Journal of Applied Polymer Science

Calcium ion-mediated grafting of a phosphate-containing monomer

Shuko Suzuki,[1,2,3] Edeline Wentrup-Byrne,[2] Adrienne Chandler-Temple,[1] Nevil Shah,[1] Lisbeth Grøndahl[1]

[1]School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland 4072, Australia
[2]School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, Brisbane, Queensland 4001, Australia
[3]Queensland Eye Institute, South Brisbane, Queensland 4101, Australia
Correspondence to: L. Grøndahl (E-mail: l.grondahl@uq.edu.au)

ABSTRACT: Grafting of monoacryloxyethyl phosphate onto expanded polytetrafluoroethylene was achieved using simultaneous grafting with the aim of improving the membrane wettability and mineralization capacity. This study explored the effect of adding calcium ions to the grafting solution and observed increased graft yield and wettability when compared with samples grafted in the absence of calcium ions. Fourier transform infrared spectroscopy mapping found the graft copolymer to be distributed in a patchy manner across the surface as well as throughout the membrane. Through X-ray photoelectron spectroscopy analysis, it was found that calcium ions were incorporated into the graft copolymer and could be extracted using a basic ethylenediaminetetraacetic acid solution without reduction in graft yield. This implies that the presence of calcium ions is affecting the graft yield by increasing the local concentration of monomer near the surface during the grafting process. Investigation of the mineralization capacity of the grafted membranes in simulated body fluid revealed that the increased wettability of the membranes rather than the presence of the calcium ions affected the mineralization outcome. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42808.

KEYWORDS: grafting; irradiation; surfaces and interfaces

Received 20 May 2015; accepted 4 August 2015
DOI: 10.1002/app.42808

INTRODUCTION

Radiation-induced graft copolymerization is a well-established, attractive technique commonly used to introduce desirable surface properties, such as hydrophilicity, adhesion, and biocompatibility, into a pre-existing polymer without altering the bulk properties. Radiation-initiation creates the necessary free radicals on the substrate and hence neither initiator nor catalysts are required. The resulting free radicals lead to the initiation of the graft copolymerization of the relevant monomer(s). Overall, this is considered a "clean" method which is particularly attractive for biomedical applications. Various parameters are known to affect the degree of grafting. These include the solvent, the monomer concentration, and their radiation dose.[1,2] The judicious use of additives can also increase the grafting yield which means that the desirable level of grafting can be achieved using reduced amounts of monomer and/or a reduced radiation dose. This leads to a more economical process and is also preferable when the polymer substrate is sensitive to irradiation, such as is the case for polytetrafluoroethylene (PTFE).[1]

Two types of additives commonly used are homopolymer inhibitors [such as ammonium ferrous sulfate (Mohr's salt), ferric chloride, and copper chloride] and grafting enhancers (such as acids and neutral salts).[1,2] In the simultaneous irradiation method, in addition to the substrate radicals, monomer radicals are also formed and these may initiate undesirable homopolymer formation. This in turn hinders monomer diffusion to the grafting sites and reduces the degree of grafting. As the addition of an inhibitor can also inhibit the graft copolymerization, the amount, typically 1 wt % or below, needs to be judiciously optimized to obtain desirable grafting levels.[2] There are reports of where the addition of inhibitors has increased the graft yields of acidic monomers such as acrylic and methacrylic acids onto various substrates including PET,[3] PFA,[4] PE,[5,6] and PTFE.[7] Grafting enhancers such as acids and neutral salts are believed to enhance the graft yield by "salting-out" the monomers from the solution into the grafting region of the polymer substrate.[8] This mechanism satisfactorily explains the enhancement when grafting nonpolar monomers onto nonpolar substrates. However, several studies have shown that the graft yield of acidic monomers such as acrylic acid is also affected by the addition of HCl in both post-irradiation[9] and simultaneous irradiation methods.[10] This in turn was attributed to the fact that the pH value of the monomer solution was altered.

Table I. Preparation and Graft Yield (GY) of the Grafted ePTFE Membranes

Sample	Dose (kGy)	[MAEP] (%)	[MAEP] (mol L^{-1})	[Ca^{2+}] (mmol L^{-1})	GY (%) ± 2
M(30)	72	30	1.5	-	9
M(40)-50	72	40	2.0	50	17
M(30)-50	72	30	1.5	50	19
M(20)-50	72	20	1.0	50	7
M(10)-50	72	10	0.5	50	5
M(30)-5	72	30	1.5	5	15
M(30)-0.5	72	30	1.5	0.5	13
M36(30)-50	36	30	1.5	50	13
M9(30)-50	9	30	1.5	50	4

The fully-fluorinated polymer PTFE is very hydrophobic, thermally and chemically inert, as well as having anti-frictional properties.[1,11] The expanded form of PTFE [expanded PTFE (ePTFE)] has a highly fibrillated microporous structure, and although soft and very flexible, is very strong and extremely resistant to tearing.[12] For clinical applications, ePTFE is known for its bioinertness and has been successfully used as a nonbiodegradable biomaterial in a range of applications such as vascular grafts, catheters, sutures, and facial augmentation.[12–14] However, when used as a facial prosthesis, due to the lack of surface functional groups needed to interact with the cellular environment, the *in vivo* fixation of the ePTFE is delayed which may cause micro-movement of the implant. This in turn can lead to fibrous encapsulation and ultimate failure of the implant. Phosphorous-containing polymers have shown much potential in a wide range of medical applications and devices,[15,16] and the presence of phosphate groups on the implant surfaces have been shown to increase the capability of nucleating calcium phosphates and improving the bone-bonding ability *in vivo*.[17–19] In a previous study, we grafted phosphate-containing monomers onto ePTFE by simultaneous irradiation, and demonstrated a clear improvement in *in vitro* mineralization and bioactivity of the modified surfaces.[20–23]

This study investigates the effect of calcium ions as an additive on the radiation-induced graft copolymerization of monoacryoxethyl phosphate (MAEP) onto ePTFE. As mentioned above, residual impurities are less than ideal especially for products intended for medical applications; however, the toxicity of calcium ions as additives is not a concern. In fact, as our aim was to improve the bone-bonding ability of ePTFE for craniofacial applications, the presence of residual calcium ions could potentially improve the calcium phosphate mineral formation and hence ultimately benefit the implant. In this study, a series of biomaterials consisting of PMAEP-grafted ePTFE either with or without calcium ions was prepared and the effects of the residual calcium ions, as well as other surface parameters, on the *in vitro* mineralization were investigated.

MATERIALS AND METHODS

Materials

ePTFE Sumitomo Poreflon 20-80 or 045-80 membranes from Sumitomo Electric, Japan, had a thickness of 70 μm. The degree of crystallinity was approximately 18%.[24] Monoacryloxyethyl phosphate (MAEP) was obtained from Polysciences (Warrington, PA). All chemicals were analytical grade, purchased from Sigma, and used as supplied. Ultrapure water from a Hi-Pure Water System was used in all experiments. Revised simulated body fluid (r-SBF, in this manuscript referred to simply as SBF) was prepared according to the published procedure[25] from reagent grade chemicals that had been dried in a desiccator for at least 3 days. Sodium chloride (≥99.5%), sodium hydrogen carbonate (99.7%), sodium carbonate (99.8%), potassium chloride (≥99.0%), dipotassium hydrogen phosphate trihydrate (≥99.0%), magnesium chloride hexahydrate (≥99.0%), calcium chloride dihydrate (≥99.0%), sodium sulfate (99.0%), and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid were from Sigma-Aldrich, sodium hydroxide was from Lab Scan Analytical Services.

Graft Polymerization

The 10-mm diameter pieces of ePTFE membrane were washed in methanol at 40°C for 1 hour and subsequently dried in a desiccator. Each polymer piece was placed in a glass test tube containing aqueous solutions of MAEP at concentrations 10%–40 %. In some samples, $CaCl_2$ was added at concentrations of 0.5, 5, or 50 mmol L^{-1} (details are given in Table I). Nichrome wire was used to keep the membranes from floating on top of the solution. The tubes were sealed with a Suba cap and dissolved oxygen in the monomer solution containing the polymer substrate was removed by bubbling nitrogen gas through it for 5 minutes. Samples were then subjected to ^{60}Co gamma rays at a dose rate of 3.7 kGy h^{-1} and a total dose varying between 9 and 72 kGy. The ^{60}Co gamma cell was a 220 Nordian Gammacell (Canada). Following graft copolymerization, membranes were washed with methanol at 40–45°C to remove any residual monomer and loose homopolymer occluded onto the membrane. Washing was continued until constant weight and samples were finally dried under vacuum.

Post-Treatment with EDTA

Selected samples (1–2 mg) were immersed in either 1 mL water or in 1 mL 0.1 M ethylenediaminetetraacetic acid (EDTA) (pH 10) with stirring for 3 hours. Each sample was subjected to this

treatment three times and the combined water or EDTA solutions analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) for Ca and P. The treated membrane samples were analyzed by mass and X-ray photoelectron spectroscopy (XPS).

In Vitro Mineralization

Calcium phosphate formation on a selection of surface-modified ePTFE membranes was evaluated by immersing the membranes in SBF for 7 days. Individual membrane pieces were placed in polystyrene containers: 25 mL SBF was added (membrane pieces were held in the centre of the container by means of inert plastic netting) and placed in a water bath maintained at 36.5 ± 0.2 °C. The SBF solution was renewed every 3 days. pH measurements on the exchanged solutions showed that the pH did not differ from the original solution of pH 7.4 ± 0.1. After 1 week the membranes were removed from the solutions, washed thoroughly with water and dried in a vacuum desiccator. Total calcium phosphate mineral (CaP) deposition was measured using the weight increase in the membranes.

Characterization

Graft Yield. Values for graft yield (GY) were obtained gravimetrically as the percentage of weight increase in the ePTFE membrane using the following equation:

$$\text{Graft yield}\,(\%) = \frac{w_g - w_o}{w_o} \times 100\%$$

w_g and w_o are the weights of grafted and original ePTFE membranes, respectively.

X-ray Photoelectron Spectroscopy. XPS analysis of the grafted membranes was performed on a Kratos Axis Ultra XPS system, using a 165-mm, 180 degree hemispherical analyzer with 8 channel trons (Kratos Analytical, Manchester, England). AlK_α radiation (1486.6 eV), typically run at 150 W (15 kV, 10 ma) was used for all spectra. The survey scan range of 0–1200 eV with a pass energy of 160 eV and the multiplex scans with a pass energy of 20 eV were carried out. The binding energy of the spectra was calibrated to that of the F(1s) peak (688 eV).[26] The XPS graft extent (GE) of PMAEP was obtained by using the areas of the carbon peaks as follow:

$$\text{Graft extent}\,(\%) = \frac{A(C-C)}{A(C-C)+A(C-F)} \times 100\%$$

The carbon peak which is not C—F is written as C—C, although it may also contain other carbons such as C═O and C—O.

Scanning Electron Microscopy. Scanning electron microscopy (SEM) analysis of platinum- or carbon-coated samples was performed on a JEOL 6460 LA, JEOL 6400F, or JEOL 2200 instrument.

FTIR Spectroscopy. Attenuated total reflectance (ATR)/Fourier transform infrared (FTIR) spectra of the surface layer of the grafted membranes were measured with a Nicolet Nexus 870 FTIR instrument equipped with a Smart endurance diamond accessory. After immersion in SBF, all samples were analyzed at two different points (64 scans, 4 cm^{-1} resolution).

Micro-ATR/FTIR spectra were recorded using a Nicolet 870 Nexus FTIR system with a Continuμm™ IR microscope equipped with a liquid nitrogen-cooled MCT detector and an ATR objective incorporating a Si internal reflection element. Mapping-spectra (128 scans, 8 cm^{-1} resolution, wave number range 4000–650 cm^{-1}) were collected in a square grid pattern covering the sectioned surface of the sample (2 mm × 2 mm area) with an aperture of 100 μm × 100 μm and a step size of 100 μm. For FTIR map imaging, peak area ratios of C═O at 1720 cm^{-1} and C—F at 1149 cm^{-1} in the respective spectral regions were used. Using the same mapping settings, (64 scans, 4 cm^{-1} resolution, wave number range 4000–650 cm^{-1}) micro-FTIR transmittance spectra were recorded using the above instrument in transmission mode. The FTIR map imaging was created using the intensity of the C═O band at 1720 cm^{-1}.

Contact Angle Measurement. Contact angle measurements were performed on a custom-built instrument which has been described in detail previously.[27] MilliQ water drops (5 μL) were placed on the surface of the sample using a 50-μL glass flat-tipped syringe and images of the drop captured and analyzed by Scion Image processing software. The advancing angles (θ_A) were measured by successive 5 μL additions of water to a total of 20 μL. Receding contact angle θ_R measurements were obtained by drawing back part of water drop from the surface of the membrane. Two spots on each membrane were evaluated and the data reported as the mean ± SD.

RESULTS AND DISCUSSION

Graft Polymerization

Grafting the phosphate-containing acrylate monomer MAEP was performed in aqueous solution using a procedure based on previous work[24] and similar degrees of grafting were found. For example, we previously reported that 20% MAEP at a dose of 50 kGy yielded 80 μg mg^{-1}, which is similar to the 9% GY (90 μg mg^{-1}) obtained for sample M(30)in this study (prepared from 30 % MAEP at a dose of 72 kGy). The focus of this study was to evaluate the effect of added calcium ions to the grafting solution. In all samples, the monomer concentration was at least 10 times larger than that of the calcium ion concentration (experimental conditions given in Table I). It was observed that the presence of calcium ions increased the graft yield from 9% to 13%, 15%, and 19% for calcium ion concentrations of 0.5, 5, and 50 mmol L^{-1}, respectively (samples M(30)-0.5, M(30)-5, and M(30)-50 in Table I). The effect of the calcium ions on enhancing the graft yield cannot be attributed to a salting out effect as this phenomenon occurs only for nonpolar monomers,[28] neither can it be acting as a homopolymer inhibitor as it is not redox active.[29] It is therefore proposed that the calcium ions interact specifically with the phosphate groups of the monomer. This theory is supported by the high affinity of calcium ions for phosphate.[30]

For a series of samples which all contained 50 mmol L^{-1} calcium ions, the effect of monomer concentration and dose were also evaluated. It was observed that the graft yield increased with monomer concentration until a value of 30% was attained after which no significant change was observed (samples M(10)-50, M(20)-50, M(30)-50, and M(40)-50 in Table I). Furthermore, an increase in graft yield was observed upon increasing the dose from 9 to 36 to 72 kGy (samples M9(30)-50, M36(30)-

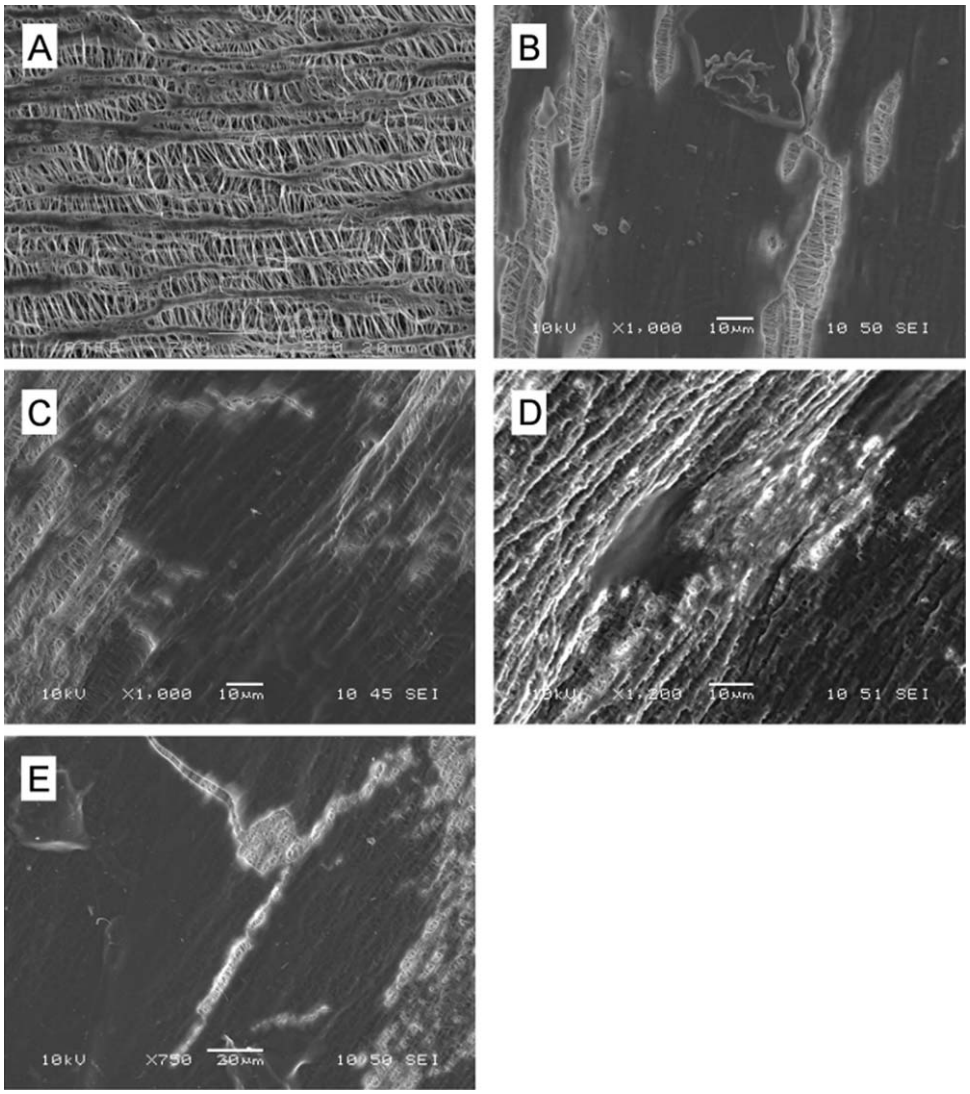


Figure 1. SEM images of (A) Untreated ePTFE; (B) sample M(30); (C) sample M(30)-50; (D) sample M(10)-50; and (E) sample M(30)-5. Please note different magnitudes of the scale bar in image (E).

50, and M(30)-50 in Table I). The increase in graft yield with increasing monomer concentration has previously been observed in a number of studies.[31–36] It can be rationalized based on radical polymerization kinetics where it has been shown that the monomer concentration affects the chain length. A plateau (observed in this study for the 30% and 40 % MAEP concentrations) has similarly been observed previously.[37,38] This observation has been attributed to an increase in solution viscosity at high monomer concentration. The higher degree of grafting which has been frequently observed with a higher dose[32,33,39] can be attributed to a higher concentration of substrate radicals which in turn leads to a higher number of grafted chains.

The morphology of the ePTFE membrane shown in Figure 1(A) is a porous anisotropic structure incorporating dense regions of 2–10 μm in diameter. These dense regions are connected by fibrils which are responsible for the porosity. After grafting, a clear morphological change is observed with large non-porous regions now visible [evident in Figure 1(B–E)]. EDX analysis (data not shown) verified that those regions that appear dark in the images are in fact the graft copolymer (the elements O and P are observed) while only the elements F and C were found in those regions that resemble the underlying membrane. There was no observable difference in the morphology of the graft copolymer in the absence or presence of calcium ions. All samples [including those of relatively high graft yields of 19% and 15%, Figure 1(C,E), respectively] showed patchy graft copolymer coverage on a relatively large size scale (for regions of view of up to 200 × 150 μm). When SEM images from multiple areas of each sample were evaluated a qualitative correlation between GY and coverage was apparent.

FTIR Spectroscopic Characterization of Grafted Membranes

The ATR/FTIR spectrum of the non-grafted PTFE membrane shows characteristic C—F stretching vibrations at 1203 and 1149 cm^{-1} [Figure 2(A)].[40] Some typical micro-ATR/FTIR spectra obtained at different points for sample M(30)-50are

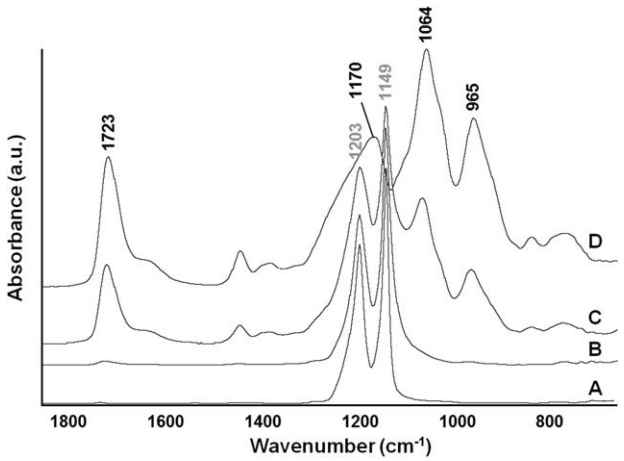


Figure 2. ATR/FTIR spectra of (A) untreated ePTFE and three regions of sample M(30)-50 with different degrees of grafting; (B) low, (C) medium, and (D) high. Black numbers refer to band positions for the graft copolymer while grey numbers refer to bands of the ePTFE substrate.

displayed in Figure 2. Examples with (B) low, (C) medium, and (D) high grafting degrees are shown. The additional PMAEP bands observed in these spectra are in agreement with those previously reported for MAEP grafted to ePTFE[24] and PMAEP produced from RAFT polymerization.[41] New vibrations corresponding to C═O, C—H, P—O—(H), and P—O—(C) are seen in the grafted membranes at 1723, 1451–1388, 1064, and 965 cm^{-1}, respectively.[21] In the case of the high grafting region [Figure 2(D)], the C—O—(C) band is also observed at 1170 cm^{-1}.[41] When comparing the FTIR spectra of the grafted membranes with or without Ca^{2+} ions, there were no significant peak shifts or new bands appearing due to the presence of the ions (data not shown). This confirms that the trace amounts of Ca^{2+} ions in the grafted PMAEP (see below) is not sufficient to cause a detectable difference in the FTIR spectra.

FTIR microspectroscopy was used to construct semi-quantitative mapping images of PMAEP-grafted PTFE substrates. The membrane sample M(30)-50 was characterized using a combination of two IR techniques, micro-ATR/FTIR

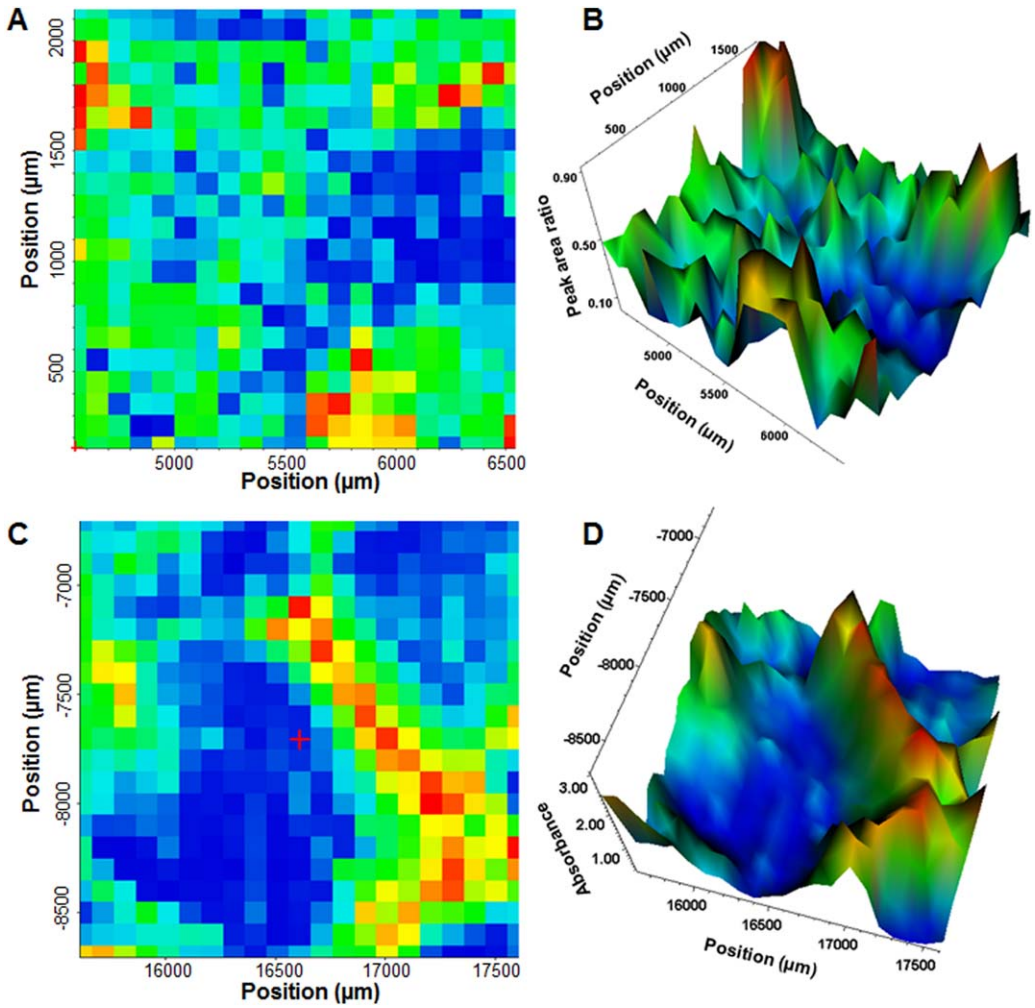


Figure 3. FTIR maps of sample M(30)-50. Micro-ATR/FTIR maps showing the ratio of the C═O to C—F vibrational modes displayed in (A)2D and (B) 3D views, and micro-FTIR transmittance maps showing the intensity of C═O peaks in (C) 2D and (D) 3D views. Each map evaluates an area of 2 × 2 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Characterization of Selected Grafted Membranes Treated with EDTA Solution

Sample	Treatment	Ca 2p (%) ± 0.05	P 2p (%) ± 0.05	GE[a] (%) ± 5	Δm[b] (%)	[Ca^{2+}] (mg L^{-1})	Ca/PMAEP (μg Ca/mg polymer)
M(40)-50	None	1.39	4.83	100	-	-	
	Water	1.43	3.62	100	4.7	0.48	5
	EDTA sol	0.20	2.75	92	1.0	8.77	90
M36(30)-50	None	0.84	3.35	85	-	-	
	Water	0.76	2.56	91	2.4	0.40	5
	EDTA sol	0.00	2.25	73	2.5	7.22	120

[a] G.E. from survey scan.
[b] Change in mass relative to initial mass as a result of treatment with either water or EDTA solution.

and micro-FTIR transmittance. The aim was to evaluate the grafting distribution differences on the submicron surface and throughout the membrane, respectively. For the micro-ATR/FTIR map, the image was constructed based on a carbonyl index calculated by taking the area ratio of the carbonyl band of grafted PMAEP at 1720 cm^{-1} to that of the C—F band of PTFE substrate at 1149 cm^{-1}. The index is generally used in preference to a single band area or intensity to allow for any variations which may occur due to the differences in the optical quality of the contact between the internal reflection element of the ATR objective and the sample surface. Figure 3(A,B) show the heterogeneity of the PMAEP grafting on the sub-micron surface of sample M(30)-50 in 2D and 3D images, respectively. This map evaluates a 2 × 2 mm region with each pixel in the map representing a 100 × 100 μm area. This is much lower resolution than that of the SEM images displayed in Figure 1; indeed, one SEM image is roughly equivalent to one pixel. By combining the information from the two techniques it is evident that the grafting is not only patchy on the micro-scale but also on the milli-scale. Such patchiness of the PMAEP graft-copolymer has been observed previously for grafting onto ePTFE substrates. Based on micro-ATR spectroscopy (where several regions on the sample were evaluated) our previous grafting study of MAEP in water onto Sumitomo membranes, which is similar to this study, found that the graft copolymer was also distributed in a patchy manner.[24] The results of grafting MAEP in methanol onto a Gore ePTFE membrane found that while the graft copolymer was distributed in a patchy manner, as evaluated by micro-ATR mapping, ToF-SIMS revealed that the grafting had occurred both on the fibril and island regions.[27] In all of these studies, it is clear that the graft copolymer never completely covers the membrane surface.

To generate the micro-FTIR transmittance map the spectra were measured through the full 70 μm thickness of the sample. Such an experiment is only possible when the material thickness is sufficiently small so not as to saturate the region of interest to such an extent that discrimination between different levels of grafting cannot be discerned (in this case the carbonyl band at 1720 cm^{-1}). The carbonyl band intensity was used to generate an image of a 2 × 2 mm region which represents grafting through the entire depth of sample M(30)-50 [Figure 3(C,D)]. Because the ePTFE substrate is highly porous, grafting can potentially occur all the way through the membrane. However, it appears that some regions are virtually ungrafted since a patchy grafting distribution of PMAEP through the membrane

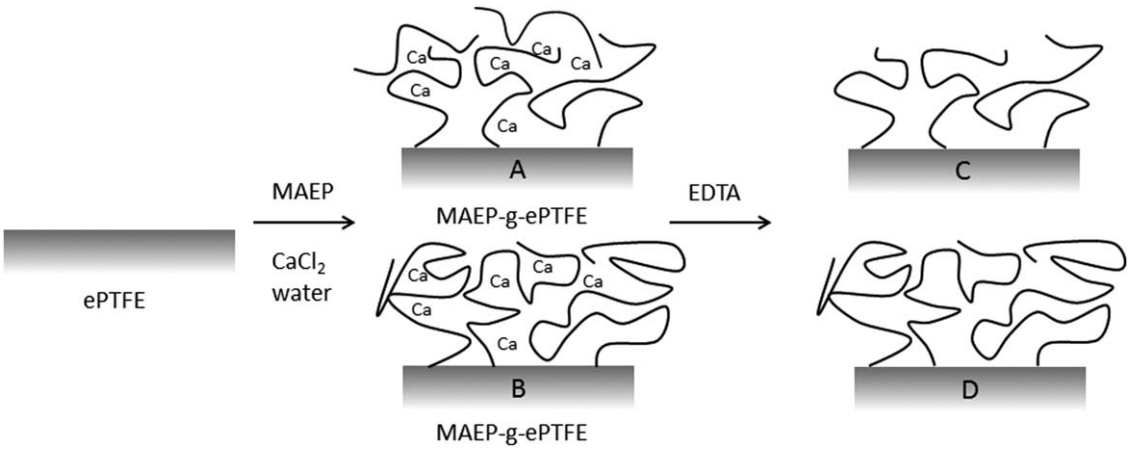


Figure 4. Schematic diagram illustrating two possible mechanisms of grafting as well as the outcomes of treating the grafted samples with EDTA solution. Grafting of ePTFE with MAEP in the presence of calcium chloride may lead to (A) the incorporated calcium ions causing electrostatic attraction of homopolymer or (B) the incorporated calcium ions increasing the amount of covalently linked graft copolymer. Treatment with EDTA would result in (C) loss of homopolymer as calcium ions are removed or (D) only removal of calcium ions with no change to the graft copolymer.

was clearly observed. The penetration of the PMAEP graft copolymer in a Gore ePTFE membrane (thickness 1 mm) has previously been evaluated using MRI.[27] Regardless of whether grafting was done in water or in methanol, the graft copolymer was only observed on the outer surface of the membrane. This was attributed to the poor wetting of ePTFE by the acrylate monomer solution.[27] The fact that we do see some grafting throughout the membrane in this study indicates that grafting can, at least to some extent, occur in thin membranes most probably by a grafting front-like mechanism.

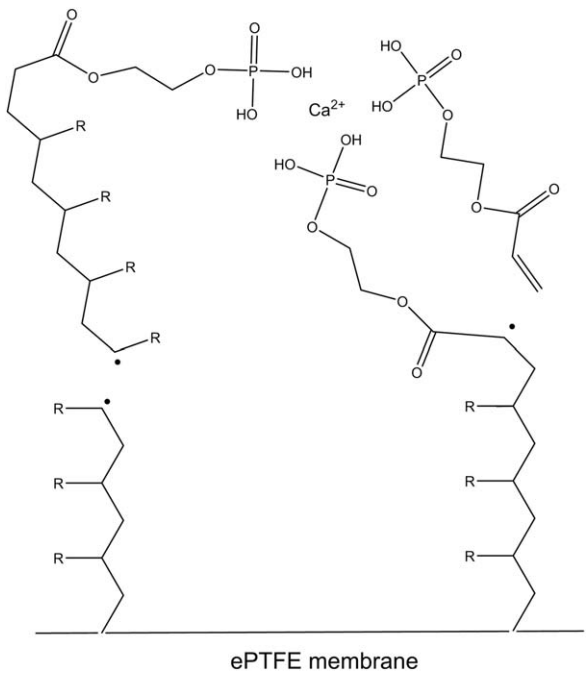
Extraction of Calcium Ions from the Graft Copolymer

As described in the Graft Polymerization section and shown in Table I, calcium ions were found to increase the graft yield of MAEP-grafted membranes. Furthermore, XPS analysis of the grafted membranes (data shown in Table II) showed that calcium ions were actually incorporated into the grafted layer whereas no chloride ions could be detected. This is in agreement with the strong affinity of calcium ions for phosphate groups where the binding constant (log*K* values) of Ca^{2+} for HPO_4^{2-} and chloride are 2.74 and −0.11, respectively.[30] Considering that an increase in graft yield is observed in the presence of calcium ions for samples where the dose was the same (Table I), the same number of initiation sites is expected in the ePTFE substrate and a higher graft yield is therefore likely due to longer grafted chains or occluded homopolymer at the surface. Two possible mechanisms, as outlined in Figure 4, were therefore considered for the observed increase in the graft yield. One of these, depicted in Figure 4(A), involves the electrostatic attraction of homopolymer, formed concomitantly in the grafting solution, by the calcium ions which are incorporated during the grafting process. The electrostatic attraction of polyelectrolytes to surfaces is a well-known phenomenon and forms the basis of layer-by-layer assembly processes.[42] The other mechanism shown in Figure 4(B), suggests that the presence of the incorporated calcium ions results in a larger concentration of reactive species near the surface thereby increasing the chain length and thus the graft yield. This can occur through the electrostatic attraction of the monomer/homopolymer radicals and/or by charge-shielding.

Since the grafted chains on the ePTFE substrate are covalently linked through C—C bonds, it is not possible to cleave them from the substrate to determine their chain length and thereby directly evaluate which of the two mechanisms is more likely to explain the observation. Therefore, to evaluate which of the two mechanisms best describes our system, treatment of the grafted membranes with a basic EDTA solution was performed with the aim of removing the calcium ions. Included was also a control treatment with water. Two grafted samples, M(40)-50 and M36(30)-50, prepared in the presence of high calcium ion concentrations were used in this part of the study. XPS analysis of these samples showed high grafting extents (100 and 85 %, respectively, see Table II) as well as significant amounts of Ca incorporation (1.39 and 0.84 atomic %, Table II). For both samples it was found that the Ca/C(monomer) ratio was 0.13 while the Ca/P ratio was 0.25–0.29.

Treatment of the grafted membranes with either water or the EDTA solution resulted in a significant change in the phosphorous content of the samples (i.e., change in P atomic %, Table II). Furthermore, phosphorous was detected in both washing solutions by ICP (data not shown). This indicates that the graft copolymer is undergoing hydrolysis of the phosphate moiety. We have previously shown that acid hydrolysis can completely remove phosphorous from the graft copolymer and that this can be used to evaluate the degree of grafting.[24] In addition, we have previously shown that, for the acrylate monomer MAEP, this hydrolysis takes place predominantly at the ester bond and occurs to some extent during the polymerization reactions which results in formation of a co-polymer graft of acrylic acid and MAEP.[43]

As seen from the results presented in Table II, treatment of the grafted membranes with water did not significantly change the calcium content in the samples as evidenced by the data showing that there was no change in the Ca atomic %. Furthermore, ICP analysis revealed only trace amounts of calcium in the water. However, treatment of the membranes with the EDTA solution resulted in near complete removal of the calcium ions. This was clearly shown by XPS and in addition, ICP confirmed significant amounts of calcium in the solution. This indicates that EDTA successfully competed for the calcium ions which is of course in agreement with the binding constants (log*K* = 10.6 for Ca^{2+} binding to $EDTA^{4-}$).[30] Although the GE of sample M36(30)-50 appears to be reduced after immersion in EDTA solution, we attribute this to the patchiness of the grafted membranes as described above rather than to a reduction in graft copolymer since the treatment of either of the grafted membrane samples with water or EDTA solution did not change the dry mass (Table II). We can therefore safely conclude that while the calcium ions were successfully removed by the use of the



Scheme 1. Schematic representation of Mechanism B where the local concentration of reactive species is enhanced in the presence of calcium ions. R = $C(O)OCH_2CH_2OPO_3H_2$.

Table III. Characterization of Selected Grafted Membranes Subjected to SBF Treatment

Sample	θ_A (°)	θ_R (°)	GE (%) ± 3	Ca 2p (%) ± 0.05	Δm (%) ± 0.3	Δm/PMAEP (%)
U	120	105	-	0	0	0
M(30)	78	59	26	0	3.0	40 ± 8
M(30)-5	75	60	82	0.83	4.4	34 ± 4
M(30)-50	61	45	85	0.91	8.2	51 ± 1

G.E. from narrow scan; errors estimated from repeat samples.

EDTA solution, this did not result in the removal of significant amounts of graft copolymer. Of the two mechanisms proposed, mechanism B [Figure 4(B)] correlates better with our data. As stated above, according to this mechanism the presence of the incorporated calcium ions results in an increase in the local monomer concentration and/or enhancement of the concentration of homopolymer radicals at the sample surface, and this is illustrated in detail in Scheme 1.

SBF Immersion

Four samples were selected for the evaluation of mineralization in SBF: the untreated ePTFE membrane (sample U), the membrane grafted in the absence of calcium ions (sample M(30)) and the membranes grafted in the presence of two different concentrations of calcium ions (samples M(30)-5 and M(30)-50). Prior to SBF immersion these samples were characterized by contact angle measurements and XPS and the data is displayed in Table III. We have previously verified, that even for porous ePTFE substrates, contact angle measurements, which probe the outmost molecular layer, give a relative evaluation of surface coverage.[27] The untreated ePTFE was found to be highly hydrophobic with an advancing contact angle of 120°. This is similar to that previously reported for ePTFE membranes from Sumitomo[21] and Pall Corporation[7] but significantly smaller than that reported for the more porous membrane from Gore[27,31] highlighting that substrate porosity does affect the apparent wettability. It is therefore only valid to use contact angle measurements as a comparative measure for porous samples such as those of this study. Grafting of the hydrophilic monomer MAEP caused a reduction in the contact angle with the extent of the reduction depending on the sample. Sample M(30)-50 displayed the lowest advancing and receding angles of 61° and 45°, respectively. It was observed that the contact angle and graft extent did not correlate and this can be attributed to the different probing depths: contact angle probes the top molecular layer whereas XPS probes to a depth of approximately 10 nm.[27] The amount of Ca detected on the samples grafted in the presence of calcium ions did not differ significantly (0.83 and 0.91 atomic % for samples M(30)-5 and M(30)-50, Table III). Sample M(30), grafted in the absence of calcium ions, displayed a significantly lower GE than the other samples; however, it had similar contact angles to sample M(30)-5. Samples M(30)-5 and M(30)-50 had similar Ca content and GE; however, M(30)-50 was significantly more hydrophilic. These three samples thus allow independent evaluation of Ca content and hydrophilicity on mineralization outcome.

Immersion of the four samples in SBF for one week resulted in mass increases for the grafted samples only. The control untreated ePTFE membrane showed no mineralization which is in agreement with previous observations[20] whereas the grafted membranes did show mineralization. This implies that mineralization occurs only where the graft copolymer is present and as such will be predominantly on the surface as this is where the graft copolymer is predominantly located [Figure 3(C,D)].The absolute mass increase (Δm in Table III) as well as the mass increase relative to the mass of the grafted PMAEP (Δm/PMAEP in Table III) both displayed significantly higher values for sample M(30)-50. This difference in mineralization capacity was also clearly reflected in the ATR/FTIR spectra of the samples. Spectra were obtained from two areas of all the SBF-treated samples. All mineralized samples displayed bands in the same positions, however, the spectral features associated with the mineral phase obtained for samples M(30) and M(30)-5 were of significantly lower intensity than for sample M(30)-50 (data not shown). Furthermore, only sample M(30)-50 had band intensities which were consistent at the two locations measured. The spectra from the two locations of samples M(30) and M(30)-5 exhibited varying band intensities which strongly suggests patchy mineralization.[20] In our previous study on grafting MAEP to ePTFE, we noted a correlation between the GE and the mineralization capacity.[20] Results from that study strongly supported the conclusion that a GE greater than 36%

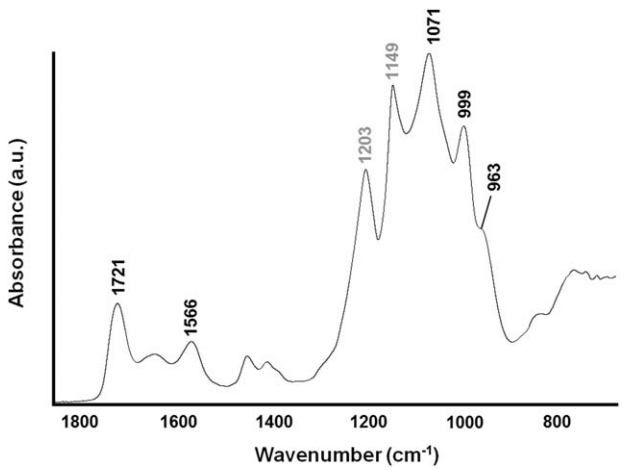


Figure 5. ATR/FTIR spectrum of sample M(30)-50 after immersion in SBF. Black numbers refer to band positions for the graft copolymer and mineral deposit while grey numbers refer to bands of the ePTFE substrate.

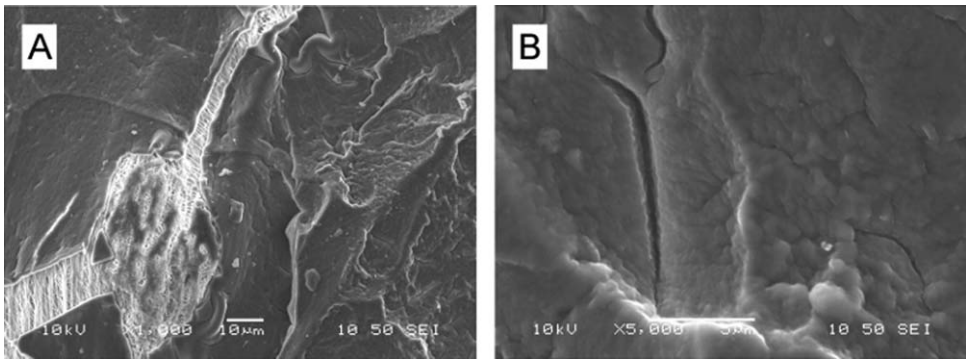


Figure 6. SEM images of sample M(30)-50 after immersion in SBF (A) low magnification (scale bar = 10 µm) and (B) high magnification (scale bar = 5 µm).

is a requirement before any mineralization occurred. Even for a sample with a graft extent of 44% only scarce mineral growth was found. However, based on this study we can now conclude that it is the *surface coverage* of the graft copolymer (as evaluated from the wettability)[27] rather than the *graft extent* which most strongly affects the mineralization outcome. Strongly supportive of this argument is the fact that sample M(30) with a graft extent of only 26% displayed equal mineralization to sample M(30)-5 (graft extent of 82 %) and most significantly these two samples displayed similar wettabilities (Table III). Furthermore, samples M(30)-5 and M(30)-50 which have similar GE values do differ significantly in wettability and in agreement with our hypothesis this leads to higher mineralization for sample M(30)-50. Furthermore, based on the results from this study we can conclude that it is the sample wettability (as a measure of surface coverage) rather than the presence of calcium ions that affects the mineralization outcome.

The ATR/FTIR spectrum of sample M(30)-50 is shown in Figure 5 where the bands at 1071 and 999 cm^{-1} are attributed to the calcium phosphate mineral layer. The band observed in the grafted sample (Figure 2) at 965 cm^{-1} remains as a shoulder at 963 cm^{-1} after mineralization while the band at 1064 cm^{-1} appears to lie under the main mineral band at 1071 cm^{-1}. The overall spectral features of this sample after mineralization closely resemble that of a previously reported PMAEP film[41] as well as MAEP-grafted ePTFE[20] where these particular spectral features were attributed to the mineral monetite ($CaHPO_4$) or brushite ($CaHPO_4.2H_2O$). In the present study EDX was used to find the Ca/P ratio of 1.1–1.2 while the (Ca+Mg)/P was found to be 1.2–1.3. These values are in good agreement with those reported from earlier studies.[41] In addition to bands attributed to the mineral, there is a new band at 1566 cm^{-1} which corresponds to the carbonyl vibration of a carboxylate group. This strongly suggests that large amounts of the PMAEP side chains were hydrolyzed at the ester bonds, and the carboxylic acid groups were deprotonated by the binding of the Ca^{2+} ions present in SBF. This phenomenon has previously been observed in PMAEP gels and films[41] and correlates with the hydrolysis of PMAEP taking place predominantly at the ester bond resulting in a co-polymer graft of acrylic acid and MAEP.[43]

SEM was used to evaluate the mineralization outcome of sample M(30)-50. Most of this sample displayed complete coverage by a mineral layer with the underlying ePTFE membrane clearly visible in only very few regions. This is illustrated in Figure 6(A) where some sample cracking due to sample drying and attachment to the SEM stub is also evident. The mineral deposit observed at this magnification is similar to that previously found for MAEP-grafted ePTFE with high graft yield.[20] A high magnification image of the mineral coating formed on the membrane, however, reveals globules of around 1 μm in diameter. In previous studies we have generally observed a tile-like mineral morphology for samples where the FTIR spectra indicated the presence of monetite or brushite[20,41] In contrast, as evaluated by FTIR, a globular mineral morphology has previously been correlated with an apatite mineral.[21,22,41] It should be noted, however, that globular mineral deposits have also been observed for samples where FTIR analysis was unable to identify the exact nature of the mineral.[21,22] It must be acknowledged, therefore, that the mineral morphology may not always be a good indicator of the mineral type that forms in SBF.

CONCLUSIONS

This study demonstrates that the presence of low concentrations of calcium ions in the monomer solution results in increased graft yield while other factors such as radiation dose and monomer concentration lead to expected grafting trends. Results confirm that calcium ions were incorporated into the graft copolymer and could be extracted using a basic EDTA solution without any changes to the graft yield. This indicates that improving the graft yield by introducing calcium ions involves a mechanism that increases the local concentration of monomer and/or homopolymer radicals near the surface during the grafting process. There is strong evidence that the mineralization capacity of the grafted membranes is dependent on the sample wettability rather than any direct effect of the incorporated calcium ions. The nature of the deposited mineral was not affected by the presence of calcium ions and based on FTIR spectra was identified as monetite or brushite, albeit with a globular mineral morphology as observed by SEM. Evaluation of the effect of other non-redox active metal ions on the graft yield for both phosphate and carboxylate-containing monomers may be an avenue for future study.

ACKNOWLEDGMENTS

The authors thank Brendan Miller and Anne Schacher who recorded the FTIR maps; Dr. Llew Rintoul (QUT) for assistance with FTIR spectroscopy; and Dr Barry Wood (CMM, UQ) with XPS data collection. This work was carried out in part in the Centre for Microscopy and Microanalysis, University of Queensland node of Australian Microscopy and Microanalysis Research Facility (AMMRF).

REFERENCES

1. Dargaville, T. R.; George, G. A.; Hill, D. J. T.; Whittaker, A. K. *Prog. Polym. Sci.* **2003**, *28*, 1355.
2. Nasef, M. M.; Hegazy, E.-L. A. *Prog. Polym. Sci.* **2004**, *29*, 499.
3. Kattan, M.; El-Nesr, E. *J. Appl. Polym. Sci.* **2006**, *102*, 198.
4. Taher, N. H.; Dessouki, A. M.; Khalil, F. H.; El-Arnaouty, M. B. *Polym. Int.* **1996**, *41*, 383.
5. Kaji, K. *J. Appl. Polym. Sci.* **1986**, *32*, 4405.
6. Sidorova, L. P.; Aliev, A. D.; Zlobin, V. B.; Aliev, R. E.; Chalykh, A. E.; Kabanov, V. Y. *Radiat. Phys. Chem.* **1986**, *28*, 407.
7. Hidzir, N. M.; Hill, D. J. T.; Martin, D.; Grøndahl, L. *Polymer* **2012**, *53*, 6063.
8. Garnett, J. L.; Hankiewicz, S. V.; Sangster, D. F. *Radiat. Phys. Chem.* **1990**, *36*, 571.
9. Zu, J.; Hu, Z.; Wang, W.; Zhang, J.; Pino, E. S.; Gu, J.; Tong, L. *J. Radioanal. Nucl. Chem.* **2007**, *273*, 479.
10. Deng, B.; Li, J.; Hou, Z.; Yao, S.; Shi, L.; Liang, G.; Sheng, K. *Radiat. Phys. Chem.* **2008**, *77*, 898.
11. Sperati, C. A.; Starkweather, Jr H. W. *Adv. Polym. Sci.* **1961**, *2*, 465.
12. Liu, F.; Grainger, D. W. In Biomaterials Science: An Introduction to Materials in Medicine; Ratner, B. D., Hoffman, A. S., Schoen, F. J., Lemons, J. E., Eds.; Elsevier/Academic Press: Amsterdam, **2012**; p 92.
13. Wentrup-Byrne, E.; Grøndahl, L.; Chandler-Temple, A. In Biointegration of medical Implant Materials: Science and Design; Sharma, C. P., Ed.; Woodhead Publishing: Cambridge, **2010**; p 51.
14. Cassady, A. I.; Hidzir, N. M.; Grøndahl, L. *J. Appl. Polym. Sci.* **2014**, *131*, 40533.
15. Wentrup-Byrne, E.; Suzuki, S.; Grøndahl, L. In Phosphorus-Based Polymers: From Synthesis to Applications; Monge, S., David, G., Eds.; Royal Society of Chemistry: Cambridge, **2014**; p 167.
16. Monge, S.; Canniccioni, B.; Graillot, A.; Robin, J.-J. *Biomacromolecules* **2011**, *12*, 1973.
17. Kamei, S.; Tomita, N.; Tamai, S.; Kato, K.; Ikada, Y. *J. Biomed. Mater. Res.* **1997**, *37*, 384.
18. Tretinnikov, O. N.; Kato, K.; Ikada, Y. *J. Biomed. Mater. Res.* **1994**, *28*, 1365.
19. Kato, K.; Ikada, Y. *Biotech. Bioeng.* **1996**, *51*, 581.
20. Grøndahl, L.; Cardona, F.; Chiem, K.; Wentrup-Byrne, E.; Bostrom, T. *J. Mater. Sci. Mater. Med.* **2003**, *14*, 503.
21. Suzuki, S.; Grøndahl, L.; Leavesley, D.; Wentrup-Byrne, E. *Biomaterials* **2005**, *26*, 5303.
22. Wentrup-Byrne, E.; Suzuki, S.; Suwanasilp, J. J.; Grøndahl, L. *Biomed. Mater.* **2010**, *5*, 045010.
23. Chandler-Temple, A.; Kingshott, P.; Wentrup-Byrne, E.; Cassady, A.; Grøndahl, L. *J. Biomed. Mater. Res. A* **2013**, *101*, 1047.
24. Grøndahl, L.; Cardona, F.; Chiem, K.; Wentrup-Byrne, E. *J. Appl. Poly. Sci.* **2002**, *86*, 2550.
25. Oyane, A.; Kim, H. M.; Furuya, T.; Kokubo, T.; Miyazaki, T.; Nakamura, T. *J. Biomed. Mater. Res. A* **2003**, *65*, 188.
26. Beamson, G.; Briggs, D. High Resolution XPS of Organic Polymers: The Scienta ESCA300 Database; Wiley: West Sussex, **1992**.
27. Chandler-Temple, A.; Wentrup-Byrne, E.; Griesser, H. J.; Jasieniak, M.; Whittaker, A. K.; Grøndahl, L. *Langmuir* **2010**, *26*, 15409.
28. Garnett, J. L.; Jankiewicz, S. V.; Sangster, D. F. *Radiat. Phys. Chem.* **1990**, *36*, 571.
29. Gupta, B.; Grover, N.; Singh, H. *J. Appl. Polym. Sci.* **2009**, *112*, 1199.
30. Martell, A. E.; Smith, R. M. Critical Stability Constants; Plenum Press: NY, **1974**.
31. Chandler-Temple, A.; Wentrup-Byrne, E.; Whittaker, A. K.; Grøndahl, L. *J. Appl. Polym. Sci.* **2010**, *117*, 3331.
32. Grøndahl, L.; Chandler-Temple, A.; Trau, M. *Biomacromolecules* **2005**, *6*, 2197.
33. Shin, Y. M.; Kim, K.-S.; Lim, Y. M.; Nho, Y. C.; Shin, H. *Biomacromolecules* **2008**, *9*, 1772.
34. Shin, Y. M.; Shin, H.; Lim, Y. M. *Macromol. Res.* **2010**, *18*, 472.
35. Luk, J. Z.; Cooper-White, J. J.; Rintoul, L.; Taran, E.; Grøndahl, L. *J. Mater. Chem. B* **2013**, *1*, 4171.
36. Li, S.; Wei, J.; Yang, H.; Wang, L.; Wang, A.; Zhang, Y.; Nie, Y. *Polym.-Plast. Technol. Eng.* **2013**, *52*, 427.
37. Yang, Q.; Xu, Z.-K.; Hu, M.-X.; Li, J.-J.; Wu, J. *Langmuir* **2005**, *21*, 10717.
38. Gupta, B.; Anjum, N. *Adv. Polym. Sci.* **2003**, *162*, 35.
39. Grasselli, M.; Navarro del Cañizo, A. A.; Camperi, S. A.; Wolman, F. J.; Smolko, E. E.; Cascone, O. *Radiat. Phys. Chem.* **1999**, *55*, 203.
40. Liang, C. Y.; Krimm, S. *J. Chem. Phys.* **1956**, *25*, 563.
41. Suzuki, S.; Whittaker, M. R.; Grøndahl, L.; Monteiro, M. J.; Wentrup-Byrne, E. *Biomacromolecules* **2006**, *7*, 3178.
42. Caruso, F. *Adv. Mater.* **2001**, *13*, 11.
43. Grøndahl, L.; Suzuki, S.; Wentrup-Byrne, E. *Chem. Commun.* **2008**, 3314.