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Nonfouling Characteristics of Dextran-Containing Surfaces

Surangkhan Martwiset, Anna E. Koh, and Wei Chen*

Chemistry Department, Mount Holyoke College, South Hadley, MA 01075

Abstract

Hydroxyl groups in dextrans have been selectively oxidized to aldehyde groups by sodium periodate in a controlled fashion with percentage of conversion ranging from 6% to 100%. Dextrans (10 k, 70 k, 148 k, 500 k, and 2 000 kDa) and oxidized 10 k dextrans have been successfully grafted to functionalized silicon surfaces. The effect of molecular weight on protein adsorption is not nearly as striking as that of the extent of oxidation. When ~ 25% of the hydroxyl groups have been converted to aldehyde groups, there is negligible protein adsorption on surfaces containing the oxidized polysaccharides. Conformations of grafted polymers depend strongly on their chemical structures, i.e. the relative amounts of –OH and –CHO groups. That the dependence of the chain conformation as well as the protein resistance on the balance of the hydrogen bond donors (–OH) and the acceptors (–OH and –CHO) implies the importance of chemical structure of surface molecules, specifically the interactions between surface and surrounding water molecules on protein adsorption. Oxidized dextrans are potential poly(ethylene glycol)-alternatives for nonfouling applications.

Introduction

Researchers in the biomaterials field have expended a great amount of effort in designing materials that are biocompatible. Poly(ethylene glycol) (PEG) has been shown to be resistant to adsorption and adhesion;^{1,2} incorporation of PEG to surfaces has been the most popular approach in designing biomaterials.³ More recently, phosphorylcholine-containing materials, such as poly(2-methacryloxyethylphosphorylcholine) (PMPC), have been designed and prepared to mimic biomembranes.⁴ PMPC polymers have been demonstrated to be biocompatible; PMPC-containing surfaces are prone to phospholipid adsorption but are resistant to protein adsorption and cell adhesion.^{5–7} PEG and PMPC have obviously different molecular structures (the former is neutral and the latter contains zwitterions) and they interact with biological entities (proteins, cells, phospholipids, and etc.) differently upon contact with body fluid. At first glance, it is surprising that the two of them are among the handful of molecules that are known to be biocompatible. What do these molecules have in common? There is relatively little known about the molecular basis of fouling resistance, i.e. how does the molecular structure of materials govern the adsorption and adhesion of biological entities.

A review by Morra summarized the two schools of thought on the physico-chemical basis of fouling resistance.⁸ The prevailing “physical view” stems from the Alexander-De Gennes theory of polymer interfaces^{9–11} and attributes fouling resistant characteristics of PEG to solely entropic effects.^{12–17} The picture of a tethered and freely fluctuating chain of PEG that is resistant to adsorption and adhesion due to its large conformational freedom, however, does not take into account its molecular structure and its interaction with aqueous media.

The “chemical view” recognizes that interactions, e.g. hydrogen bonding, between water molecules in the media and specific chemical functional groups on the surface-tethered

*Email: weichen@mtholyoke.edu; Tel: 413–538–2224; Fax: 413–538–2327.

molecules cause perturbation of the water's three-dimensional structure and increase the free energy of the system, which may lead to protein adsorption from the media. Such interaction between surface functional groups and surrounding water is dictated by the electron-donor/electron-acceptor characteristics of the species involved.¹⁸⁻²² Due to the complexity of the system which consists of a three dimensional array of hydrogen-bonded water and surface molecules, theoretical evaluation¹⁸⁻²² of the chemical view has been a challenging task. Recently, some experimental reports have shed light on the governing effect of molecular interactions on biocompatibility, mostly protein resistance. In the first set of reports, surfaces containing oligo(ethylene glycol) (OEG) were shown to be protein resistant, which was attributed solely to the interaction between OEG and water since short OEG chains do not possess much conformational freedom.²³⁻²⁶ Reports on the interactions between water molecules and some water soluble polymers indicate that hydrogen bonding is electrostatic in nature so that most polyelectrolytes associate strongly with water molecules and alter the three-dimensional water structure at the interface while neutral molecules (such as PEG) and zwitterion-containing polyelectrolytes (such as PMPC) do not perturb the structure of the surrounding water molecules.²⁷⁻²⁸ Even though the more recent studies have been biased toward the chemical view of nonfouling, it is a complicated phenomenon and one should have an open mind in evaluating various factors at play whether it is entropy, interaction, or both (one being more critical than the other or being equally important).

In this study, different molecular weight dextrans and oxidized dextrans of molecular weight 10 kDa were grafted to functionalized silicon wafer surfaces in order to study the molecular variants that affect protein adsorption. The selection rationale is based on the fact that dextrans are nontoxic, water soluble, and neutral, which are characteristics favorable for biomaterials. They are natural polysaccharides and inexpensive choices. The wide molecular weight range that is commercially available (10 to 2 000 kDa) allows probing the entropic effect. Furthermore, the oxidation of polysaccharides using periodate to convert –OH groups to –CHO/hemiacetal groups is well understood.²⁹⁻³¹ By controlling the extent of oxidation, the relative amount of electron acceptors and donors on oxidized dextrans thus the interaction between water and the polymer chains was varied and studied. By evaluating both physical and chemical views of nonfouling in the same system for the first time, we have a better understanding of its molecular basis. Such exploration of the fundamental basis of biocompatibility will open up new opportunities for the design of the next generation biomaterials and better and less expensive alternatives.

Experimental

General

Silicon wafers were obtained from International Wafer Service (100 orientation, P/B doped, resistivity 1–10 Ωcm , thickness 450–575 mm). Dextrans (M_w = 10, 70, 148, 500, and 2 000 kDa), phosphate buffered saline (PBS) tablets, albumin (bovine, essentially fatty acid free), lysozyme (from chicken egg white), and collagen solution (type I, from calf skin) were purchased from Sigma. 3-aminopropyltrimethoxysilane (APDES) was purchased from Gelest, Inc. House purified water (reverse osmosis) was further purified using a Millipore Milli-Q system that involves reverse osmosis, ion-exchange and filtration steps (18.2 $M\Omega\text{cm}$). Other reagents and solvents were obtained from Aldrich or Fisher and used as received. Ellipsometric measurements were made with a Microphotonic EL X-01R ellipsometer. The light source was a He-Ne laser with λ = 632.8 nm. The angle of incidence (from the normal to the plane) was 70°. The thicknesses of the layers were calculated using a single layer model (silicon substrate/silicon oxide + graft layer/air) with the following parameters: air, n_0 = 1; silicon oxide + graft layer, n_1 = 1.457; silicon substrate, n_s = 3.882, k_s = 0.019 (imaginary part of the refractive index). X-ray photoelectron spectra (XPS) were recorded with a Physical Electronics Quantum 2000 ESCA Microprobe with Al K_{α} excitation. Spectra were obtained at a 45° take-off angle

(between the plane of the surface and the entrance lens of the detector optics). XPS atomic composition was determined from peak areas and sensitivity factors of individual elements; at least three samples and two areas on each sample have been analyzed to obtain the average atomic compositions (C, N, Si, and O contents) with errors of less than $\pm 5\%$. AFM images were obtained with a Veeco Metrology Dimension 3100 atomic force microscope operated in tapping mode. Contact angle measurements were made with a Ramé-Hart telescopic goniometer and a Gilmont syringe with a 24-gauge flat-tipped needle. The probe fluid used was water, purified as described above. Dynamic advancing (θ_A) and receding angles (θ_R) were recorded while the probe fluid was added to and withdrawn from the drop, respectively. The values reported are averages of 3–5 measurements made on different areas of the sample surface.

Functionalization of Silicon Wafers

Silicon wafers were cut into 1.5×1.5 cm pieces and cleaned by submerging in a freshly prepared mixture of 7 parts concentrated sulfuric acid containing ~ 2 wt% sodium dichromate and 3 parts 30% hydrogen peroxide for 1 h. Wafers were then removed and rinsed with copious amounts of water and dried in a clean oven at 110°C for 30 min. Thickness of the native oxide on clean wafers was determined to be 22 ± 1 Å by ellipsometry. Silanization with APDES was performed in the vapor phase at 70°C for 72 h using ~ 0.5 mL of silane. There was no contact between the liquid silane and the wafers. After silanization, wafers were rinsed with toluene (3x), ethanol, and Milli-Q water. The samples were then dried at reduced pressure for 30 min.

Oxidation of Dextrans

A desired amount of NaIO_4 was dissolved in 60 mL of Milli-Q water. The solution was added to another solution containing 4 g of dextran and 30 mL of Milli-Q water. The reaction was stirred in the dark for 2 h at room temperature. At the end of the reaction, the solution was dialyzed (molecular weight cutoff = 1 kDa) extensively against Milli-Q water for 4 days. A powdery sample was obtained via lyophilization and stored at 4°C in the dark. The aldehyde contents of oxidized dextran samples were quantified via titration. 40.0 mg of a dextran/oxidized dextran sample was dissolved in 15.0 mL of 0.100 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ solution. The solution was sealed with parafilm and stirred overnight. Titration was carried out using a 0.100 M NaOH solution and monitored using a pH meter.

Grafting of (Oxidized) Dextrans

0.1 g of a dextran/oxidized dextran sample was dissolved in a 10 mL pH = 5.2 buffer solution containing 0.87 g of K_2SO_4 and 0.028 g of NaCNBH_3 . The solution mixture was filtered through a $0.45\ \mu\text{m}$ Nylon filter and introduced to a flask containing amine-functionalized silicon wafer samples. The reaction was carried out at 40°C for 96 h. The samples were then rinsed with copious amounts of water and dried at reduced pressure for 30 min.

Protein Adsorption

Protein solutions were freshly prepared by dissolving proteins or diluting protein solutions with PBS solution (0.01 M, pH 7.4) to give a final concentration of typically 0.1 mg/mL for albumin and lysozyme, and 30 $\mu\text{g/mL}$ for collagen. Substrates were incubated in PBS solution for 2 h prior to adsorption. The adsorption experiments were carried out in a closed flask containing 10 mL of protein solution at 37°C for 1 h. Rinsing was accomplished by six successive dilutions using protein free PBS solution without exposing the samples to the air-water interfaces. Samples were subsequently rinsed with water four times to dissolve adsorbed salt and were dried at reduced pressure for 30 min before analysis.

Results and Discussion

Dextrans produced from *Leuconostoc mesenteroides* are 1,6-glucans with some glucose side chains, which are mostly connected to the main chain by 1,3 linkages. The strategy for grafting dextrans of different molecular weight and oxidized dextrans of varying extent of oxidation is illustrated in Scheme 1. Single silicon surfaces were functionalized initially with an aminosilane in the vapor phase. Covalent attachment of dextrans/oxidized dextrans was accomplished via reactions between the surface amine groups and the aldehyde groups of dextrans (derived from the reducing chain ends) and oxidized dextrans. Finally, to evaluate the molecular basis of nonfouling, protein adsorption as a function of dextran molecular weight and the extent of oxidation was carried out to shed light on the relative importance of the entropic effect of tethered fluctuating surface molecules and interactions between surface molecules and surrounding water.

Silicon wafers were chosen as the substrates because they can be chemically modified by a wide range of silanes. Preparation of covalently attached monolayers on silicon wafer surfaces has been reviewed and delineated by Fadeev and McCarthy.^{32,33} Modification of silicon oxide surfaces using reactive alkylsilanes can be done either in solution or in the vapor phase. The vapor phase is preferred because it appears to be the cleanest and easiest method to obtain uniform monolayers. Single silicon oxide surfaces were functionalized with APDES at 70 °C for 72 h in the vapor phase. The resulting thickness increase of $\sim 6 \text{ \AA}$ as measured by ellipsometry corresponds to the attachment of the amine-containing silane monolayer. Shorter reaction times resulted in smaller thickness increases indicating incomplete monolayer formation. Advancing and receding water contact angles are $70^\circ/34^\circ$ (θ_A/θ_R) and XPS at 45° take-off angle (sampling depth is $\sim 30 \text{ \AA}$) indicates the presence of 2.3% nitrogen on the amine surface. The results are summarized as entry 1 in Table 1. Atomic force microscopy images of silicon wafers before (not shown) and after silanization (Figure 1a) show little difference; both are very smooth. All of these results indicate that the formation of the covalently attached monolayer was complete and the surface functionalization was successful using the amine-containing silane under the conditions used.

Oxidation of dextrans using periodate to convert $-\text{OH}$ groups to $-\text{CHO}$ /hemiacetal groups is well understood,²⁹⁻³¹ and was taken advantage of here to tune the molecular interactions between dextrans/oxidized dextrans on surfaces and surrounding water molecules: $-\text{OH}$ groups are hydrogen bond acceptors and donors and $-\text{CHO}$ groups are only hydrogen bond acceptors. It is important to note that the polysaccharide chain length is not affected by the oxidation process as shown in Scheme 2. In order to quantitatively oxidize the $-\text{OH}$ groups, a molar ratio of 2 between NaIO_4 and dextran repeat units is required. Molar ratios were varied between 0 (pristine dextrans) and 2 (fully oxidized dextrans), at ratios of 0, 1/8, 1/4, 1/2, 1, 3/2, and 2 for each molecular weight sample of dextrans. For ease of further reference, $^{\text{ox}}10\text{k}^{1/2}$ represents a 10k molecular weight sample that is oxidized by using a molar ratio of NaIO_4 to dextran repeat unit of 1/2. Oxidized dextrans of each molecular weight and each extent of oxidation were prepared and water solubility of these samples decreases with molecular weight and extent of oxidation; this limited the scope of the study. As a result, only the 10k molecular weight samples (10k , $^{\text{ox}}10\text{k}^{1/8}$, $^{\text{ox}}10\text{k}^{1/4}$, $^{\text{ox}}10\text{k}^{1/2}$, $^{\text{ox}}10\text{k}^1$, and $^{\text{ox}}10\text{k}^{3/2}$) had sufficient solubility to be evaluated; the fully oxidized sample, $^{\text{ox}}10\text{k}^2$, was also excluded due to poor water solubility. IR and ^1H NMR spectra of dextrans and oxidized dextrans show decreased intensities of $-\text{OH}$ peaks but insignificant $-\text{CHO}$ signals after oxidation. This is consistent with an earlier report of the formation of cyclic hemiacetals from aldehydes upon dextran oxidation in aqueous media.³⁰ The aldehyde/hemiacetal content of oxidized samples was quantified by adding an excess amount of $\text{NH}_2\text{OH}\cdot\text{HCl}$ and titrating the generated HCl with a NaOH solution while monitoring the pH using a pH meter using a reported procedure.³¹ That the aldehyde contents

obtained from titration are consistent with the theoretical predictions (not shown) indicates that the oxidation reaction is quantitative under the conditions used.

Five different molecular weight samples, 10k, 70k, 148k, 500k and 2 000k, and five oxidized 10k samples, $^{ox}10k^{1/8}$, $^{ox}10k^{1/4}$, $^{ox}10k^{1/2}$, $^{ox}10k^1$, and $^{ox}10k^{3/2}$, were grafted to amine-functionalized silicon wafers. NaCNBH_3 and K_2SO_4 were added to reduce the unstable imines to amines and to collapse polymer chains to increase grafting density, respectively.³⁴ After 96 h, surfaces containing $\sim 7 \text{ \AA}$ thick grafted layers of dextran and oxidized dextran were obtained with decreased surface nitrogen contents and lower water contact angles due to the hydrophilic nature of the polysaccharides. Grafting results of 10k and $^{ox}10k^{1/2}$ are shown as representative data in Table 1. Low grafting yield is the most common issue associated with the “grafting to” technique. Our objective here, however, is not to strive for thick grafted layers, rather it is to evaluate how sensitive protein resistant characteristics are affected by the chemical structure and molecular weight of the grafted polysaccharides.

AFM images of surfaces before and after grafting with 10k dextran and oxidized dextrans are shown in Figure 1. Despite similar grafting thicknesses, the topographical appearances are drastically different upon oxidation: the pristine dextran chains appear to be collapsed, which is a common feature for all molecular weight dextrans studied (not shown), and the oxidized chains become more spread out as the extent of oxidation increases. The “transition” occurs when the molar ratio of NaIO_4 to dextran repeat unit is 1/2 or at the extent of oxidation of 25%. The most obvious explanation is that oxidation provides more grafting sites, which affects chain conformations upon grafting: each unmodified dextran chain contains one reducing chain end, which acts as the only surface attachment site to give rise to the “tail” conformation; on the other hand, aldehyde groups along the oxidized chains offer more anchoring sites to form the “loop” and “train” conformations. Another possible reason is the difference in molecular interactions between pristine dextran-water and oxidized dextran-water systems. $-\text{OH}$ groups are both hydrogen bond acceptors and donors thus can interact with each other strongly; however, $-\text{CHO}$ groups are only hydrogen bond acceptors so they do not hydrogen bond with each other but can hydrogen bond with surrounding water molecules. Oxidation changes the donor/acceptor balance on the surface molecules and their hydrogen bonding interactions with the surrounding water molecules. The collapse of dextran chains is likely due to strong intramolecular hydrogen bonding interactions among the $-\text{OH}$ groups. The relatively extended oxidized chains (smooth topographies shown in Figure 1d and 1e) exhibit stronger intermolecular hydrogen bonding interactions between the $-\text{CHO}$ groups and water molecules. Drying apparently preserves the aqueous solution conformations as observed by AFM. The lower water contact angles observed for the oxidized dextran surfaces in comparison to those of the pristine dextran surfaces (Table 1), support the argument of more favorable interactions between oxidized chains and water.

Protein adsorption studies were carried out on substrates before and after grafting. Because protein adsorption is followed by cell adhesion in undesirable biological responses, we used protein adsorption as a criterion for nonfouling. Three proteins, albumin, collagen and lysozyme were chosen because they represent proteins of different size, shape, composition, and biological functions. Collagen (285 kD, $15 \text{ \AA} \times 15 \text{ \AA} \times 3\,000 \text{ \AA}$, $\text{pI} = 4.5$) has a long, stiff, and triple stranded helical structure. As a major component of skin and bone, it is the most abundant protein in mammals. Bovine serum albumin (BSA) (68 kD, $40 \text{ \AA} \times 40 \text{ \AA} \times 140 \text{ \AA}$, $\text{pI} = 4.8$) is one of the most abundant blood proteins and its biological functions are transport and maintenance of colloid osmotic pressure. Lysozyme (14 kD, $30 \text{ \AA} \times 30 \text{ \AA} \times 45 \text{ \AA}$, $\text{pI} = 11$) is a natural antibiotic that catalyzes the degradation of polysaccharide chains in the cell walls of bacteria.

Protein adsorption experiments were carried out in 0.01 M PBS buffer at 37 °C. 1 h was sufficient for the adsorption to reach completion. After adsorption, the protein solution was partially withdrawn and the remaining solution was diluted with the PBS buffer, and the process was repeated several times. During the dilution/rinsing process, samples were immersed in the solution to prevent the transfer of Langmuir-Blodgett-like protein films onto samples at the air-solution interface.

The surfaces containing adsorbed protein were characterized by XPS, ellipsometry, and AFM. During the time of XPS analysis (less than 10 min per sample), no beam damage of adsorbed protein was observed based on both peak shapes and atomic compositions. Both amine-functionalized and dextran/oxidized dextran-grafted surfaces contain small amounts of nitrogen, 2.3% (entry 1 in Table 1) and ~1.3% (entries 2 and 3 in Table 1), respectively, and protein adsorption is marked by an increase in the peak area of N_{1s} at 400 eV, thus the difference in nitrogen content before and after protein adsorption is used to quantify the amount of protein adsorbed. Carbon, oxygen, and silicon come from multiple sources: the silicon substrate, the silane monolayer, the grafted (oxidized) dextrans, and the adsorbed proteins; their contents do not vary as significantly as the amount of protein adsorbed and are not presented here. Figure 2 shows nitrogen content differences upon adsorption of collagen, lysozyme, and albumin obtained at 45° take-off angle, as a function of molecular weight of grafted pristine dextrans. The adsorbed amounts vary somewhat for the three proteins, however, they follow the same trend: protein adsorption decreases as the molecular weight of dextrans increases and rises slightly at the highest molecular weight studied. Longer chains are relatively more flexible and extended (higher entropy) and are more effective at repelling incoming proteins. Because samples with identical thickness (7 ± 1 Å) were studied with regard to protein adsorption, the surface grafted with the highest molecular weight dextran has the lowest grafting density and proteins can adsorb in between chains. Even though protein adsorption is reduced as dextran molecular weight increases, the adsorbed amount is significant for all pristine dextrans independent of molecular weight. When protein adsorption is studied as a function of the extent of oxidation for the 10k molecular weight set of samples, the reduction in adsorbed amount is much more drastic when the extent of oxidation reaches 25% (Figure 3). The slight increase at the highest extent of oxidation (75%) is perhaps due to the more hydrophobic nature of the aldehyde groups, reaction between the aldehyde groups and amine functionality in proteins, and/or decreased polysaccharide chain mobility due to the increased number of anchoring sites.

Comparing the AFM images in Figure 1 and protein adsorption trend in Figure 3, the transition from the “collapsed” to “spread out” conformations and the dramatically increased protein resistance occur at the same extent of oxidation. We cannot help but consider this concurrence as more than coincidental. Two explanations are offered above for the observed change in conformations of grafted chains as the conversion of –OH groups to –CHO groups taking place: one is the difference in the number of anchoring sites that gives rise to the “tail” to “loop” and “train” transition and the other is the change in the interactions of surface polymers with surrounding water molecules when some –OH groups are converted to –CHO groups. The increase in the number of anchoring sites alone, which gives rise to decreased chain mobility/entropy, should have promoted protein adsorption to some extent if the “physical view” alone were correct; this is opposite of what we observed. The chemical transformations along the dextran backbone, thus the change of interactions between surface functionalities, –OH vs. –CHO groups, and water molecules must then contribute significantly to the observed resistance to nonspecific protein adsorption. This demonstrates that the chemical nature of surface molecules dictates their interactions with surrounding water molecules, which significantly perturbs their interactions with other entities, such as proteins, in the environment.

Ellipsometric thickness changes after protein adsorption have also been quantified, which correlates well with the XPS data in general. Some representative data are shown in Table 1.

Collagen adsorption results are shown here because among the three proteins studied, the adsorbed amounts are the highest for collagen (Figures 2 and 3). Clearly, grafting pristine dextrans to surfaces does not improve the nonfouling characteristics (entries 4 and 5), however, grafting oxidized dextrans drastically reduces protein adsorption to surfaces, e.g. the thickness increase is only 2 Å and nitrogen content increase is only 1% upon collagen adsorption on moderately (25%) oxidized 10k dextran (entry 6). AFM images of some collagen-adsorbed surfaces are shown in Figure 4. It is apparent that collagen adsorbs extensively on the amine- and dextran-containing surfaces but negligibly on the oxidized-dextran surface, which is consistent with the XPS and ellipsometry results.

The protein resistance of $^{ox}10k^{1/2}$ as a function of graft thickness has also been carried out. Oxidized dextrans are protein repellent as long as the grafted layer thickness is at least 4 Å, which is extremely effective. This specific sample of oxidized dextran is comparable to PEG³⁴ in the amount of nitrogen detected by XPS on their surfaces after protein adsorption and should be evaluated further as a biocompatible material.

Conclusions

Dextrans of different molecular weights and oxidized 10k dextrans containing varying ratios of –OH and –CHO groups have been grafted to functionalized silicon surfaces. Even though the grafted layer thickness is only on the order of angstroms, reduction in protein adsorption, which is a function of the extent of oxidation, is striking. The most protein resistant surfaces contain moderately oxidized dextrans. The effect of molecular weight on protein adsorption is not as dramatic. AFM images reveal that the chain conformations of the grafted polymers depend strongly on their chemical structures, i.e. the relative amounts of –OH and –CHO groups. This study sheds light on the relative importance of the fundamental variants in the molecular design of nonfouling materials and points out the importance of surface chemical structures and the interactions between surface molecules and surrounding water. Oxidized dextrans have been shown to be viable alternatives to PEG for nonfouling applications.

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Figure 1. AFM images ($1 \times 1 \mu\text{m}$) of surfaces before (a) and after grafting with 10k (b), $^{ox}10k^{1/8}$ (c), $^{ox}10k^{1/2}$ (d), and $^{ox}10k^{3/2}$ (e).

Figure 2. Nitrogen content difference before and after adsorption of collagen, lysozyme, and albumin, obtained at XPS 45° take-off angle, as a function of the molecular weight of grafted pristine dextrans.

Figure 3.

Nitrogen content difference before and after adsorption of collagen, lysozyme, and albumin, obtained at XPS 45° take-off angle, as a function of the extent of oxidation of grafted 10k dextran and oxidized dextrans.

Figure 4. AFM images ($1 \times 1 \mu\text{m}$) of collagen-adsorbed surfaces: (a) containing aminosilane-monolayer, (b) grafted with $10^{1/8}$, and (c) grafted with $10^{1/2}$.

Scheme 1.

Grafting of dextran/oxidized dextrans to functionalized silicon surfaces

Scheme 2.
Oxidation of dextrans

Table 1

Ellipsometric thickness of the top layer (ΔT), advancing and receding water contact angles (θ_A/θ_R), and nitrogen content determined by XPS at 45° take-off angle of some surfaces prepared.

	ΔT (Å)	Water contact angles (θ_A/θ_R)	Nitrogen content (%)
Si-(NH ₂)	6	70°/34°	2.3
Si-NH ₂ -(10k)	7	60°/12°	1.3
Si-NH ₂ -(^{ox} 10k ^{1/2})	7	51°/13°	1.3
Si-NH ₂ -(coll)	31	-	8.1 [*]
Si-NH ₂ -10k-(coll)	33	-	10.1 [*]
Si-NH ₂ - ^{ox} 10k ^{1/2} -(coll)	2	-	1.0 [*]

* : difference of nitrogen content before and after collagen adsorption