

Analysis of photoinitiated polymerization in a membrane mimetic film using infrared spectroscopy and near-IR Raman microscopy

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Abstract

A method has been developed to investigate the extent of polymer cross-linking that results following in situ photopolymerization of an acrylate-functionalized phospholipid assembly adsorbed onto a stabilized, membrane-mimetic film produced from a polyelectrolyte multilayer (PEM) on polytetrafluoroethylene (PTFE) grafts. The acrylate phospholipid monomer was synthesized, prepared as a unilamellar vesicle, and fused onto closed-packed acyl chains that make up the PEM membrane-mimetic barrier on the PTFE graft. Both broad band white light and 514.5 nm laser radiation were used as excitation sources for photoinitiation; eosin Y was used as the photoinitiator. The use of 514.5 nm excitation reduced the time for maximum polymerization of the acrylate lipid from 60 min to 240 s. Infrared spectroscopy was successfully used to analyze the extent of photopolymerization in simplified model acrylate lipid systems; however, this method could not be used to analyze acrylate polymerization in heterogeneous, multicomponent PEM membrane-mimetic barriers on PTFE grafts. A near-infrared Raman microscopy method based on the ratio of the integrated areas of the C=C and C–N vibrations was shown to provide equivalent information to the IR method for analysis of the extent of polymerization efficiency in acrylate lipids. In addition, it proved feasible to extend this near-IR Raman method to the in situ analysis of the extent of polymerization in a stabilized acrylate lipid membrane on a PEM film in a PTFE vascular graft. This work describes a new approach for generating and analyzing the robustness of a membrane-mimetic coating on biomaterial surfaces, and may improve our ability to predict the long-term stability of polymeric membrane-mimetic films on implantable medical devices.

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1. Introduction

It is believed that a clinically viable artificial blood vessel may be constructed by incorporation of physiologically-relevant antithrombogenic mechanisms at the blood-material interface [1–6]. In this regard, investigators have noted for nearly two decades that the phosphorylcholine headgroup seems to limit the induction of blood clot formation on synthetic surfaces [7–9]. Based on this observation, there has been considerable

interest in the use of supported lipid membranes for biosensor applications, as biofunctional coatings to protect transplanted cells, or as blood contacting films for artificial organs, including prosthetic blood vessels [1,2,10]. Indeed, continued interest in the formation of supported lipid membranes on polyelectrolyte multilayers (PEM) has been driven by the potential to further enhance the versatility of layer-by-layer thin films by offering an additional mechanism for modulating both surface physiological properties and biological activity [11–15].

Supported lipid membranes applicable for these purposes have been constructed by either: (i) assembling a layer of closely packed hydrocarbon chains onto an underlying substrate followed by controlled dipping through an organic amphiphilic monolayer at an air-water interface [16], or (ii) by exposure to a dilute solution of emulsified lipids or unilamellar vesicles [17]. However, a significant barrier to the development of such

Abbreviations: ARPC, 1-palmitoyl-2-[1,2-(acryloyloxy)dodecanoil]-sn-glycero-3-phosphocholine; ATR, attenuated total reflectance; EY, eosin Y; FT-IR, Fourier transform infrared; HATR, horizontal attenuated total reflectance; PTFE, polytetrafluoroethylene; TEA, triethanolamine; VP, vinyl pyrrolidone

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a device has been limited stability outside of a laboratory environment. In particular, non-covalently associated planar lipid assemblies, in and of themselves, are insufficiently robust for medical implant applications [18–20]. One method that has been examined to increase the stability of a planar lipid film has been to polymerize the substrate-supported lipid film. In this regard, we have found that room temperature photoinitiated polymerization is preferential to heat initiated polymerization due to the potential to inactivate membrane-associated proteins at temperatures typically required for thermal decomposition of the initiator [6].

A critical parameter that will dictate the long-term stability of a supported polymeric membrane-mimetic film is the extent of polymerization within the lipid assembly. Infrared spectroscopy is a particularly useful spectroscopic method for deducing the degree of polymerization in a photo-irradiated polymer sample [21]. The use of real-time infrared spectroscopy for the measurement of polymerization efficacy relies on the decrease of the integrated intensity of the C=C vibration that occurs with increased cross-linking after an irradiation period [22–24].

Although real-time IR spectroscopy has been widely used for quantifying the extent of cross-linking in polymerization studies, practical difficulties exist in implementing this method for planar membrane assemblies on PEM supports. For example, IR spectroscopy is physically based on a net change in the permanent dipole moment during a molecular vibration, which fundamentally provides only a very weak IR intensity for the homonuclear diatomic C=C bond [25]. Thus, it can be problematic to quantitatively measure the decrease in the C=C vibrational intensity, particularly for multicomponent samples such as the multilayered membrane mimetic samples of interest to our group, in which this mode may be partially or completely overlapped.

Raman spectroscopy offers an alternative approach to the more established IR methodology for this type of analysis. While IR activity is based on a net change in the dipole moment of a molecule, Raman activity is based on a change in the vibration's polarizability [26]. Raman is therefore more applicable than IR to the analysis of homonuclear stretching vibrations such as the C=C stretch that is used in the analysis of polymerization efficacy. Raman can also be used to more readily study aqueous samples, since water is a relatively weak Raman scatterer.

In the present work, we first evaluate the utility of the standard real-time IR spectroscopy method for determining the extent of polymerization of the outermost lipid layer on a PEM-supported membrane-mimetic film after photoinitiated polymerization in minimally hydrated thin films. We then demonstrate that a method based on near-IR Raman microscopy produces equivalent results to the standard IR method for quantifying photo-induced polymerization in these model systems. We show that the use of a laser as the irradiation source substantially decreases the time needed for maximum polymerization as well as increasing the maximum polymerization efficiency in these samples. Finally, we demonstrate that this Raman method can be successfully applied in situ to deduce the extent of photo-induced polymerization of PEM-supported membrane mimetic films when they are coated onto the interior walls of 4 mm i.d. polytetrafluoroethylene vascular grafts.

2. Materials and methods

2.1. Reagents

Deuterium oxide was obtained from Sigma Aldrich (St. Louis, MO). Monoacrylate-PC (1-palmitoyl-2-[1,2-(acryloyloxy)dodecano-yl]-*sn*-glycero-3-phosphocholine) (ARPC) was synthesized as previously described [1].

2.2. Preparation of ARPC for photoinitiation

Monoacrylate-PC (1-palmitoyl-2-[1,2-(acryloyloxy)dodecano-yl]-*sn*-glycero-3-phosphocholine) (ARPC) samples were prepared for photopolymerization as a minimally hydrated lipid solution. A volume of 3.19×10^{-3} mL of deuterium oxide was added to 7.48 mg of ARPC and heated to a temperature of 40 °C and then vortexed, in three cycles, to form multilamellar vesicles. The photoinitiator, consisting of 1 µL of eosin Y (EY), 225 mM triethanolamine (TEA), and 37 mM vinyl pyrrolidone (VP) in water, was added to the vesicle solution. This was placed on a 45° trapezoidal shaped germanium horizontal ATR crystal that was cleaned by sonicating for 15 min in a 6:3:1 mixture of chloroform, methanol, and water followed by a further 15 min in distilled deionized water.

2.3. Sample irradiation

White light irradiation was carried out using a Dyna-Lite (Scientific Instruments, Inc., Skokie, IL) source with an intensity of ~10 mW at the sample. The source was placed ~7.5 cm above the sample. A Coherent Radiation Innova 90 Series Ar⁺ laser (Coherent, Santa Clara, CA) provided excitation at 514.5 nm. Typical intensity for photoinitiation was ~100 mW at the sample. Light intensity was measured with a Coherent Model 10 power meter.

2.4. Infrared spectroscopy

Spectra were acquired using a Digilab/BioRad FTS-60 Fourier Transform infrared (FT-IR) spectrometer (Randolph, MA) equipped with a narrow-band, LN₂-cooled HgCdTe detector. Sample solutions of ARPC were placed on trapezoidal shaped Ge ATR crystals (Spectral Systems, Hopewell Junction, NY). A horizontal attenuated total reflectance (HATR) accessory (CIC Photonics, Albuquerque, NM) was employed for both sample irradiation and spectral acquisition. ATR-IR spectra were collected using the following instrumental parameters: 512 background scans, triangular apodization, and 4 cm⁻¹ resolution.

2.5. Raman microscopy

Near-IR Raman spectra were acquired using a fiber-optic interfaced Kaiser Optical Systems confocal Raman microscope (Kaiser Optical Systems, Inc., Ann Arbor, MI). The microscope unit is based on an Olympus BX-60 research light microscope with an integrated, single-mode (Ø ~ 7 µm), fiber-optic

input/output module (HoloLab Series 5000, Kaiser Optical Systems) that delivers the excitation laser light to the microscope and collects the Raman scattered radiation. A 50× objective (Carl Zeiss, Jena, Germany) was used on the microscope head to excite/collect the scattered radiation. The Raman scattered light is delivered to a *f*/1.8 holographic imaging spectrograph (HoloSpec *f*/1.8i, Kaiser Optics Systems) using fiber optics. The detector on this spectrograph is a liquid-N₂ cooled CCD detector (1024EHRB, 1024 × 256 pixels, Princeton Instruments, Princeton, NJ) that is back-illuminated and is specifically optimized for high QE in the near-IR spectral region (~35% at 1000 nm). The holographic grating in the spectrograph simultaneously measures the spectral range of 100 to 3450 cm⁻¹ at an excitation wavelength of 785 nm. The 785 nm excitation wavelength was produced using a Coherent Radiation (Coherent, Santa Clara, CA) 899 Ti:Sapphire ring laser pumped by a Coherent Innova 300 Series Ar⁺ laser.

2.6. Software

IR and Raman spectra were collected using instrument software supplied by the manufacturers. Post-processing of all collected spectra was conducted using the Grams32/AI spectral software processing package (Galactic Industries, Nashua, NH). All spectra presented here were baseline corrected for clarity.

2.7. Vascular graft coating procedure

Polytetrafluoroethylene (PTFE) grafts (WL Gore, Flagstaff, AZ, 4 mm i.d.) were initially impregnated with an aqueous solution of gelatin (6 wt.%) followed by overnight crosslinking in 1.5% (v/v) glutaraldehyde. The luminal surface was then coated with a series of five alternating layers of alginate (0.15 wt.%) and poly-L-lysine (0.10 wt.%) by serial perfusion of the prosthesis at a flow rate of 1 mL/min for 120 s. After each perfusion, the prosthesis was rinsed with deionized water at 2 mL/min. The luminal surface was then perfused with a 0.1 mM terpolymer solution (1% DMSO/20 mM NaH₂PO₄, pH 7.4) at a flow rate of 0.1 mL/min for 10 min. A 1.2 mM solution of monoacrylate lipid vesicles (20 mM NaH₂PO₄, pH 7.4, 750 mM NaCl) containing eosin Y as a photoinitiator was then prepared and perfused through the prosthesis at a flow rate of 1 mL/h for 5 h at 43 °C. The graft was then irradiated with a 360°, 1.6 cm side-firing probe attached to a 200 mW argon ion laser (Melles Griot). Approximately 50–75 mW of laser power was brought into the graft and polymerization was performed by irradiating each 3 cm graft segment for 10 min. The graft was then rinsed with water at 2 mL/min for 10 min.

3. Results and discussion

3.1. Analysis of photopolymerization in a model lipid sample using infrared spectroscopy

The most widely accepted method for the analysis of the degree of cure, or percent polymerization, in polymer films is through real-time infrared spectroscopy [22–24]. This method

relies on the use of the ratio of the integrated intensity of the C=C vibration to an internal standard to determine the percent polymerization occurring over a given irradiation period. A widely used internal standard is the C=O vibration [21], although any band that retains constant integrated intensity during irradiation may be used as the standard by which to compare intensity changes occurring in the C=C vibration.

In order to determine whether real-time IR was applicable to the membrane-mimetic system under study, we initially analyzed a simplified homogeneous system composed solely of the minimally hydrated monoacrylate ARPC lipid (Fig. 1B) with eosin Y photoinitiator. This allowed direct analysis of the IR spectroscopic regions of interest and yielded results that could be used in the study of in situ photopolymerization in the more heterogeneous model consisting of the ARPC lipid supported on a polyelectrolyte multi-layer (Fig. 1).

A minimally hydrated ARPC lipid solution was employed in these studies since the C=C stretching vibration at 1621 cm⁻¹ in a sample composed of 12 mM lipid vesicles is completely masked by bulk water (see, e.g. Fig. 2A). Minimum hydration allows the lipid to retain its full thermodynamic properties [27], yet provides for less interference from bulk water. In addition, deuterium oxide was used as the solvent in place of water in these model lipid samples in order to shift the remaining solvent bands away from the 1600 to 1750 cm⁻¹ region of the spectrum (Fig. 2B).

The minimally hydrated ARPC sample was placed on the surface of a Ge crystal in a horizontal ATR cell within the sample compartment of the FT-IR spectrometer and a broad band, white light source was used for the initiation of photopolymerization [1,2,4,6,18–20,28]. The light source was directed through a custom-made quartz window fitted into the top cover of the sample compartment; the power of the incident energy was approximately 6 mW, measured at the sample.

In order to determine the percent polymerization by HATR FT-IR, the ratio of the integrated area of the C=C vibration to the ester carbonyl band was determined:

$$\text{degree of cure} = \frac{A_0 - A_t}{A_0} \quad (1)$$

where A_0 is the ratio at $t=0$ s of the area of the monoacrylate C=C peak at 1621 cm⁻¹ to that of the lipid ester carbonyl band centered at 1734 cm⁻¹, while A_t is the identical peak area ratio at a specified time t .

The spectrum of the ARPC lipid taken prior to commencing irradiation was denoted as the $t=0$ sample. The sample was then irradiated for 15, 30 and 60 min using a white light source with an intensity of ~6 mW. After each irradiation period, spectra were collected and the integrated areas of the C=C stretch at 1621 cm⁻¹ and the lipid ester carbonyl band at 1734 cm⁻¹ were measured (Fig. 3). The ratio of the two bands was equal to 7.8×10^{-4} ($t=0$ min), 2.8×10^{-4} ($t=15$ min), 1.7×10^{-4} ($t=30$ min), and 1.4×10^{-4} ($t=60$ min); these ratios corresponded to average percent polymerization 0, 62.9, 77.5, and 85.5%, respectively (Fig. 4). These results confirmed that real-time IR could be used to monitor polymerization in a simplified monoacrylate PC model system.

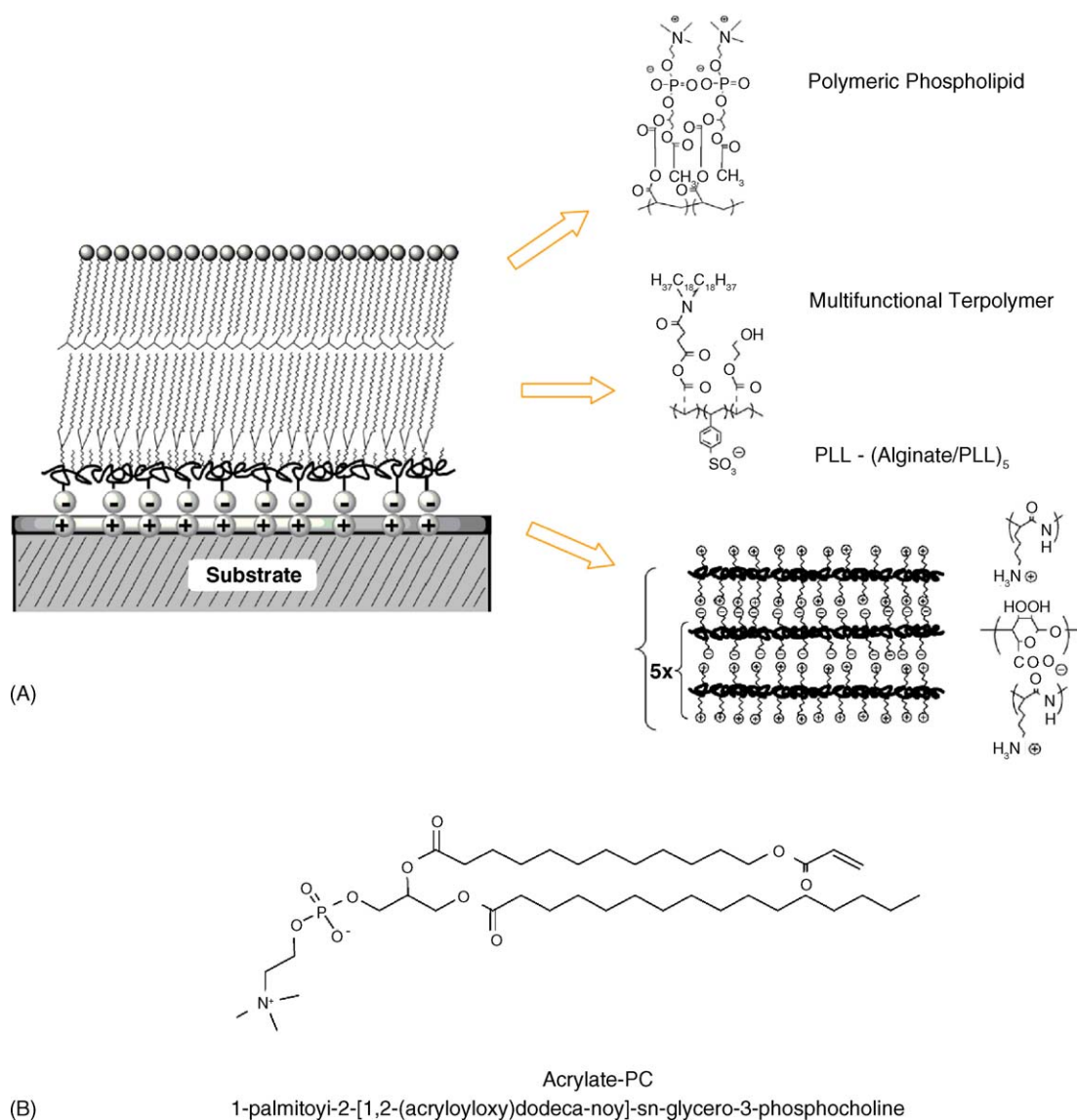


Fig. 1. (A) Polymeric phospholipid monolayer supported by a polyelectrolyte multi-layer and hydrated cushion. (B) Structure of acrylate phospholipid.

3.2. Analysis of photopolymerization in a model lipid sample using Raman spectroscopy

Although real-time IR proved feasible for a simplified model ARPC system, we were unable to conduct a similar analysis using an ARPC lipid membrane on a polyelectrolyte multilayer. The relatively weak C=C vibration could not be quantitatively resolved using IR spectroscopy in this heterogeneous, multilayered, membrane mimetic system.

In order to overcome this limitation, we have evaluated Raman spectroscopy as an alternative method for quantifying the extent of polymerization in these samples. As previously noted, Raman is an attractive option to IR spectroscopy for these analyses since homonuclear stretching vibrations (such as the C=C bond) are fundamentally much stronger in Raman than in IR [26]. Moreover, Raman can also be used to readily study aqueous samples, since water is a relatively weak Raman scatterer [29].

A correlative study between Raman and real-time IR was performed on simplified system composed solely of the minimally hydrated monoacrylate ARPC lipid with eosin Y photoinitiator. Near-IR Raman microscopy was performed using a 785 nm excitation wavelength with approximately 60 mW of incident power measured at the sample position. The ratio of the integrated area of the C=C stretching vibration at 1638 cm^{-1} versus the C–N stretch at 715 cm^{-1} was used to determine the percentage of polymerization in the sample after irradiation. The C–N stretch was chosen as the internal standard because it provides a sharp well-defined peak for analysis and did not change intensity following irradiation.

Fig. 5A illustrates the decrease in intensity for the Raman C=C stretching vibration of the ARPC lipid after irradiation. We observed decreases in the integrated area for the C=C peak similar to those observed during infrared measurements (cf. Figs. 5A and 3A) while there was virtually no change in the C–N integrated peak area (Fig. 5B). The decrease in

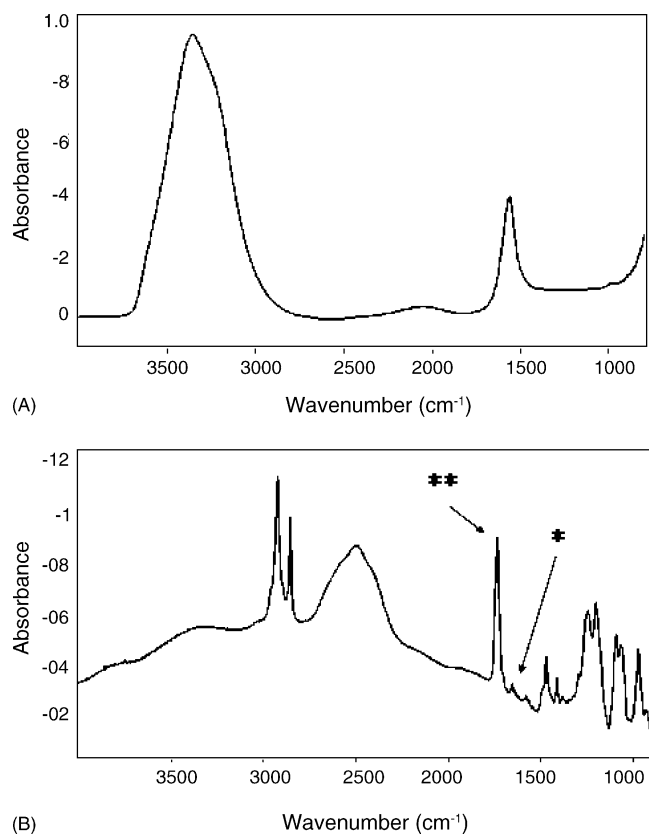


Fig. 2. (A) FT-IR spectrum of a 12 mM ARPC lipid sample, illustrating the effects of bulk water in the sample. The C=C and C=O bands are completely obscured. (B) The same sample prepared with deuterium oxide. The shifted bulk water no longer interferes with analysis of the region in question. The band denoted by the single asterisk (*) is the C=C vibration at 1616 cm⁻¹ while the band denoted by the double asterisk (**) is the C=O vibration at 1734 cm⁻¹.

the intensity of the C=C vibration observed using Raman is qualitatively similar to that obtained from the HATR FT-IR measurements.

The percent polymerization was determined using the ratio of the integrated areas of the C=C stretch at 1638 cm⁻¹ versus the C–N stretch at 715 cm⁻¹. The ratio of these two bands was equal to 6.7×10^{-2} ($t=0$ min), 2.4×10^{-2} ($t=15$ min), 1.5×10^{-2} ($t=30$ min), and 1.2×10^{-2} ($t=60$ min). These ratios corresponded to average polymerization percentages of 0, 73, 77, and 86%, respectively (Fig. 4). At 30 and 60 min, the differences in percent polymerization obtained by IR and near-IR Raman measurements were less than 0.7%. These results indicate that Raman spectroscopy is a feasible method for monitoring polymerization in a simplified monoacrylate PC model system.

3.3. Analysis of photopolymerization in a model lipid sample using Raman spectroscopy with a 514.5 nm excitation source

The experiments described above were performed using a broad-band, white light source for sample irradiation. Since the absorbance maximum of the xanthene dye eosin Y used here as a photoinitiator occurs at ~ 517 nm [30], experiments were repeated using the 514.5 nm wavelength from an Ar⁺-ion laser

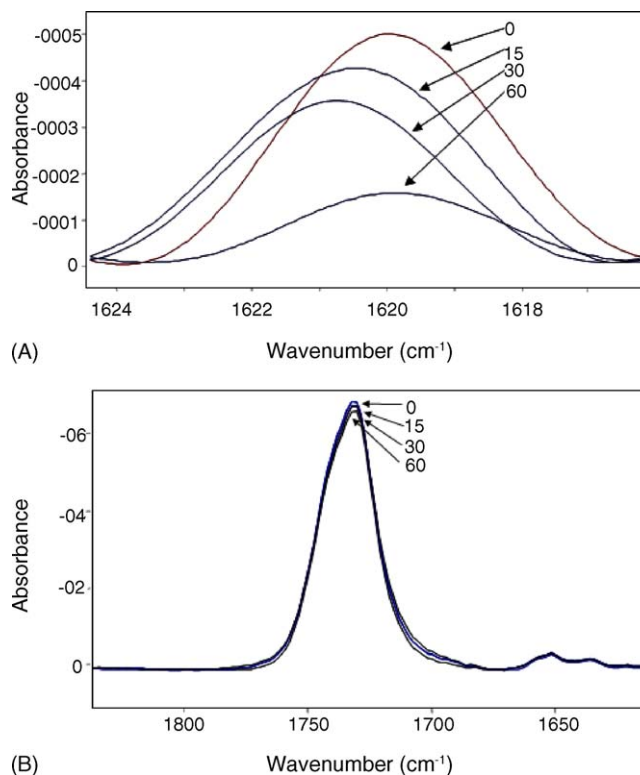


Fig. 3. (A) Decrease in C=C band intensity for the given irradiation times measured by HATR FT-IR spectroscopy using white light irradiation at ~ 6 mW power. (B) Decrease in the ester carbonyl band intensity for the given irradiation times measured by HATR FT-IR spectroscopy using white light irradiation at ~ 6 mW power.

as the excitation source in order to assess its impact on polymerization efficiency [31,32].

Minimally hydrated ARPC lipid samples were prepared and irradiated with ~ 100 mW of 514.5 nm radiation. The laser output was delivered to the horizontal sample using a 200 μ m core silica multi-mode optical fiber (Anhydroguide G, Fiberguide Industries, Stirling, NJ). The output of the laser from the optical fiber describes a cone defined by the numerical aperture of

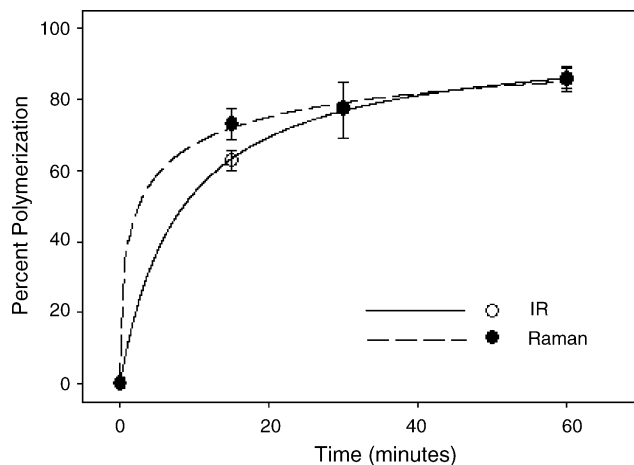


Fig. 4. Percent polymerization of ARPC samples for a given irradiation period as measured using both near-IR Raman microscopy and IR HATR measurement methods after samples were irradiated with white light at ~ 6 mW power.

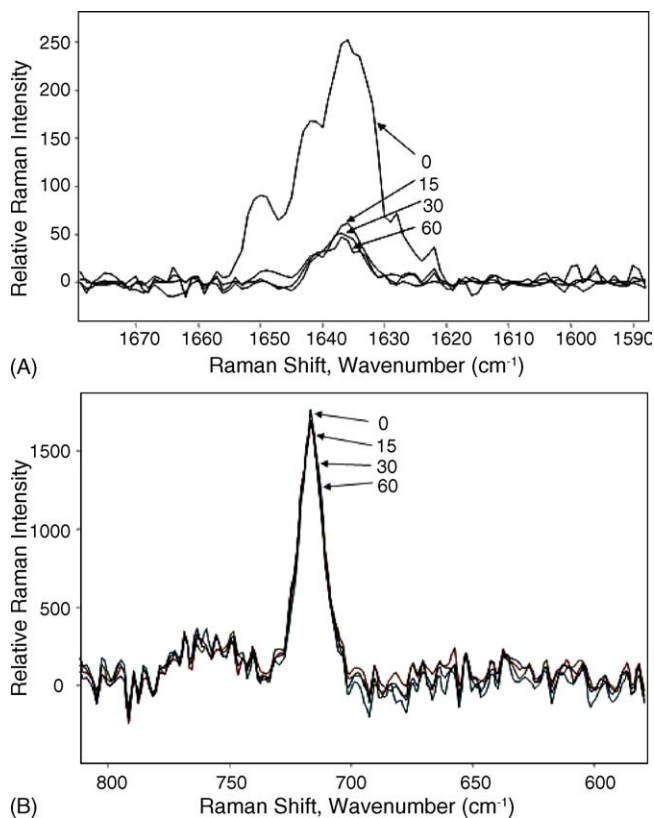


Fig. 5. (A) Decrease in integrated area of C=C bond during irradiation for given periods measured with near-IR Raman microscopy using white light irradiation at ~ 6 mW power. (B) Constant integrated area of C-N bond during irradiation under above conditions.

the fiber [33–35]. The optical fiber was positioned such that the entire area of the lipid film, which consisted of several cm², was irradiated by the output cone of the fiber probe. Near-IR Raman microscopy confirmed that the irradiation times required for maximum polymerization were substantially shorter (240 s versus 60 min) and the polymerization efficiency significantly greater (100% versus 86%) when the 514.5 nm laser wavelength was used as the excitation source (Fig. 6).

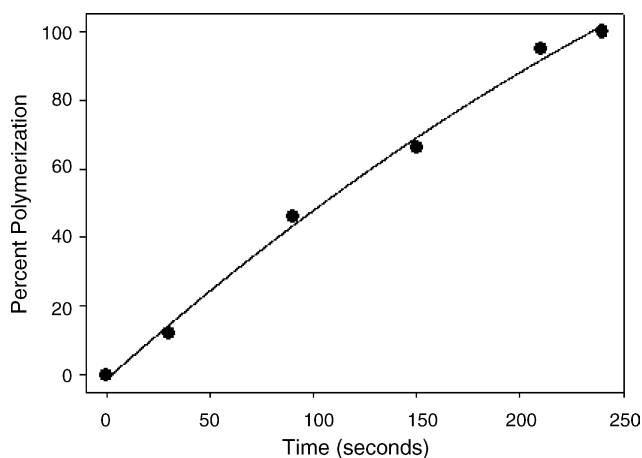


Fig. 6. Percent polymerization of monoacrylate-PC samples for given irradiation periods measured by near-IR Raman microscopy after samples were irradiated using 514.5 nm light at ~ 100 mW power.

3.4. In situ analysis of photopolymerization in a vascular graft using Raman microscopy

Based on the results described here, near-IR Raman microscopy was used to analyze the photopolymerization of a multicomponent membrane-mimetic film formed directly onto a PTFE vascular graft (Fig. 1A). Graft samples were irradiated for various time periods up to 300 s using 514.5 nm light from a laser-coupled optical fiber inserted into the center of the 4 mm i.d. PTFE graft. Subsequent to irradiation, the PTFE graft was cut lengthwise and the interior of the graft was examined using near-IR Raman microscopy. A non-irradiated sample was used as a baseline reference to determine zero percent polymerization.

Due to several overlapping bands from the underlying PEM film components in the 1580–1730 cm⁻¹ region, spectral subtraction was performed in order to isolate the C=C stretching vibration for analysis. This was accomplished using the spectrum obtained from a PTFE graft sample that included all of the PEM film constituents with the exception of the outer monoacrylate-PC and photoinitiator. Representative Raman spectra for non-irradiated and irradiated samples are shown in Fig. 7 and illustrates the loss of intensity of the monomer ARPC bands in the region 1800–1400 cm⁻¹ following photoinitiated polymerization.

Fig. 8 presents the results of the polymerization efficiency for the ARPC-coated, PEM-supported membrane-mimetic film on the interior of the vascular graft after irradiation by 514.5 nm radiation (open circles) as well as the polymerization efficiency

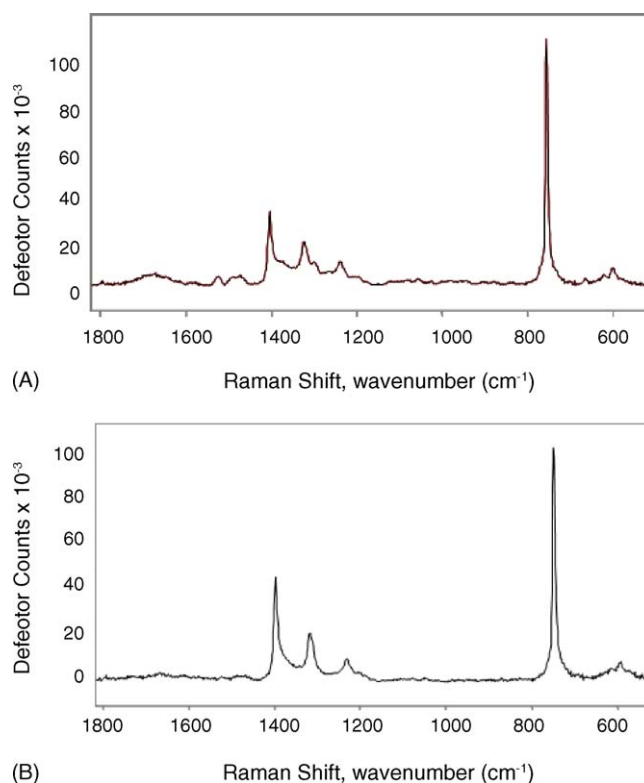


Fig. 7. (A) Near-IR Raman spectrum of PTFE graft prior to irradiation. (B) Near-IR Raman spectrum of PTFE graft after samples were irradiated using 514.5 nm light for 60 s at ~ 100 mW power.

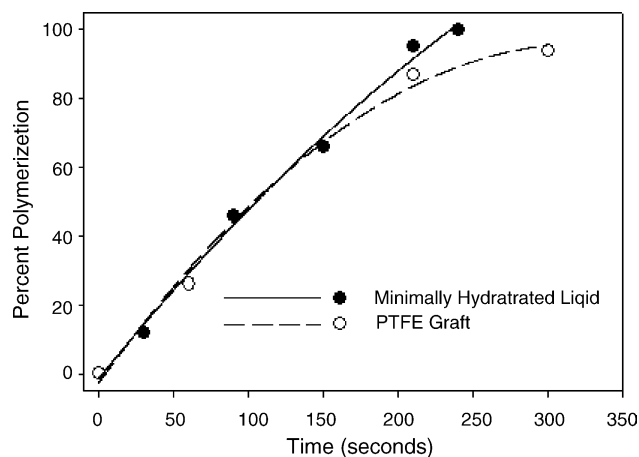


Fig. 8. Percent polymerization comparison between minimally hydrated monoacrylate-PC samples and PTFE graft samples for given irradiation periods measured by Raman microscopy. The values indicated by squares represent the PTFE graft samples.

values obtained from near-IR Raman microscopy of the minimally hydrated ARPC lipid model systems (filled circles). It is clear from this figure that the in situ ARPC lipid adsorbed onto a membrane-mimetic PEM film achieves a polymerization efficiency of greater than 90%, with nearly identical irradiation efficiencies as that of the simplified planar model system.

4. Conclusions

We have demonstrated that near-IR Raman microscopy is a viable technique for the in situ analysis of photopolymerization efficiency in membrane-mimetic lipid assemblies coated onto the interior surfaces of PTFE vascular grafts. While IR spectroscopy has long been the spectroscopic method of choice for the analysis of polymer cross-linking, practical difficulties exist in the use of IR for the in situ analysis of photoinitiated polymer cross-linking on biomaterial surfaces. The results presented here established that near-IR Raman microscopy produced equivalent results to standard IR methods for quantitating photopolymerization in simplified polymerizable lipid systems. The use of a visible laser as the excitation source for photoinitiated polymerization reduced the time needed for complete polymerization of the membrane-mimetic film to less than 300 s. Significantly, it was possible to extend the near-IR Raman method to the in situ analysis of polymerization efficiency in a stabilized lipid membrane on a chemically complex polyelectrolyte multilayer film. This work describes a new approach for analyzing polymerizable coatings on biomaterial surfaces, and may improve our ability to predict the long-term stability of polymeric thin films on implantable medical devices.

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References

- [1] K.G. Marra, T.M. Winger, S.R. Hanson, E.L. Chaikof, *Macromolecules* 30 (1997) 6483.
- [2] K.G. Marra, D.D.A. Kidani, E.L. Chaikof, *Langmuir* 13 (1997) 5697.
- [3] J.H. Chon, K.G. Marra, E.L. Chaikof, *J. Biomater. Sci. Polym. Ed.* 10 (1999) 95.
- [4] J.M. Orban, K.M. Faucher, R.A. Dluhy, E.L. Chaikof, *Macromolecules* 33 (2000) 4205.
- [5] J. Feng, P.-Y. Tseng, K. Faucher, J.M. Orban, X.-L. Sun, E.L. Chaikof, *Langmuir* 18 (2002) 9907.
- [6] H. Liu, K.M. Faucher, X.L. Sun, J. Feng, T.L. Johnson, J.M. Orban, R.P. Apkarian, R.A. Dluhy, E.L. Chaikof, *Langmuir* 18 (2002) 1332.
- [7] J.A. Hayward, D. Chapman, *Biomaterials* 5 (1984) 135.
- [8] B. Hall, R.L. Bird, M. Kojima, D. Chapman, *Biomaterials* 10 (1989) 219.
- [9] K. Ishihara, T. Tsuji, T. Kurosaki, N. Nakabayashi, *J. Biomed. Mater. Res.* 28 (1994) 225.
- [10] E. Sackmann, *Science* 271 (1996) 43.
- [11] K. Katagiri, F. Caruso, *Macromolecules* 37 (2004) 9947.
- [12] K. Katagiri, F. Caruso, *Adv. Mater.* 17 (2005) 738.
- [13] U.Y. Wang, M. Schonhoff, H. Möhwald, *J. Phys. Chem. B* 108 (2004) 4767.
- [14] S. Moya, W. Richter, S. Leporatti, H.B. Baumier, E. Donath, *Biomacromolecules* 4 (2003) 808.
- [15] R. Georgieva, S. Moya, E. Donath, H.B. Baumier, *Langmuir* 20 (2004) 1895.
- [16] T. Kunitake, in: A. Baszkin, W. Norde (Eds.), *Physical Chemistry of Biological Interfaces*, Marcel Dekker, Inc., New York, 2000, p. 283.
- [17] H. Ringsdorf, in: A. Baszkin, W. Norde (Eds.), *Physical Chemistry of Biological Interfaces*, Marcel Dekker, Inc., New York, 2000, p. 243.
- [18] A.W. Mauk, E.L. Chaikof, P.J. Ludovice, *Langmuir* 14 (1998) 5255.
- [19] T.M. Winger, E.L. Chaikof, *Langmuir* 14 (1998) 4148.
- [20] T.M. Winger, P.J. Ludovice, E.L. Chaikof, *Langmuir* 15 (1999) 3866.
- [21] C.T. Sanderson, B.J. Palmer, A. Morgan, M. Murphy, R.A. Dluhy, T. Mize, I.J. Amster, C. Kutal, *Macromolecules* 35 (2002) 9648.
- [22] N.S. Allen, S.J. Hardy, A.F. Jacobine, D.M. Glaser, B. Yang, D. Wolf, *Eur. Polym. J.* 26 (1990) 1041.
- [23] N.S. Allen, S.J. Hardy, A.F. Jacobine, D.M. Glaser, B. Yang, D. Wolf, F. Catalina, S. Navaratnam, B.J. Parsons, *J. Appl. Polym. Sci.* 42 (1991) 1169.
- [24] D.B. Yang, *J. Polym. Sci. A* 31 (1993) 199.
- [25] D. Steele, in: J.M. Chalmers, P.R. Griffiths (Eds.), *Handbook of Vibrational Spectroscopy*, John Wiley & Sons, Chichester, 2002, p. 44.
- [26] D.A. Long, *The Raman Effect*, John Wiley & Sons, Chichester, 2002.
- [27] G. Cevc, D. Marsh, *Phospholipid Bilayers*, Wiley-Interscience, Toronto, 1987, p. 57.
- [28] J.H. Chon, K.G. Marra, E.L. Chaikof, *J. Biomater. Sci. Polym. Ed.* 10 (1999) 95.
- [29] P.R. Carey, *Biochemical Applications of Raman and Resonance Raman Spectroscopies*, Academic Press, New York, 1982.
- [30] O. Valdes-Aguilera, C. Pathak, J. Shi, D. Watson, D. Neckers, *Macromolecules* 25 (1992) 541.
- [31] C.P. Pathak, A.S. Sawhney, J.A. Hubbell, *J. Am. Chem. Soc.* 114 (1992) 8311.
- [32] G.M. Cruise, O.D. Hegre, D.S. Scharp, J.A. Hubbell, *Biotechnol. Bioeng.* 57 (1998) 655.
- [33] P. Plaza, N.Q. Dao, M. Jouan, H. Fevrier, H. Saisse, *Appl. Opt.* 25 (1986) 3448.
- [34] P.J. Hendra, G. Ellis, D.J. Cutler, *J. Raman Spectrosc.* 19 (1988) 413.
- [35] C.K. Chong, C. Shen, Y. Fong, J. Zhu, F. Yan, S. Brush, C.K. Mann, T.J. Vickers, *Vib. Spectrosc.* 3 (1992) 35.