

Surface modification, characterization and biofunctionality of pegylated titanate films obtained by the sol-gel method

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Pegylated titanates have been prepared as biomedical supports in the form of thin films. The independent preparation of a polyethylene glycol (PEG) and a titanium isopropoxide (TIPT) precursor was followed by mixing at different molar ratios and spin casting onto Si (100) substrates to form the films. Activation of the hybrid structure was induced by annealing at temperatures right below the PEG melting point. Structural and compositional changes during these steps were followed by Fourier transformed infrared (FTIR) spectroscopy, XPS and water contact angle (CA) measurements. Biofunctionality of the pegylated titanates as antifouling ophthalmic films was tested on the one hand by determination of optical constants using genetic algorithms. On the other hand, indication of surface biocompatibility was provided by seeding mesenchymal stem cells. The results show that PEG-rich surfaces are less biocompatible than mild inorganic surfaces as derived from the inhibition of cytoskeleton polarization. Copyright © 2008 John Wiley & Sons, Ltd.

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Introduction

The sol–gel processing of hybrid materials containing an inorganic matrix bearing a molecular structure is currently in expansion in the field of biomaterials. In this configuration, the created structures benefit from the stability of the inorganic matrix and the biological response determined by the molecular groups. This approach has been generally applied to produce hybrid films with a wide spectrum of functionalities including biomedical, structural, anticorrosion, optical and electronic applications.^[1]

In the biomedical field, the organic phase may principally bear two kinds of molecules. On the one hand, those providing chemical bonds destined to enhance cell proliferation or initiate the adhesion cascade of a target biomolecule or, on the other hand, molecular structures with an antifouling character aiming at the inhibition of unspecific interactions at the interface. Biofunctional hybrid films that modified cell adhesion^[2] and allowed the controlled molecular immobilization of DNA^[3] have been already proposed by our group. These films were prepared by the sol–gel process using titanium isopropoxide (TIPT) as precursor leading to the inorganic phase, which has previously led to optically functional films.^[4]

Materials used for the fabrication of ophthalmic lenses (both intra and exo) require a complex equilibrium of optical, surface antifouling and mechanical properties to become functional. In this work we propose the use of poly(ethylene-glycol) (PEG), a recurrent polymer used in antifouling applications at biomolecular and cellular level,^[5] to form hybrid films with TIPT as potential enhancers of optical and surface properties of ophthalmic lenses in view of the reputed antifouling properties of PEG and the above-mentioned optical properties of TIPT.

Experimental

For film preparation, TIPT and PEG precursors were prepared in a two-step process from TIPT (Puurum, Fluka) and PEG (MW 6000, Fluka). Firstly, we prepared a 0.4 M TIPT solution in ethanol containing a precise TIPT/water ratio ($r_w = 0.82$) and controlled pH (pH = 1.27).^[4] Secondly, a selected quantity of PEG was dissolved in toluene to form a 5% w/v solution. Mixtures were prepared to obtain 95, 90, 80, and 60% molar PEG precursors plus two reference precursors for 100% TIPT and 100% PEG. Homogeneous optical quality films were obtained by spin coating at 2000 rpm during 45 s on Si (100) and subjected to final thermal treatment at 60 °C during 30 min.

The Fourier transformed infrared (FTIR) characterization was performed in vacuum to reduce adsorbed solvent effects. Measurements were carried out in transmission mode in a Bruker Vector 22 (resolution 8, 4000–400 cm⁻¹, 32 scans at 10 KHz).

The surface composition of PEG-TIPT films was studied by XPS. Spectra were acquired with an Ultra photoelectron spectrometer XPS, (KRATOS Analytical, UK) equipped with a

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150-W monochromatic Al K α source and obtaining the spectra at 90° take-off angle and pass energy of 20 eV. The chamber background pressure was 6×10^{-10} Torr and slightly increased up to 2×10^{-9} Torr during operation. A flood gun was used to compensate the surface charge helping to refer all peak energies to the binding energy of the hydrocarbon peak (285 eV). The spectra were peak fitted with a Gaussian/Lorentzian function after a linear background subtraction (Vision 2 software, Kratos, UK) and quantitative analyses were performed taking into account appropriate sensitivity factors.

In order to evaluate morphological modifications on the hybrids, the different films produced were evaluated by SEM, Philips XL-30). Water contact angle (CA) measurements onto the PEG-TIPT hybrid films were carried out as a first antifouling test in a DGD Fast/60 (GBX technologies) with automated drop formation and deposition of milli-Q water drops (18 KOhm cm). Optical evaluation was performed by registering film reflectance in the UV-vis wavelength range using a Jasco V-560 double-beam spectrophotometer, equipped with an integrating sphere to avoid scattering losses. Optical constants were obtained by simulating the spectra using genetic algorithms as previously reported in detail.^[6]

Human mesenchymal stem cells (hMSCs) from bone marrow were used to test the biocompatibility of the PEG-TIPT coatings. About 15 000 cells were seeded on each sample (1 cm²) and incubated with 2 cm³ DMEM-LG plus 10% foetal bovine serum (FBS) during 1 day. As control surfaces, hMSCs were plated on glass coverslips. Cells were washed with phosphate buffer saline (PBS) and incubated in DMEM-LG with 10% FBS during 24 h at 37 °C in 5% CO₂. Then, the cells were washed with PBS, fixed in 3.7% formaldehyde in PBS during 15 min at room temperature (RT). In order to analyze the cytoskeleton, cells were permeated in 0.1% Triton X-100 in PBS and immunofluorescent reactions were used to overcome the material opacity so as to visualize cytoskeleton proteins. The surfaces were incubated for 1 h with sera from bGH-transgenic mice (1/100). After washing,

the surfaces were incubated in dark conditions for 1 h with phalloidin Alexa 594 (1/200) (Molecular Probes). After incubation, the cells were visualized by a fluorescence vertical microscope (Axioskop2 plus) coupled to a charged-coupled devices (CCD) color camera.

Results and Discussion

FTIR

The different TIPT doses introduced in the PEG structure induced notable changes in the hybrid films as deduced from FTIR spectra. With regard to the original PEG films, (Fig. 1) no drastic differences can be derived with respect to PEG standards,^[7] particularly well characterized by the intensity of the highly absorbing 2950, 2880 and 2820 cm⁻¹ bands ascribed to CH₂ modes.

In fact, all the hybrid films present the characteristic PEG profile with very similar slight deviations, mainly related to deactivation of bands acting parallel to the polymer chains. For 90% PEG sample, it can be observed that the intensity of the band at 1235 cm⁻¹ (downward arrow, acting parallel) suffers a mean relative decrease of 25% after the hybridization with TIPT with respect to the band at 1280 cm⁻¹ (upward arrow, acting perpendicular). The original intensity ratio of this band (untreated sample) with the neighboring band at 1280 cm⁻¹ gives a value of 0.84, which is reduced to 0.62 after hybridization. This is a constant effect for other hybridized films. This preferential deletion of a selected parallel acting band indicates a modification of the original linear polymer as an issue to the formation of the adjacent TIPT structure.

XPS

The surface analysis of the hybridized films demonstrated that remarkable changes take place in the surface chemistry. Both survey and high-resolution spectra provided relevant information

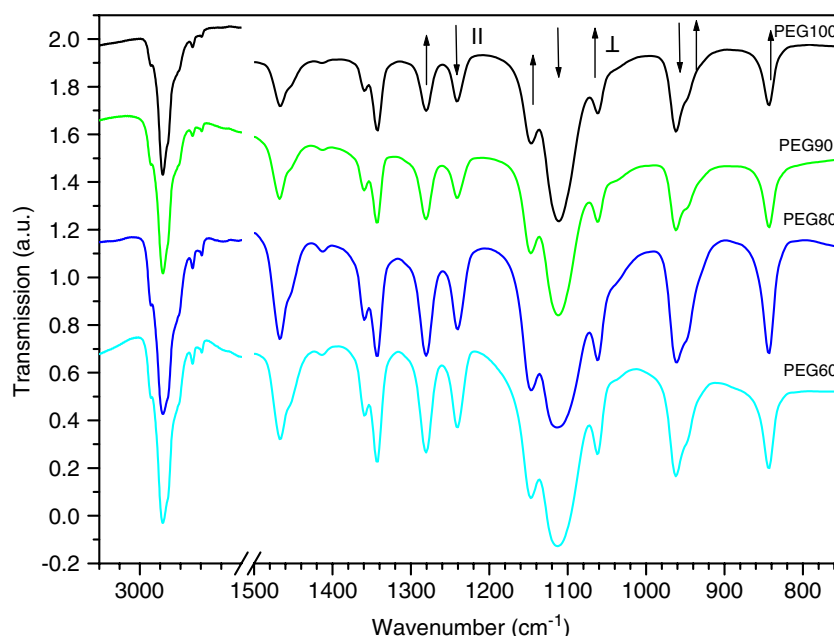


Figure 1. FTIR spectra of the PEG-TIPT hybrid films with 100, 90, 80, and 60% molar PEG. Downward arrows: Modes parallel to PEG linear structure. Upward arrows: Modes perpendicular to PEG linear structure. This figure is available in colour online at www.interscience.wiley.com/journal/sia.

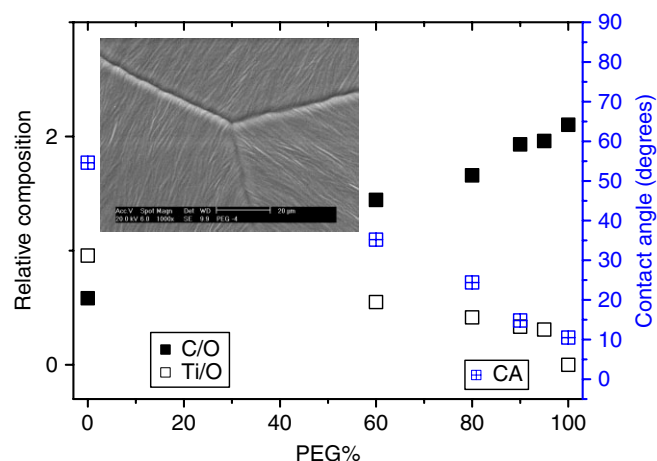


Figure 2. XPS determination of C/O and Ti/O composition as a function of PEG-TIPT hybrid molecular incorporation (left axis). Related water contact angles to the hybrid PEG-TIPT films (right axis). Inset: SEM image of the typical grain boundary structure observed in PEG-containing films (PEG90). This figure is available in colour online at www.interscience.wiley.com/journal/sia.

to interpret the chemical modification. Figure 2 (left axis) shows the evolution of the C/O and Ti/O elemental ratio for increasing PEG molecular content. Oxygen was chosen as reference element for these ratios since the film composition of this element is not strongly affected by hybridization (5% maximum difference). The results show that the controlled insertion of TIPT in the precursor allows a control of the final Ti composition of the films (inorganic component). At the same time, it can be observed that the higher the Ti composition, the less organic incorporation is allowed in the film.

High-resolution C 1s spectra (not shown) of the hybrid films allowed to derive information related to the antifouling behavior. We observed that the original antifouling PEG presents a dominant monocomponent peak at 286.5 eV assigned to C–C–O. The incorporation of TIPT induced an absolute increase of hydrocarbon (C–C) at 285 eV with respect to C–O. A relative increase of C=O at 288 eV with respect to C–O was also observed, though total C=O remained below 3% of total carbon content. Though not linearly, the common weight of the C contribution increased coherently with the Ti content as determined from the survey analysis.

Film morphology

In order to probe the morphology and optical homogeneity of the PEG-TIPT hybrids a SEM study was performed. Only pure TIPT films appeared to be completely homogeneous and flat at the studied magnifications. PEG-containing films presented a clearly hexagonal multidomain structure. The inset in Fig. 2 shows the confluent point of three neighboring PEG90 structures. Furthermore, it can be observed that the inner structure of the domains is composed of parallel fibers with a clear radial distribution. The increase of the TIPT composition up to 40% did not alter this hexagonal domain structure of the hybrid films.

Water contact angle

The water CA measurements were performed to assay the hydrophilic-hydrophobic character of the produced hybrid films.

As expected from references, measurements on pure PEG samples led to very low water CAs (Fig. 2 right axis). Measurements of pure PEG were however followed by irregular dispersion of the water droplets due to film instability. This induced relatively higher error values on measurements of these films. After the incorporation of TIPT the CAs were observed to increase as the TIPT content increased. Droplets remained well confined on the surfaces. Final CAs for 100% TIPT films were more than five times higher than those measured for pure PEG. From the plot, it can be observed that the relative increase correlates well with C/O and Ti/O composition measured by XPS.

Optical properties

The optical parameters of the different films were obtained by simulating the experimental reflectance spectra using genetic algorithms. The results for low (PEG90) and highly (PEG60) TIPT doped samples show a relative change in the optical properties as a function of composition. First efforts were devoted to determine film thickness, which happened to be approximately 25% higher for PEG90 with respect to PEG60 (810 nm face to 613 nm) as an issue of higher precursor viscosity. Inset in Fig. 3 shows the light modulation induced by the optical PEG90 films in the form of an experimental and a simulated spectrum. The results from the simulation show that, in the 400–800 nm range, the real part n presents a very similar behavior for PEG90 and PEG60 with only a slight increase of n for PEG60 below 500 nm. The behaviour of the complex part k is similar in both samples, excepting for an increase of k for PEG90 at wavelengths below 500 nm. These results are coherent with previous analyses that underline the high n , low k values for TIPT derived films, especially below 500 nm.^[4] Particularly, the very low absorption measured for every sample at wavelengths above 500 nm makes the films compatible with the ophthalmic applications.

Cell behavior

The behavior of hMSCs on the PEG-TIPT surfaces was evaluated by determining actin cytoskeleton with rhodamine staining. In the PEG surfaces (Fig. 4) it can be observed that hMSCs present spread cytoskeleton organization and irregular distributions, which may arise from the PEG instability. The introduction of small TIPT molecular ratios does not modify substantially the cell cytoskeleton and cell distribution, although cell coverage increases noticeably. High TIPT insertion in the hybrid films (PEG60) induced a fibroblastic-like morphology with the characteristics described in proliferating cells.^[8] We observe slightly Y-shaped cells with a homogeneous distribution on the surface. In parallel, a huge increase in cell density is observed with respect to the pure PEG film. This behavior was again observed for cells cultured on pure TIPT surfaces, though cell coverage presented not-so remarkable increase in cell density. These results suggest that cell density follows a related trend with surface wettability, with increasing cell density for less hydrophilic films. Although no morphometric determination was carried out, an increased cell area in cells growing on PEG-rich hybrid films was observed in comparison with the more bipolar cells observed on TIPT-rich films. From a general biomedical point of view, PEG60 would be more biocompatible than the other samples showing higher cell density and more surface-adapted cells.

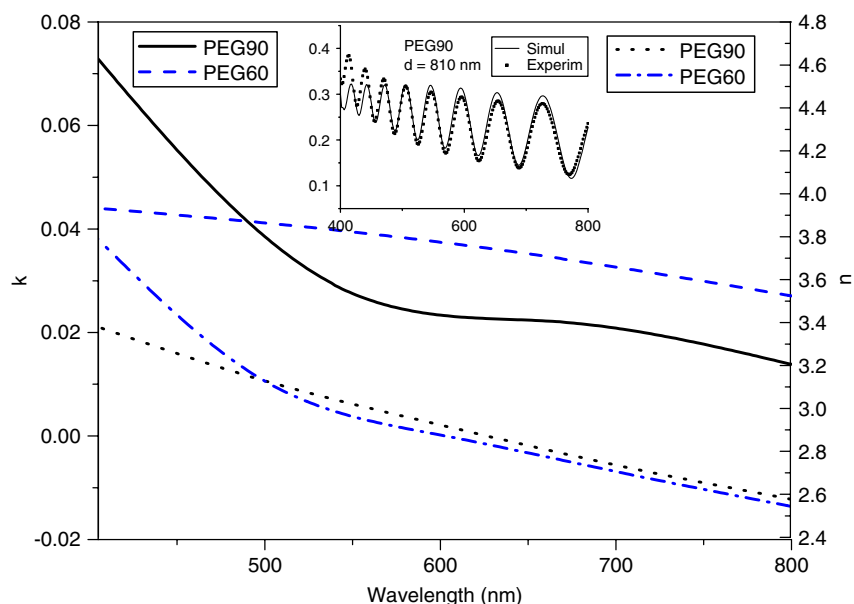


Figure 3. Variation of optical parameters n (right axis) and k (left axis) for a 90% PEG film and a 60% PEG film. Inset: Typical modulated reflectance spectrum with experimental (dots) and simulated behavior (straight). This figure is available in colour online at www.interscience.wiley.com/journal/sia.

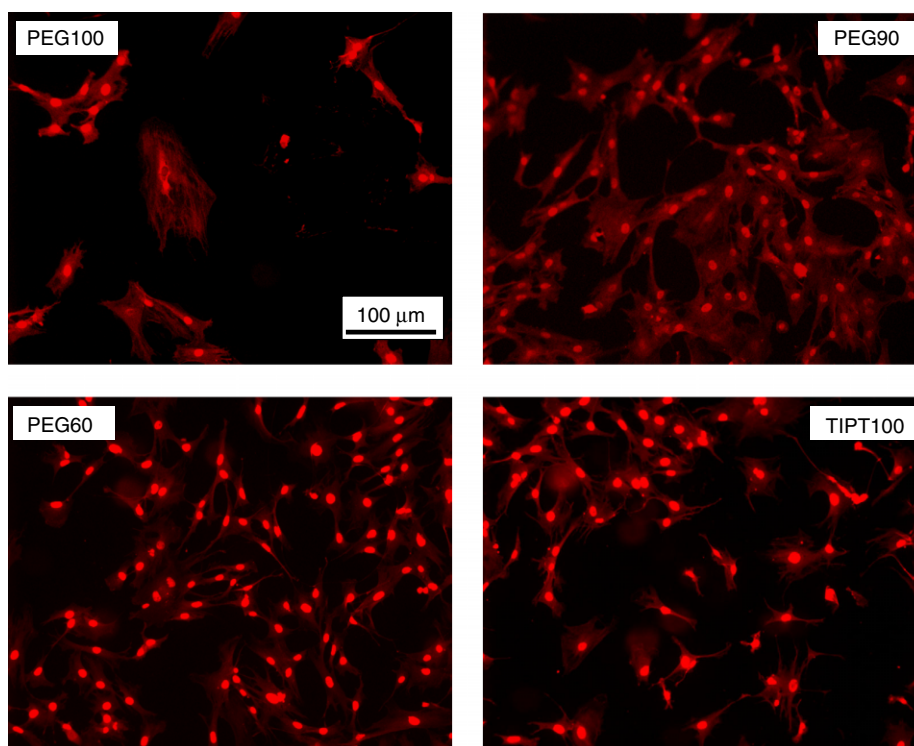


Figure 4. Fluorescence images showing the behaviour of hMSCs on the surfaces of PEG-TIPT hybrids. All images presented at identical scales. This figure is available in colour online at www.interscience.wiley.com/journal/sia.

Conclusions

Formation of hybrid PEG-TIPT films is possible by the preparation of sol–gel sols and spin coating at 2000 rpm. Such films present, in the molecular range explored, a morphology and structure that remind that of pure PEG films, i.e., a homogeneously thick film composed of hexagonal domains with an internal radial fiber decoration. The composition of such films increases in inorganic

content (Ti–O network) by increasing the TIPT molecular fraction of the starting sol. The formation of the TIPT network modifies the PEG molecular behavior inhibiting preferentially linear vibrations. This phenomenon is also related to increasing hydrocarbon and double carbon oxygen bonds, which may benefit PEG film stability but also induce an increase of water CAs (reduced antifouling character). The optical parameters of the films were obtained by using genetic algorithms to simulate experimental reflectance

spectra in the 400–800 nm range, which denote the benefit of TIPT insertion for higher real part and lower complex part refractive index (specially in the 400–500 nm range). Biocompatibility assays show that PEG-rich surfaces are less biocompatible than mild inorganic surfaces as derived from the inhibition of cytoskeleton polarization. All these features suggest that PEG-TIPT hybrids may be of interest as coating materials for ophthalmic materials. Further work is in progress to determine film adhesion and performance on traditional polymeric contact lenses.

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