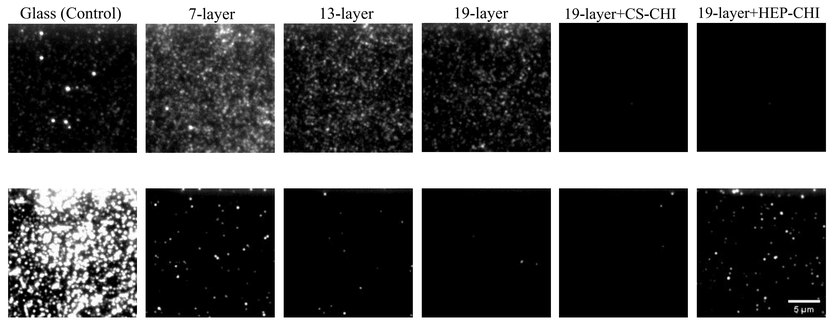


Figure 7. Averaged 1000 fluorescence video frames of protein adsorption after 10 min with 1 nM concentration of albumin (top) and fibrinogen (bottom)

Conclusions, Future Directions

- Glycocalyx mimetic surface coatings are prepared by combining layer-by-layer assembly of PEMs with GAG-rich PCNs.
- XPS confirms adsorbed PCNs from the relative amount of sulfur in the sulfate-containing nanoparticles.
- AFM confirms that PCN swell in solution and present a GAG-rich polymer brush. The PCN features representing dense glycocalyx mimetic brush-like regions on these surfaces cover the surfaces of the PEMs. A dense GAG brush is presented, with height features from 100 to 150 nm high, indicating stretched polymer chains.
- The modulus of the PCN is different from the modulus of the underlying PEM, providing further evidence of the brush-like nature of the PEM.
- A remarkable result is the observation that blood protein adsorption is greatly inhibited on the PEM+PCNs surfaces compared to the PEM and bare substrate.
- These new surfaces provide a tunable platform for studying how blood proteins and blood cells interact with polyanionic polymer brushes composed of different GAGs.
- By discerning how the features of the polymer brush influence blood-surface interactions, we can better design blood contacting materials.