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Dextran Grafted Silicon Substrates: Preparation, Characterization And Biomedical Applications

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ABSTRACT

Biodevices used in the cardiovascular system suffer from well-known problems associated with surface-induced gas embolism and thrombosis. In order to improve the biocompatibility of these devices, biomimetic coatings show good promise. We recently 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 was performed on coatings obtained both from commonly used polydisperse dextrans and low-dispersity dextrans in the 1-100 kDalton molecular weight range.

We have combined standard surface analysis techniques, such as ellipsometry, contact angle measurements and AFM, with less traditional vibrational spectroscopy techniques in the characterization of our biomimetic coatings. FTIR, micro-FTIR and micro-Raman spectroscopies were utilized to correlate the conformational and molecular aspects of the grafted poly- and monodisperse dextran chains to their attractive biological properties.

INTRODUCTION

The polysaccharide dextran exhibits outstanding biocompatibility [1], and in recent years, a large number of studies have highlighted surface-bound dextran as a protein-binding and cell-resistant coating for biomaterials. Many different anchoring methods have been investigated with the aim of obtaining highly controllable attachment strategies that require a small number of steps during synthesis and avoid toxic reagents [2,3].

We used the methods proposed by Dai *et al*. for coating silicon wafers with dextran [4] which involves low residual toxicity reagents and mild aqueous reaction conditions. In this method, the oxidation of glucose units by periodate produces hemialdals capable of reacting with amine groups previously anchored on silicon wafers through the deposition of a 3-aminopropyltriethoxysilane (APTES) self-assembled monolayer. This Schiff-base linkage is then reduced by sodium cyanoborohydride to a secondary amine.

The thickness, wettability, roughness and gas bubble adhesion properties of the coatings were investigated as a function of dextran molecular weight (in the range 1-100 kDa) and polydispersity index [5]. We found that glass tubes coated with low-dispersity dextran with a

molecular weight of 100 kDa showed similar gas bubble adhesion properties to real excised blood microvessels.

It is necessary to further improve our scheme for coating silica surfaces with dextran films. Further optimization of the process requires improved capabilities for the characterization both of the different grafting steps and the final coatings. Additional techniques should be combined with standard surface analysis techniques, such as ellipsometry, contact angle measurements, AFM and XPS to better understand the properties of our biomimetic coatings. Infrared (IR) and Raman spectroscopies, especially in their micro- forms, are perhaps natural tools for the study of layered, multi-component polymeric surfaces, however, these techniques have been largely neglected to date.

In this study, we performed detailed analyses both on the physical structure of the poly- and low-dispersity dextrans and the interfacial bonding and structure of the coatings on Si wafers by means of FTIR, micro-FTIR and micro-Raman. Our novel micro-Raman studies showed significant differences in interfacial bonding and structure between the poly- and low-dispersity dextrans and the APTES SAMs.

EXPERIMENTAL DETAILS

The details regarding the preparation of our dextran coatings and their characterization (ellipsometry, contact angle measurements, AFM, gas bubbles adhesion tests) can be found in [5]. The reaction scheme for the immobilization of oxidized dextran on amine-functionalized Si surfaces is shown in Fig. 1. It will be used as a guideline for the interpretation of micro-FTIR and micro-Raman spectra recorded on the coatings.

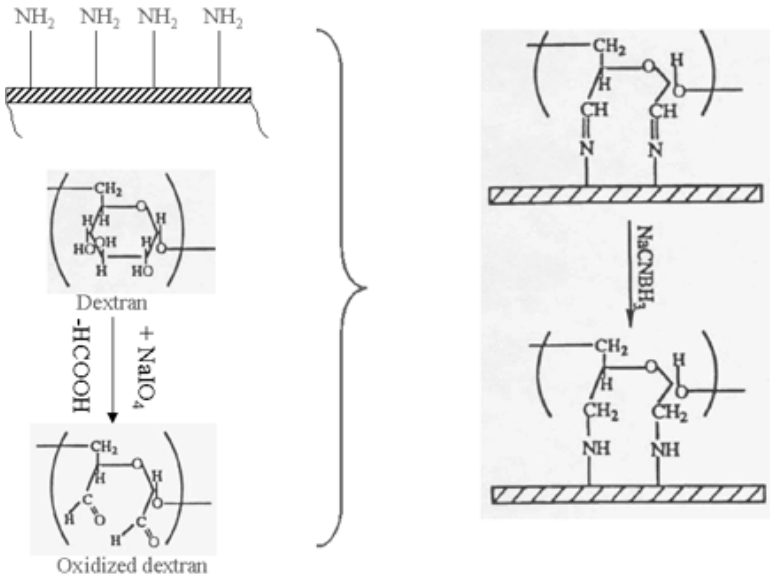

Figure 1. Reaction scheme for the immobilization of oxidized dextran on APTES amine-functionalized Si surfaces (modified from [4]).

FTIR spectroscopy. A Bruker IFS113V spectrometer was used which was equipped with a Globar source, a Ge/KBr beamsplitter and a DTGS (deuterated tryglycine sulfate) detector. The spectra were recorded in the 400-4000 cm^{-1} range with a spectral resolution of 2 cm^{-1}. In order to obtain a good *S/N*, 50 scans were averaged before being Fourier transformed. Dextran powders were dispersed in spectroscopic grade CsI (Aldrich). The powder was pressed at 10 tons to get a pellet. Background spectra of the vacuum were also obtained as reference signals.

Micro-FTIR spectroscopy. Infrared microscopic measurements of the coatings were obtained with an IRT-30 microscope coupled to an FT/IR-470Plus Fourier Transformed Infrared Spectrometer (JASCO Corporation). The infrared microscope was equipped with a high-sensitivity mercury-cadmium-tellurium (MCT) detector, with liquid nitrogen (LN_2) and a "direct through system" observation system using CCD camera. 6 areas of interest for each sample were isolated using variable rectangular apertures (50 μm × 50μm). FT-IR spectra were acquired in the range 600-4000 cm^{-1} at 2 cm^{-1} resolution averaging 300 scans.

Micro-Raman spectroscopy. Raman spectra were recorded with Jasco Ventuno micro-Raman spectrometer, using a compact green light second-harmonic-YAG (SHG-YAG) laser of 532 nm wavelenght at 100% of its power (~ 30 mW). The laser was focused on the different samples using an objective lens of 100×, allowing a spatial resolution down to 1 μm and a confocal aperture diameter (which largely determines the actual spatial resolution) of 200 μm equivalent to 8 μm spatial resolution. The grating has 1200 lines/mm, giving a spectral resolution of about 2 cm^{-1} in the whole 50-4000 cm^{-1} spectral range. Spectra with a good *S/N* ratio were obtained averaging 10 scans of 50 s exposure time each. 5 areas for each sample were analysed.

RESULTS AND DISCUSSION

The FTIR and micro-Raman spectra of polydisperse and low-dispersity (PDI respectively 5 and 1.2) dextran powders in the 1-100 kDa range of molecular weights are presented in Figs. 2 and 3.

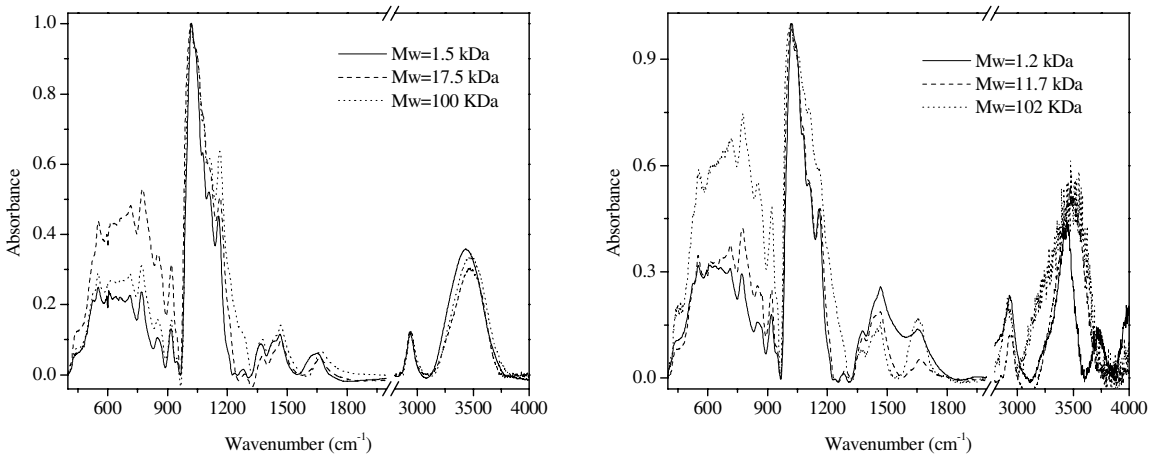

Figure 2. FTIR spectra of polydisperse (left) and low-dispersity (right) dextran powders in CsI pellets.

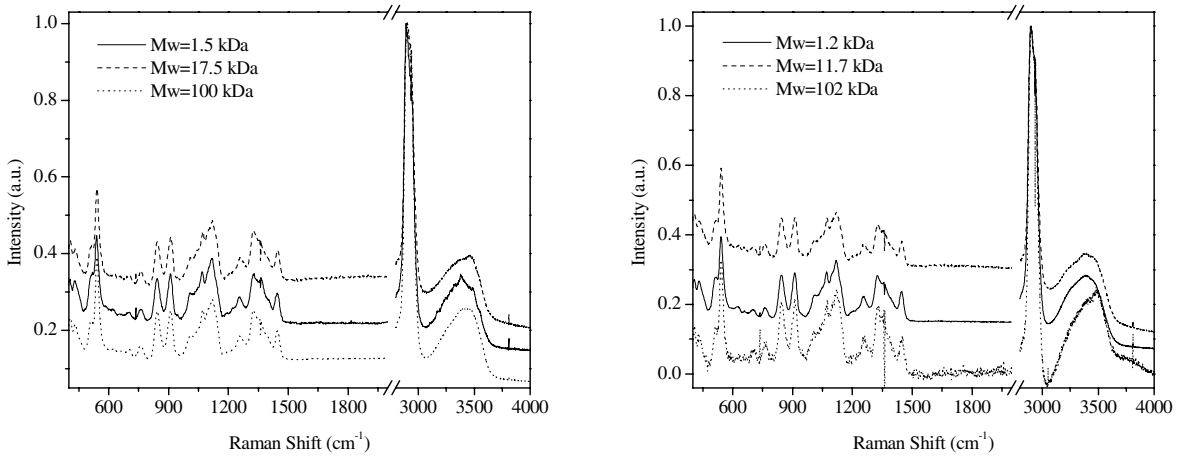

Figure 3. Micro-Raman spectra of polydisperse (left) and low-dispersity (right) dextran powders.

We have successfully obtained micro-Raman and micro-FTIR spectra from poly- and low-dispersity dextrans over a wide range of molecular weights in biomimetic coatings. These spectra have demonstrated significant structural and chemical differences between samples containing poly- and low-dispersity dextrans.

Examples of micro-FTIR spectra are shown in Fig. 4, and diagnostic regions of micro-Raman spectra are shown in Fig. 5. For clearness, spectra recorded on the coated samples are always compared to spectra obtained from pure dextran powders and bare Si wafers. It was not possible to record appreciable vibrational spectra on APTES SAMs functionalized Si substrates (no new peaks from $–NH_2$ group in the monolayers were observed). This is in agreement with previous studies where weak and broad $–NH_2$ peaks could be collected only using ATR with the APTES monolayer formed directly on a Si ATR prism [6]. The intensity of our spectra is directly related to the molecular weight and dispersity of the dextrans in the coating. This is of course expected as a larger volume of material can be analyzed in thicker samples, and the thickness of the coatings is proportional to the molecular weight and PDI of the dextrans.

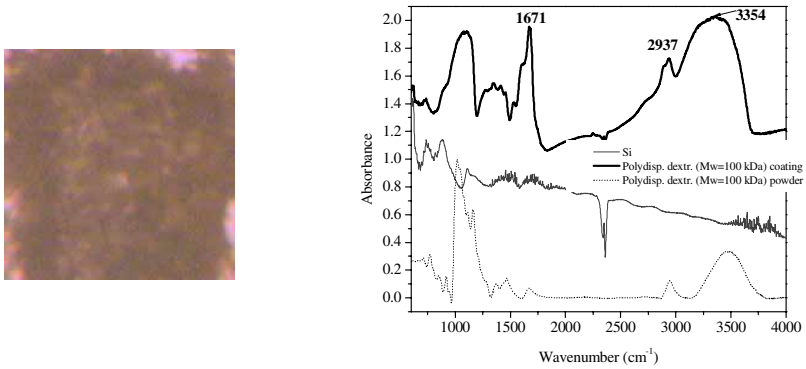

Figure 4. Monitor infrared microscope image (left) of a selected dextran coating area (50 µm ×50 µm) and FTIR spectrum acquired on it (right-black thick line).

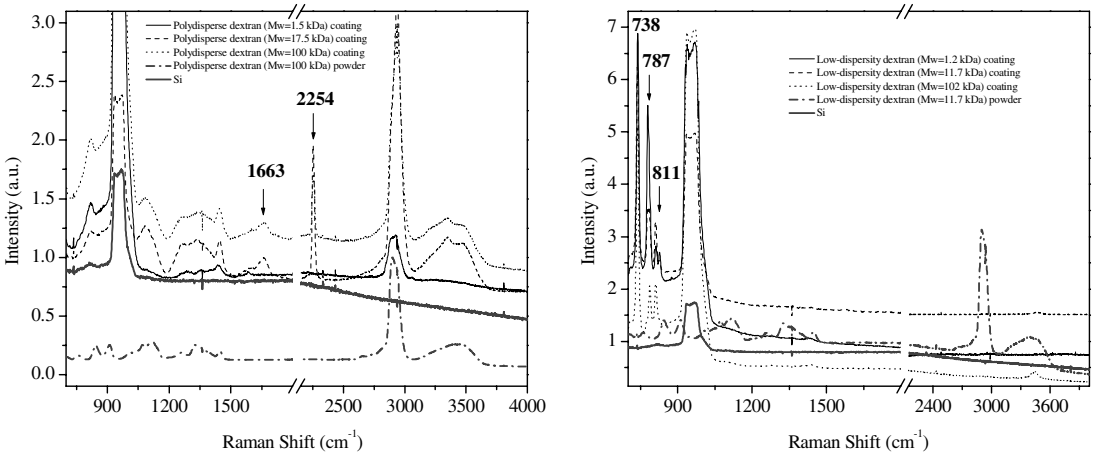

Figure 5. Micro-Raman spectra of the polydisperse (left) and low-dispersity (right) dextran coatings.

The data indicate that micro-Raman is a better technique than micro-FTIR for analysis of our polysaccharide coatings on Si substrates; the antisymmetric Si-Si stretching at about 1000 cm^{-1} being much more intense in the IR spectrum and heavily compromising the identification and location of the dextran key vibrational modes in that region. The confocal arrangement in which the Raman microscope is operated, moreover, excludes out-of-focus light from passing into the spectrograph, strictly limiting the sample volume in both *x,y* (in plane) and *z* (depth) analyzed in a single spectrum. This not only allows for greater spatial resolution, but also for the potential for depth discrimination and profiling. In our particular case we focused the incident laser radiation on the upper polymer coating so that, even not completely avoiding the intense scattering spectrum of the Si substrate, we could observe interesting diagnostic peaks from the carbohydrate coatings themselves.

Our micro-FTIR measurements provided results only for the high Mw polydisperse dextran coating, and when the selected sample area appeared particularly dark (Fig. 4, left). A dark region indicates high values of layer thickness (about 100 nm [5]) and polymer grafting density. New peaks with respect to the Si substrate may be observed at 1671, 2937 (CH_2 stretching) and 3354 (OH stretching) cm^{-1} (only the peak at 1671 being new with respect to the powder).

The micro-Raman technique provides very interesting information, displaying a clear difference between the spectra obtained on the polydisperse and low-dispersity dextran coatings. New peaks respect to the bare Si substrate and the pure powders appear at 1663 and 2254 cm^{-1} in the polydisperse coatings or at 738, 787 and 811 in the low-dispersity ones. The scattering patterns of dextran powders in the 1200-1500 cm^{-1} region can still be easily recognized in all the spectra. Even if a univocal assignment and interpretation of the new signals is difficult, they indicate the suitability of micro- vibrational spectroscopy techniques (especially micro-Raman) as characterization tools and conformational and structural probes. Differences in spectra recorded from *typical* regions in the coatings suggest that polydisperse and low-dispersity dextrans undergo different reactions during an identical grafting process leading to the formation of different chemical bonds and different structures. This is not surprising considering the differences in thickness, wettability and roughness of polydisperse and low-dispersity coatings [5]. In particular it seems that the final reduction process is not completely efficacious in the polydisperse case. Imines generally possess a Raman band near 1670 cm^{-1} for the C=N stretching

frequency [7] and the intense band at 2253 cm^{-1}, associated to a C≡N stretching [7], can only be ascribed to unreacted $NaCNBH_3$. The intense band at 738 cm^{-1} (Fig. 5, right) shown by all the low-dispersity coatings can be assigned to the *a'* N-H deformation of a secondary amine [7], so suggesting that the imine linkage was, in this case, prevalently reduced by $NaCNBH_3$ to a secondary amine (Fig. 1, right). Still unclear remains the origin of the 787 and 811 cm^{-1} bands occurring in a scarcely diagnostic region of the vibrational spectrum.

CONCLUSIONS

We performed a novel, systematic spectroscopic characterization, using FTIR, micro-FTIR and micro-Raman, both of polydisperse and low-dispersity dextrans in biomimetic coatings. Significant structural and chemical differences between coatings containing poly- and low-dispersity dextrans have been found, however the origins of these differences are not understood.

We have highlighted the suitability of these spectroscopic techniques for the characterization of multi layer and multi component polymeric coatings. We have been able to track certain functional groups in our coatings, and therefore have an accurate method for determining the quality of the synthesis route and coatings.

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