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Abstract
There has been much recent interest in polysaccharide coatings for biotechnology applications. We obtained highly wettable dextran coatings applied to flat silicon wafer surfaces through a two-step process: in the first step, the silicon is aminated by the deposition of a selfassembled monolayer of 3-aminopropyltriethoxysilane (APTES); in the second step, polydisperse and low dispersity dextrans with molecular weights ranging from 1 kDa to 100 kDa are covalently grafted along the backbone to the surface amino groups to achieve strong interfacial anchoring. The effect of dextran concentration on film thickness and contact angle is investigated. Atomic force microscopy (AFM) has been employed to characterize surface roughness and coverage of the dextrans as well as the APTES monolayers. The synthetic surfaces were also tested for gas bubble adhesion properties.

Keywords
physical adhesion, contact angle, self-assembled monolayers, surface roughness, wetting

Biomimetic Dextran Coatings On Silicon Wafers: Thin Film Properties And Wetting

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ABSTRACT

There has been much recent interest in polysaccharide coatings for biotechnology applications. We obtained highly wettable dextran coatings applied to flat silicon wafer surfaces through a two-step process: in the first step, the silicon is aminated by the deposition of a self-assembled monolayer of 3-aminopropyltriethoxysilane (APTES); in the second step, polydisperse and low dispersity dextrans with molecular weights ranging from 1 kDa to 100 kDa are covalently grafted along the backbone to the surface amino groups to achieve strong interfacial anchoring. The effect of dextran concentration on film thickness and contact angle is investigated. Atomic force microscopy (AFM) has been employed to characterize surface roughness and coverage of the dextrans as well as the APTES monolayers. The synthetic surfaces were also tested for gas bubble adhesion properties.

INTRODUCTION

The failure to achieve control over the chemical and biological processes at the interface between host tissues and an alien material may lead to unfavorable host responses such as thrombous formation and chronic inflammation. The biocompatibility of an implanted material in contact with biological fluids, such as blood or tears, essentially depends on the interaction between its surface and the host tissue. Extensive research is being carried out to gain a better understanding of these interfacial processes.

A trend has emerged that aims to develop material surfaces whose properties resemble or mimic the biologically compatible external glycocalyx of the cellular membrane. The glycocalyx [1] is composed of a dense array of highly hydrated polysaccharides and proteoglycans that collectively provide a steric repulsive barrier to minimize undesirable protein and platelet interactions and adsorption. Hydrophilic polysaccharides [2-4] in general and dextrans [5] in particular, covalently linked to the surfaces of biomedical devices offer increased wettability and a marked decrease of cell and bacterial adhesion. Dextrans are hydrophilic and non-charged natural polymeric carbohydrates, and are especially interesting for these purposes because of their excellent biocompatibility and the wide range of molecular weights that are readily available. The goal of the present study has been to create glycocalyx-like surfaces which inhibit the adhesion of blood components, possessing a range of both polydisperse and low-dispersity dextrans, grafting densities and thickness. Biomimetic surfaces prepared by this strategy show

highly favorable characteristics; in fact, although there is much discussion regarding the identity of crucial physical-chemical properties of immobilised macromolecules, it is widely believed that to be effective in thrombus formation reduction, the bound polymer must be highly hydrophilic, densely packed, neutral and conformationally mobile [8].

The combination of a highly controlled grafting chemistry and low dispersity dextrans, to our best knowledge demonstrated here for the first time with this biomimetic purpose, results in model surfaces for further studies. Smooth surfaces are in fact extremely desirable in all situations (surgery, endoscopic procedures, decompression sickness etc.) in which vital organ blood flow can be compromised by microvascular gas embolism where a gas bubble blocks a thin blood vessel. The molecular mechanical basis of bubble adhesion to the vessel wall causing blood flow obstruction is basically unknown; our preliminary experiments [9] suggest though that blood flow obstruction is caused by adhesion of the bubble surface to the endothelial glycocalyx. If surface-surface interactions between the endothelial glycocalyx and the bubble interface control bubble adhesion, and thus determine microcirculatory blood flow and resultant organ injury, the surface roughness of an artificial blood vessel will directly impact the contact angle of gas bubbles and will then become an essential feature.

The purposes of our experiments are to graft dextrans to planar surfaces, characterize the surfaces and then test their biomimetic behavior by measuring bubble adhesion to glass tubes grafted with dextran following the same protocol.

EXPERIMENTAL DETAILS

APTES SAMs on silicon oxide as surfaces to immobilize polysaccharides

Silicon wafers (from Silicon Quest Int'l, thickness 475-575 μm, diameter 4"), cut into pieces (≈1 cm^2) with a diamond-tipped stylus and dusted free of debris with N_2, were cleaned according to the following procedure. The wafers were heated for 20 minutes at 80 °C, and were then immersed in a mixture of H_2O_2 (30% in volume) and H_2SO_4 (70% in volume) at 80 °C for 30 minutes. After cooling, the wafers were washed with deionized water and dried by a stream of N_2. The surface of the wafers was oxidized to form a SiO_x layer by placing them in a UVO Cleaner (Model 4, Jelight Company, Inc.) for 10 minutes. The amino-terminated monolayers were obtained by dipping silicon wafers into a 5 wt% solution of APTES, (Gelest Inc., USA) in toluene for 16 hours in a glove-box. After 16 hours the samples were first sonicated for 20 minutes still immersed in the APTES-toluene solution, then for 20 minutes immersed in N,N-Dymethylformamide and finally again for 20 minutes in deionized water. After the sonication steps the samples were dried by a stream of N_2.

Grafting of dextran polysaccharides to APTES surfaces

Several studies have developed methods for covalently grafting dextran to various surfaces. Most of these reported methods require toxic and expensive sulfonyl chloride reagents, for example tresyl chloride. Furthermore, the reaction conditions for sulfonyl chloride activation of dextran require stringent anhydrous conditions and organic solvents [7]. The dextran immobilization method described here requires the use of low-toxicity reagents and mild reaction conditions that can be performed in aqueous solutions and do not require a glove-box [3].

Dextran was activated by oxidizing the anhydroglucopyranoside (Glc) subunits with sodium metaperiodate ($NaIO_4$). This reaction converts Glc subunits to cyclic hemiacetal structures. These hemiacetal-containing units could then be reacted with the amine groups that terminate APTES. All the experiments were conducted both on dextran obtained from Leuconostoc ssp. (MWs ~1500, 15-20000, ~100000, Fluka) and low dispersity dextrans purchased from Phenomenex in the same range of molecular weights.

Dextran was dissolved in deionized water (546 mg in 100 ml, 100 mg in 100 ml and 50 mg in 50 ml). An equal volume of deionized water containing 2.5 g of $NaIO_4$ was added slowly while stirring. The utilized molar ratio of $NaIO_4$ to Glc units and reaction time ensures, according to previous literature investigations, a 100% oxidation of the Glc units. The procedure of mixing the two reactant solutions was adopted to avoid possible detrimental effects due to high local reactant concentrations. The reaction mixture was then stirred at room temperature for 20 hours for the oxidation to reach completion. Throughout the oxidation reaction, the extent of photochemical decomposition of sodium metaperiodate was minimized by using an aluminum foil to exclude light. The freshly aminated silicon wafers were immersed in the unpurified reaction solution containing oxidized dextran, and 0.75 g of $NaBH_3CN$ was added. The mixture was kept in the dark at room temperature for two days to allow for completion of the surface immobilization reaction. Finally, specimens were rinsed extensively with deionized water prior to analysis.

Surface characterization

Ellipsometry. A Rudolph AutoEL II Null-Ellipsometer equipped with a He-Ne laser (λ = 632.8 nm) with an angle of incidence fixed at 70.0° was used to determine the thickness of the SiO_x and APTES (total thickness minus SiO_x layer thickness) layers assuming for both SiO_x and APTES a refractive index of n=1.462.

The thickness of the polysaccharide coatings was determined using an index of refraction n=1.5 [6]. Measurements were performed at five different spots on a given sample. The reported results are the average of five different samples, i.e., an average of 25 measurements.

Contact angles of water drops (2 μl) were measured according to a standard method at room temperature. The reported results are the average of five different samples.

Atomic force microscopy (AFM) investigations were carried out with a Digital Instruments (di) Multimode Scanning Force Microscope with the Nanoscope III controller and software.

In vitro bubble adhesion was performed both on Microvessels (2^{nd} – 3^{rd} order mesenteric arterioles) removed from adult Wistar rats and on glass capillary tubes (320 μm inner diameter, Alltech Associates) with the inner surface coated with APTES and low dispersity dextrans using the surface activation and grafting methods described above for coating flat surfaces.

Microvessels and capillary tubes were mounted between micropipettes in a perfusion chamber (Living Systems, Burlington, VT) placed on an inverted microscope (Nikon Axiovert) connected to a CCD camera. They were bathed and perfused with a 5% BSA solution. The bath fluid and perfusate were maintained at 37 °C and equilibrated with 5% CO_2, 21% O_2 and balance N_2. This maintains gas volume with bubbles by eliminating gradients for gas influx or efflux.

Preservation of endothelial- and smooth muscle-mediated vascular responses was confirmed by topical application of phenylephrine and acetylcholine in the case of the microvessels. Flow through the vessels/tubes was stopped and an air microbubble (3-4 nl) was injected using a Drummond “Nanoject” injector via a 10 μm diameter micropipette punctured through the vessel

wall adjacent to the outflow cannula and then removed. The bubble was repositioned near the inflow cannula and allowed to reside intravascularly for 10 minutes at which time the pressure on the perfusate inflow side was raised until the bubble dislodged. The differential pressure across the bubble, ΔP, was recorded at the moment of bubble movement.

The bubble length, L, and the vessel internal diameter, D, were determined for each experiment from videomicrometry. The force balance in the absence of flow and without any buoyancy, gives that the adhesion over the surface area in contact with the vessel/tube wall ($\kappa\,\pi DL$) must equal the pressure across the bubble times the cross sectional area ($\Delta P\,\pi D^2/4$). Then κ, the bubble-vessel/tube adhesion force per unit surface area, is given by **$\kappa=\Delta PD/4L$.**

RESULTS AND DISCUSSION

Ellipsometry analysis of the coated layers was used to verify that the intended interfacial bonding had taken place. The presence of hydrophilic coatings on the hydrophobic silicon wafers-APTES is also documented by air/water contact angle measurements.

We systematically investigated the effects of dextran polydispersity, molecular weight and concentration on film thickness, wettability and roughness. Table I shows thickness and contact angles of all the different coatings, while in Figure 1 we have a roughness comparison between polydisperse and low dispersity dextran coatings. The dried layer thickness increases systematically with dextran concentration and molecular weight. For polydisperse coatings the thickness increases by a factor of ten passing from 1.5 to 17.5 kDa and by a factor of thirty passing from 1.5 to 100 kDa. Lowering the concentration from 5.5 to 1 mg/ml, causes instead a decrease of the thickness by a factor of about three for each molecular weight.

For low dispersity coatings the effect of molecular weight on thickness is definitely less pronounced, since we have an increase by a factor of two passing from 1.2 to 102 kDa. A systematic increase is also observed when we compare low dispersity and polydisperse coatings with the same molecular weight and concentration.

An opposite effect can be observed on measured air/water contact angle values. The least hydrophilic polysaccharide coating is also the thickest one, and in general, contact angle values decrease with increasing molecular weight and concentration, and are lower for polydisperse dextrans at the same concentration of the low dispersity samples. Some of the polysaccharide coatings were so hydrophilic that their contact angles could not be reliably measured; which suggests highly wettable surfaces.

Table I. Dextran coatings: thickness (nm) and contact angle (°)

High dispersity dextrans	Weight Ave. Mwt (kDa)		1.5	17.5	100
	[5.5 mg/ml]	**Thickness (nm)**	***3.2 ±0.3***	***34 ±2***	***96 ±17***
		Contact Angle (°)	***12 ±4***	***12.4 ±2.8***	-
	[1 mg/ml]	**Thickness (nm)**	***1.0 ±0.2***	***8.8 ±0.9***	***45 ±3***
		Contact Angle (°)	***20 ±4***	***9 ±3***	***7 ±2***
Low dispersity dextrans	Weight Ave. Mwt (kDa)		1.2	11.7	102
	[1 mg/ml]	**Thickness (nm)**	***2.4 ±0.6***	***3.0 ±0.2***	***5.2 ±0.5***
		Contact Angle (°)	***30 ±3***	***22 ±3***	***15 ±3***

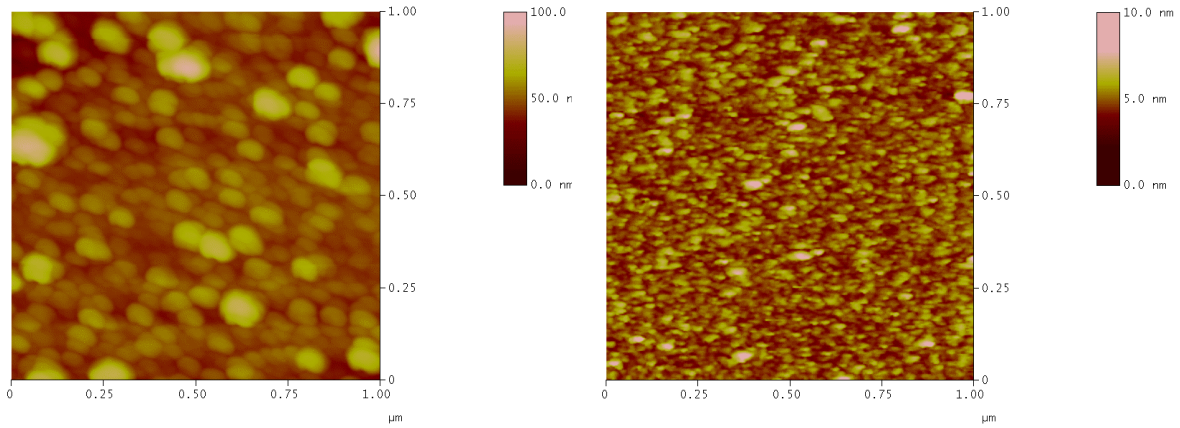

Figure 1. AFM images of grafted polydisperse, MW=1.5 kDa (left) and low dispersity, MW=11.7 kDa (right) dextrans

To test for stability, all dextran grafted surfaces were immersed in water at room temperature for three days. All coatings maintained their thickness and contact angle properties, suggesting that dextran is strongly bound to the substrate and highly stable.

AFM studies on the low dispersity dextran grafted surfaces show that the surface is very smooth relative to the polydisperse dextran grafted by the same strategy. Figure 1 (right image) shows that features are uniformly distributed across a relatively smooth 11.7 kDa dextran surface having a RMS roughness of 0.7 nm, an order of magnitude smoother than the polydisperse 1.5 kDa dextran (see Figure 1 (left image)). Note also that the height scale is an order of magnitude smaller on the right. We can reasonably explain this trend by considering that the polydisperse dextrans give a surface a “loops-and-trains” topography, whereas the low dispersity molecular weight molecules yield a “trains” topography with fewer loops. This interpretation is consistent with the measured thickness values, suggesting that the polydisperse polysaccharides, being less tightly bound to the surface, also form a thicker, more exaggerated loops-and-trains surface than the tightly packed low dispersity polysaccharides.

The results of the *in vitro* gas bubble adhesion experiments (Table II) were found to be of particular importance. Note that values of κ sensibly increase passing from the unmodified and APTES grafted tubes to the dextran grafted tubes, and also systematically increase with increasing dextran molecular weight and approach the value measured in real excised microvessels.

This initial set of results demonstrates that the longer chain dextrans are associated with an adhesion force that agrees well with adhesion measured using the more complex biological surface.

Other constructs are to be synthesized with the intent of incorporating more biochemical features of the endothelial surface.

Table II. Gas bubble adhesion on microvessels and modified glass tubes

	Microvessels	Capillary tubes coating				
		None	**APTES**	**Dex 1,2 kDa**	**Dex 11,7 kDa**	**Dex 102 kDa**
κ (dyne/cm^2)	*358*	59.5	42.8	121.1	170.4	302.3

In the context of an important pathophysiological process (i.e., gas embolism), we have a method for assessing mechanical behavior (function) associated with the molecular structure which we can perhaps then use to develop a therapy or medical intervention.

CONCLUSIONS

We synthesized a coating layer of dextran, a relatively simple and well characterized neutral polysaccharide, with the purpose of mimicking the cells' glycocalyx layer, that prevents non-specific cells-protein interactions. Systematic physical chemical characterization, with evaluation of thickness, wettability and roughness, was performed on coatings obtained both from commonly used polydisperse dextrans and low dispersity dextrans in the 1-100 kDa molecular weight range.

Low dispersity high molecular weight dextran grafted on the inner surface of glass capillary tubes can also be used as an ideal *in vitro* model for gas bubble adhesion studies, since it showed values of adhesion force/unit area for bubbles in 5% BSA very similar to those obtained in excised blood microvessels.

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