Improving Blood Compatibility of Natural Rubber by UV-Induced Graft Polymerization of Hydrophilic Monomers

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ABSTRACT: Natural rubber (NR) latex films surface-grafted with hydrophilic monomers, poly(ethylene glycol) methacrylate (PEGMA), *N*-vinylpyrrolidone (VPy), and 2-methacryloyloxyethyl phosphorylcholine (MPC), were prepared by UV-induced graft polymerization using benzophenone as a photosensitizer. The grafting yield increases of vulcanized NR latex films as a function of time and monomer concentration were of lesser magnitude than those of the unvulcanized NR latex films. This can be explained as a result of the crosslinked network generated during vulcanization acting as a barrier to the permeation of the photosensitizer and the monomer. The appearance of a characteristic carbonyl stretching in the attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra of NR latex films after the surface grafting of PEGMA and MPC indicates that the modification has proceeded at least to the sampling depth of ATR-FTIR ($\sim$ 1–2 μm). According to the water contact angle of the modified NR latex films, the surface grafting density became higher as the grafting time and monomer concentration increased. The complete absence of plasma protein adsorption and platelet adhesion on the surface-modified NR latex films having grafting yield above 1 wt % is a strong indication of improved blood compatibility. Results from tensile tests suggest that graft polymerization does not cause adverse effects on the mechanical properties of vulcanized NR latex films. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 112: 208–217, 2009

Key words: biocompatibility; graft copolymers; hydrophilic polymers; rubber; surfaces

INTRODUCTION

The blood compatibility of a material is determined by interactions at the blood-material interface that depend upon the physicochemical features of the material surface. It involves complement activation, platelet adhesion, and stimulation of plasma coagulation. In general, a material surface in contact with blood triggers a number of biological systems through the adsorption of proteins and cells. It is believed that the nature of the adsorbed protein layer determines all adverse events that impair the use of some materials as medical devices.[1,2] To suppress those actions, several means of surface modification have been proposed. Surface grafting or coating of hydrophilic polymers such as poly (acrylamide), poly(*N*,*N*-dimethyl acrylamide), poly (vinyl alcohol), poly(hydroxyethyl methacrylate), poly(ethylene glycol) (PEG), and poly(ethylene oxide) (PEO) onto material surfaces is recognized as a simple and versatile method that can effectively reduce protein adsorption and platelet adhesion.

Among the hydrophilic polymers, PEO and its derivatives are the most well known for this purpose.[3–8] The excluded volume effect and the dynamic motion of the water-soluble PEO chains on the surface are responsible for suppressing protein adsorption and platelet adhesion. Rapidly moving hydrated PEO chains and a large excluded volume induce the microflow of water, tend to repel protein molecules which approach the surface and thus prevent stagnation of the proteins on the surface. The

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repulsive forces due to the adsorbed PEO chains are generated by the loss of possible chain conformations, as the volume available to the adsorbed chains is reduced during their approach to the surfaces. The molecular weight and graft density of PEO are found to remarkably influence the effectiveness of the suppression. Poly(*N*-vinylpyrrolidone) is another hydrophilic polymer that is commonly used for improving the hemocompatibility of materials. Graft polymerization of *N*-vinylpyrrolidone (VPy) was reported on a number of synthetic polymers, such as polysulfone,[9] polypropylene,[10] and polyacrylonitrile,[11] as well as elastomeric materials such as silicone rubber.[12]

The surfaces of phospholipid assemblies, such as liposomes, are known to be quite inert for biological systems and proteins, and cells mildly interact with them. Inspired by the biomembrane surface, which is mainly constructed of neutral phospholipids (phosphorylcholines), MPC polymers were fundamentally designed as biomimetics of the biomembrane structure.[13] Polymers coated with MPC copolymers can effectively reduce protein adhesion and denaturation and inhibited cell adhesion, even when the polymer was in contact with whole blood in the absence of any anticoagulants.[14,15] Graft copolymerization of MPC has also proven successful in improving the blood compatibility of polymer surfaces.[16–19] Unlike PEO, a different phenomenon is inferred for explaining the anticoagulant activity of MPC polymers. It has been demonstrated that MPC polymers assume a hydration state when wet, with free water molecules that are not superficially bonded.[20,21] In general, there is an exchange of water molecules when a protein molecule is adsorbed onto a material surface. The superficially adsorbed protein loses its solvation water in the part that makes contact, inducing a phenomenon of conformational change as the hydrophobic part of the protein is exposed toward the material surface. If the state of a water molecule on the surface of a material is similar to that of an aqueous solution (free water), then the proteins do not need to release their solvation molecules during contact with the surface, and therefore hydrophobic interactions and conformational changes are suppressed.

Natural rubber (NR) possesses excellent elasticity, flexibility, and resistance against splitting. These properties are attractive for a wide range of applications, including medical products that, in principle, can be manufactured from NR latex, such as surgical gloves, tubing, catheters, and balloons.[22] However, the poor blood compatibility of NR in comparison with silicones and polyurethanes hampers its direct use in biomedical applications. This is due in large part to the hydrophobic nature of NR, which can induce irreversible protein adsorption. The first attempt to improve the blood compatibility of NR was reported by Razzak et al.[23,24] Radiation-induced grafting of *N,N*-dimethylacrylamide (DMAA) and *N,N*-dimethylaminoethylacrylate onto a NR tube was successfully achieved. The grafting proceeded most effectively in carbon tetrachloride, the solvent that can attain the most swelling of the NR tube. The grafting yield was dependent on irradiation time, grafting temperature, and monomer concentration. As assessed by *ex vivo* testing, the blood compatibility was determined based on visual observation of the blood clotting in the modified NR tube. A higher grafting yield provided a better blood compatibility. The clotting disappeared when the grafting yield was beyond 30%. Later, they reported the blood compatibility of NR-*g*-DMAA in comparison with silicone rubber (SiR) tubing.[25] According to the *ex vivo* loop test, testing against the circulation of blood flow, the NR-*g*-DMAA exhibited better performance than the ungrafted NR tube and also the SiR tube, even though the grafting yield of NR-*g*-DMAA was $\sim$ 19 wt %. The blood flow through the NR-*g*-DMAA tube continued smoothly for 60 min. However, the blood flow through the ungrafted NR and SiR tubes almost stopped after 40 and 30 min, respectively. They further demonstrated that the peroxidation technique can raise the degree of grafting in radiation copolymerization of DMAA onto NR to up to 42 wt %, which was even higher than for preirradiation or simultaneous peroxidation and preirradiation grafting.[26]

Kang and coworkers[27] used argon plasma treatment followed by UV-induced graft copolymerization of poly(ethylene glycol) methacrylate (PEGMA) to modify NR latex films The grafting yield depended upon the grafting time and concentration of PEGMA. The grafting of PEGMA macromonomers of differing molecular weight was also explored. An NR surface with a high density of grafted PEG was very effective in reducing protein adsorption and platelet adhesion. A lower grafting yield of high-MW PEGMA was more effective than a high grafting yield of low-MW PEGMA in improving blood compatibility.

Although there have been some publications reporting the surface modification of NR by UV-induced graft polymerization, to the best of our knowledge, only one of them was accomplished without plasma pretreatment using a vapor phase process.[27–31] Herein, we alternatively propose to utilize UV-induced graft polymerization of selected hydrophilic monomers, that is, PEGMA, MPC, and VPy, on NR latex films in the presence of benzophenone as a photosensitizer. This approach can simply be conducted in aqueous solution, therefore it is an environmentally friendly method. It is postulated that the absence of the plasma pretreatment should

minimize the surface damage to NR, which might cause a weak boundary layer on the NR surface prior to the grafting step. Comparative studies are carried out on both unvulcanized and vulcanized NR latex films. Results from blood compatibility studies of surface-modified NR latex films are addressed in terms of plasma protein adsorption and platelet adhesion. To assure that the mechanical integrity of NR is still preserved after graft polymerization, selected modified NR latex films are subjected to tensile tests.

EXPERIMENTAL

Materials

High ammonium natural rubber latex (HANR, 60% dry rubber content) was supplied by the Thai Rubber Latex Public, Chonburi (Thailand). All curing reagents, 50% sulfur dispersion, 50% zinc oxide dispersion, 50% zinc diethyldithiocarbamate dispersion, potassium laurate, and potassium oxide, were of reagent grade and supplied by the Kijphaiboon (Thailand). PEGMA (M_w = 360), VPy, bovine serum albumin (BSA), phosphate buffer solution (PBS), and 50% glutaraldehyde were purchased from Aldrich (USA) and used as received. MPC was purchased from the NOE, Japan and was used as received. Sodium dodecyl sulfate (SDS) and benzophenone were purchased from Fluka (Switzerland). Acetone, methanol, and ethanol were of analytical grade and purchased from Merck (Germany). A bicinchoninic acid assay kit (QuantiPro™ BCA assay) was purchased from Sigma Chemical (USA). Platelet-poor plasma (PPP) and platelet-rich plasma (PRP) were supplied by the Thai Red Cross Society (Thailand).

Preparation of NR latex films

Unvulcanized NR latex films were cast directly from HANR and dried in the dark for 3–4 days at ambient temperature. Vulcanized NR latex films were prepared by the accelerated-sulfur vulcanization process at 60°C. The curing formula contains the following reagents: 0.90 phr of 50% sulfur dispersion, 1.20 phr of 50% zinc oxide dispersion, 1.20 phr of 50% zinc diethyldithiocarbamate dispersion, 0.75 phr of 20% potassium laurate, and 3.00 phr of 10% potassium oxide in proportion to 100 phr HANR. The cast NR latex films were dried in the dark for 7–10 days at ambient temperature.

Surface graft polymerization of hydrophilic monomers onto NR latex films

NR latex films were washed for 30 min each with hot deionized water, methanol, and acetone in an ultrasonic bath. The NR latex films were immersed in 1% (w/v) benzophenone in acetone for a desired period of time and then dried in the dark under vacuum for 2 h. The NR latex films having absorbed benzophenone were immersed in a quartz tube containing 25 mL monomer solution in degassed deionized water. Degassing was done by boiling the deionized water for a few minutes prior to bubbling with nitrogen gas until the water was cooled down to ambient temperature. The quartz tube was then capped with a septum before exposure to UV light with $\lambda = 350 \pm 50$ nm, 500 W at 60°C. The nitrogen gas was bubbled into the monomer solution throughout the course of the reaction. After a desired reaction time, the NR latex films were stirred in water overnight and then soaked for 1 h and rinsed thoroughly with hot ethanol (50°C) to remove benzophenone and the ungrafted homopolymer before being dried under vacuum overnight. The grafting yield was calculated from the following equation:

$$\text{Grafting yield } (\%) = \frac{\text{Weight of NR}_{\text{after grafting}} - \text{Weight of NR}_{\text{before grafting}}}{\text{Weight of NR}_{\text{before grafting}}} \times 100$$

Attenuated total reflectance-Fourier transform infrared spectroscopy

All spectra were collected at a resolution of 4 cm^{-1} and for 64 scans using a Nicolet Magna 750 series II FTIR spectrometer (USA) equipped with a DTGS detector. A horizontal plate attenuated total reflectance (ATR; Spectra Tech, USA) accessory with a 45° germanium (Ge) prism was employed for all ATR spectral acquisitions.

Contact angle measurements

A droplet of deionized water was placed on the tested surface by bringing the surface into contact with a droplet suspended from a Gilmont syringe with a 24-gauge flat-tipped needle. Images of water droplets on the surface were taken with a digital camera (Sony, Model F707, Japan). The droplet images were processed with Adobe Photoshop 6.0 software to obtain contact angle data.

Determination of benzophenone residue in NR latex films

NR latex films of dimensions 1.0 × 1.0 cm^2 were put in ethanol in an ultrasonic bath for 30 min and further soaked overnight in ethanol. The absorbance of the ethanol solution was measured at 251 nm by UV spectroscopy (Techne, Specgene, England). The amount of dissolved benzophenone was calculated

from the benzophenone concentration in the ethanol solution. The data are expressed as mean ± standard deviation (SD).

Determination of total amount of adsorbed human plasma protein

The controlled and modified NR latex films of dimensions 1.0 × 1.0 cm^2 were placed into a 24-well tissue culture plate containing deionized water. The samples were allowed to stand in the wells overnight to reach an equilibrium hydration. Each sample was removed from deionized water and suspended in a well containing 2.0 mL PPP before incubation at 37°C for 3 h. Three samples were analyzed for each condition. The samples were removed from PPP and rinsed thoroughly with PBS (2×) to remove any loosely attached protein. The adsorbed protein on the sample surface was detached by soaking each film in 2.0 mL of 1% aqueous solution of SDS for 30 min. A protein analysis kit based on the BCA method was used to determine the concentration of the protein dissolved in the SDS solution. The 100 μL (0.1 mL) of SDS solution that soaked each sample was added into a well of a 96-well tissue culture plate. A 100 μL of BCA working solution was then added to each well. The well-plate was incubated at 37°C for 2 h. The absorbance of the solution was measured at 562 nm by UV–vis spectroscopy (Microtiter plate reader; model Sunrise, Tecan, Austria). The amount of protein adsorbed on each sample was calculated from the protein concentration in the SDS solution. The data are expressed as mean ± SD for each condition.

Evaluation of platelet adhesion

The controlled and modified NR latex films of dimensions 1.0 × 1.0 cm^2 were placed into a 24-well tissue culture plate containing PBS. The samples were allowed to stand in the wells overnight to reach an equilibrium hydration. Then 2.0 mL PRP was added into each well via a micropipet. The well plate was incubated for 1 h at 37°C. After the PRP was removed, the substrates were rinsed with PBS (3×). The saline solution containing 2.5% (v/v) glutaraldehyde was added to each well to fix the platelets adhered on the sample surface. The samples were rinsed with PBS (3×) followed by deionized water (3×) prior to dehydration by sequentially soaking in 30, 50, 70, 90, 99, and 100% (v/v) ethanol in water for intervals of 10 min each. The samples were dried under vacuum for 24 h, and then sputtered with gold before being analyzed by scanning electron microscopy (SEM; JEOL Model JSM-5800L, Japan).

Tensile stress–strain properties

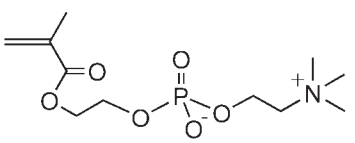
The mechanical properties of vulcanized rubber were tested according to ASTM D 412 using Tensile Machine model Lloyd 468 K. The vulcanized NR sheets were cut with a compressed air sample cutter (Model SDAP-100-N) using a dumbbell (Type IV). The stress-strain curve of vulcanized NR in the form of dumbbell test pieces was measured before and after graft polymerization using the following conditions: 25°C, 60% relative humidity, 10.00 pts/s sample rate, and 500 mm/min crosshead speed.

RESULTS AND DISCUSSION

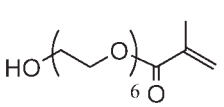
Graft polymerization

In this particular study, photo-induced graft polymerization in the presence of benzophenone, a well-known photosensitizer, was selected as an approach for grafting hydrophilic monomers onto NR latex films. The films were soaked in the solution containing benzophenone to allow the photosensitizer to permeate into the substrate before starting the graft polymerization process. The pretreatment of the substrate with the photosensitizer has been proven to be an effective way to enhance grafting efficiency as opposed to the incorporation of benzophenone together with the monomer in solution.[32] After being irradiated, benzophenones are excited to the triplet state that can abstract hydrogen atoms from polyisoprene of NR and subsequently yield polyisoprene radicals capable of initiating graft polymerization of hydrophilic monomers (Fig. 1). General mechanism in the step of initiation and propagation of polymerization of vinyl monomer sensitized by benzophenone is schematically shown in Figure 2.

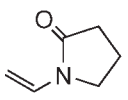
For a preliminary determination of the effect of polymerization parameters on the extent of graft polymerization, PEGMA was chosen as a representative monomer. The average grafting yield as well as the water contact angle of NR latex films as a



2-methacryloyloxyethyl phosphorylcholine (MPC)



poly(ethylene glycol)methacrylate (PEGMA) (MW=360)



N-vinylpyrrolidone (VPy)

Figure 1 Chemical structures of hydrophilic monomers used for graft polymerization.

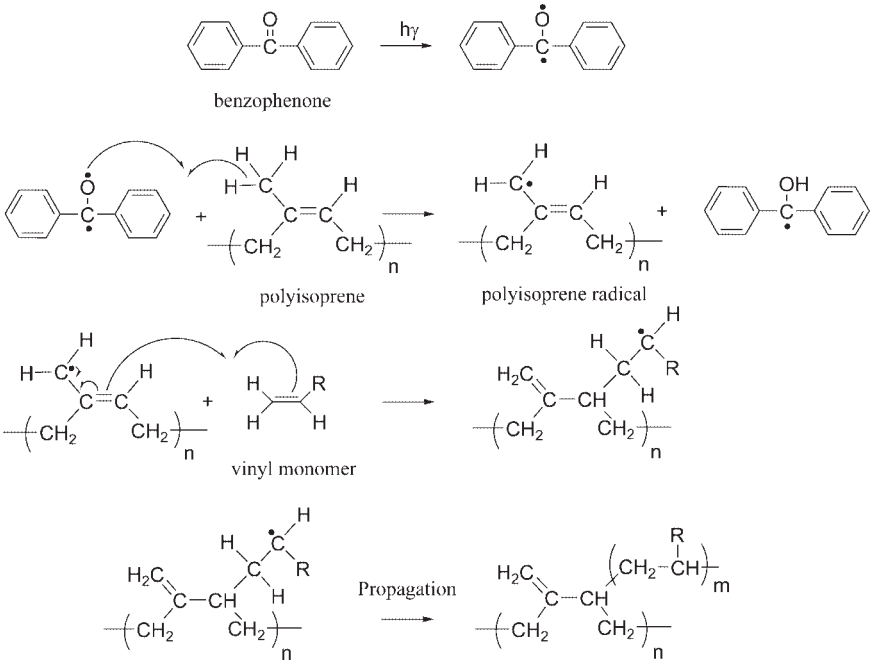


Figure 2 General mechanism for graft polymerization of vinyl monomer on polyisoprene photosensitized by benzophenone.

function of time using 0.5*M* PEGMA are plotted together in Figure 3. Apparently, the grafting yield increased as a function of grafting time and did not rise much higher after 120 min. The fact that the grafting yield of the vulcanized NR latex film was lower in magnitude than that of the unvulcanized NR latex film implies that the crosslinked network of vulcanized NR inhibits the permeation of benzophenone and the monomer and thus suppresses the extent of grafting. Similar trends were also observed for graft polymerization of VPy and MPC (data not shown). Using the grafting time of 150 min, the grafting yields of vulcanized NR latex films of ~ 0.85, 0.19, and 0.99 wt % were obtained for PEGMA, VPy, and MPC, respectively. We attribute the relatively low grafting of VPy (<0.5 wt %) to its radical being less reactive than other two methacrylate radicals derived from PEGMA and MPC, possibly due to stabilization by lone-pair electrons on the nitrogen atom of the pyrrolidone ring.

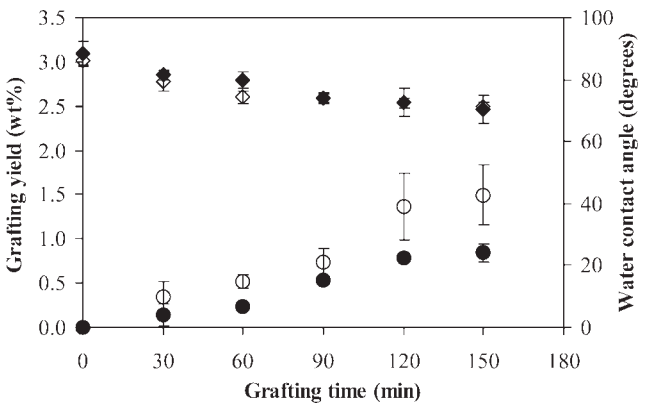


Figure 3 Grafting yield [unvulcanized (○), vulcanized (●)] and water contact angle [unvulcanized (◇), vulcanized (◆)] of NR latex films after graft polymerization with 0.5*M* PEGMA as a function of grafting time.

Water contact angle data for NR-*g*-PEGMA shown in Figure 3 revealed that the surface of modified NR latex film became more hydrophilic as the grafting yield increased for both unvulcanized and vulcanized NR latex films. Because the sampling depth of the contact angle is generally in the range of a few angstroms, the contact angle of a modified surface should mainly be dependent on the surface grafting density, not the overall grafting yield. The declining trend of water contact angle tended to level off at 120 min, suggesting that the surface grafting saturated. The fact that the contact angles of the vulcanized and unvulcanized NR latex films were indistinguishable after being graft polymerized for 90–150 min implies that both surface-modified NR latex films possessed the same surface grafting density in spite of their different overall grafting yields. According to Figure 4, the grafting yield could also be elevated as the concentration of PEGMA was raised. Once again, the contact angle of the unvulcanized NR latex film is almost superimposed on that of the vulcanized NR latex film, regardless of their different grafting yields.

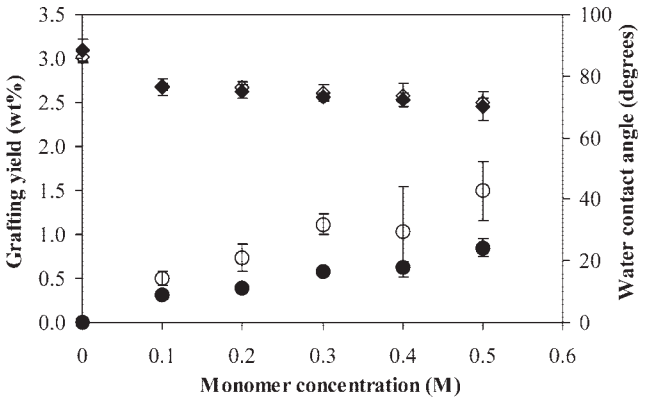


Figure 4 Grafting yield [unvulcanized (○), vulcanized (●)] and water contact angle [unvulcanized (◇), vulcanized (◆)] of NR latex films after graft polymerization for 150 min as a function of PEGMA concentration.

The success of graft polymerization was also confirmed by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) analysis. Figures 5 and 6 show ATR-FTIR spectra of unvulcanized and vulcanized NR latex films, respectively, before and after graft polymerization. The appearance of a characteristic C=O stretching at 1720 cm^{-1} in the spectra of both vulcanized and unvulcanized NR latex films after surface grafting indicated that the graft polymerization of PEGMA proceeded to the depth of at least 1 μm (the sampling depth of ATR-FTIR analysis) from the top surface of both NR substrates. The lower intensity of carbonyl stretching of vulcanized NR latex films in comparison with that for unvulcanized NR films corresponded well with the extent of grafting deduced from the grafting yield. As a consequence of its low grafting yield and perhaps very thin modified layer, the characteristic carbonyl stretching was not detected in the spectra of either vulcanized or unvulcanized NR

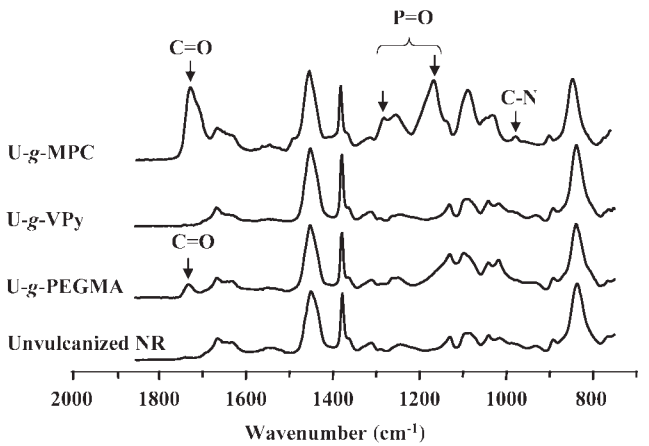


Figure 5 ATR-FTIR spectra of unvulcanized NR (U) latex films before and after graft polymerization with 0.5*M* PEGMA, VPy, and MPC for 150 min.

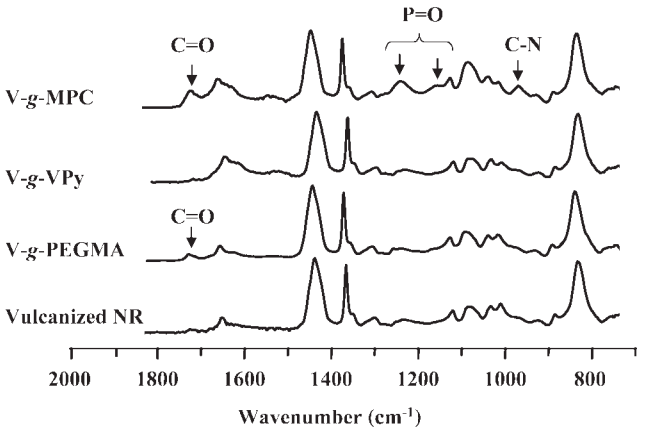


Figure 6 ATR-FTIR spectra of vulcanized NR (V) latex films before and after graft polymerization with 0.5*M* PEGMA, VPy, and MPC for 150 min.

latex films after graft polymerization with VPy. This observation suggests that the ATR-FTIR analysis is not sensitive enough to recognize such small extent of surface grafting. For the NR latex films graft polymerized with MPC, the P=O stretching due to the phosphate group at 1150 and 1240 cm^{-1}, and C—N stretching at 970 cm^{-1} due to the ammonium group ($N^+(CH_3)_3$) of MPC were noticed in addition to the C=O stretching at 1720 cm^{-1}, in both vulcanized and unvulcanized NR latex films. This result also confirms the success of graft polymerization of MPC and that the modification proceeded to the depth of the ATR-FTIR analysis.

For medical applications, it is necessary to assure that there is no photosensitizer, initially incorporated during graft polymerization, that is trapped inside and/or leaching out from the surface-modified NR latex film afterwards. The quantity of benzophenone can be determined by measuring the UV absorbance of the ethanolic solution after soaking the NR latex films before and after graft polymerization. The amount of benzophenone adsorbed and/or absorbed by the unvulcanized and vulcanized NR latex films was ~ 30 and 45 μg/cm^2, respectively. After graft polymerization, the amount of dissolved benzophenone was below 5 μg/cm^2, which indicated that most of the benzophenone was consumed after graft polymerization. It should be noted that benzophenone was no longer detected after the modified NR latex films were stirred in water overnight and rinsed thoroughly with hot ethanol (50°C).

Blood compatibility

The amount of plasma protein adsorbed on a material surface is a primary factor in evaluating the blood compatibility of the material. When the material is in contact with blood, a surface-induced thrombosis is initiated by the adsorption of plasma

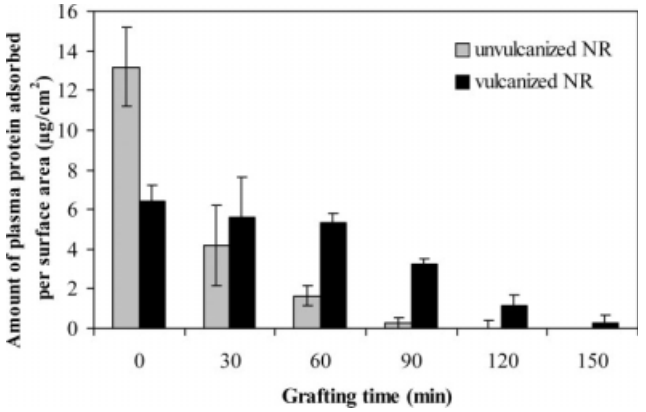


Figure 7 Amount of plasma protein adsorbed per surface area of NR latex films before and after graft polymerization with 0.5*M* PEGMA as a function of grafting time.

TABLE I
Grafting Yields and Water Contact Angles of Surface-Modified NR Latex Films Subjected to Comparative Plasma Protein Adsorption Study

Monomer	NR latex film	Grafting yield (wt %)	Water contact angle (degrees)
Virgin NR	Unvulcanized	–	86.2 ± 1.8
	Vulcanized	–	88.5 ± 3.7
PEGMA	Unvulcanized	1.50 ± 0.34	72.5 ± 4.5
	Vulcanized	0.85 ± 0.10	72.5 ± 1.6
VPy	Unvulcanized	0.35 ± 0.05	75.2 ± 1.8
	Vulcanized	0.19 ± 0.09	77.9 ± 1.5
MPC	Unvulcanized	1.67 ± 0.13	68.4 ± 1.13
	Vulcanized	0.99 ± 0.01	68.9 ± 0.61

proteins, followed by the adhesion and activation of platelets. As analyzed by immunogold assay, Ishihara et al.[16] have demonstrated that the adsorption of two human plasma proteins namely fibrinogen and γ-globulin were significantly suppressed on polyethylene membranes after being surface-modified by graft polymerization of PEGMA, VPy, MPC, and acrylamide (AAm). To mimic the actual physiological environment of blood which simultaneously involves many proteins, we desired to determine the adsorption of proteins of the modified NR latex films from PPP, which was directly separated from real blood. It is believed that the results should provide first-hand information indicating whether the surface-modified NR latex films can be used for real applications. Figures 7 and 8 illustrate the amount of plasma protein adsorbed on NR latex films after graft polymerization with PEGMA as a function of time and monomer concentration, respectively. The amount of plasma protein adsorbed was drastically decreased after surface grafting, especially in the case of unvulcanized NR film. The reduction of protein adsorption strongly depended on the grafting yield and surface hydrophilicity, which can be varied as a function of grafting time and monomer concentration. It seems that a complete absence of protein adsorption on NR latex films required as high as 1 wt % grafting yield (see Figs. 3 and 4 for grafting yield data). As a result of the lower grafting yield, the depletion of protein adsorption of the modified vulcanized NR latex films was not as effective as that of the unvulcanized NR latex films using the same grafting time and concentration. Notably, there was twice as much protein adsorbed on the unvulcanized NR latex film as on the vulcanized NR latex film before graft polymerization. Two reasons may account for this outcome: (1) the plasma protein is not only adsorbed but also absorbed by the unvulcanized NR latex film, whose crosslinking density is rather low in comparison with the vulcanized NR latex film. It is this same characteristic that permits the surface grafting of the unvulcanized NR latex film to proceed more efficiently than that of the

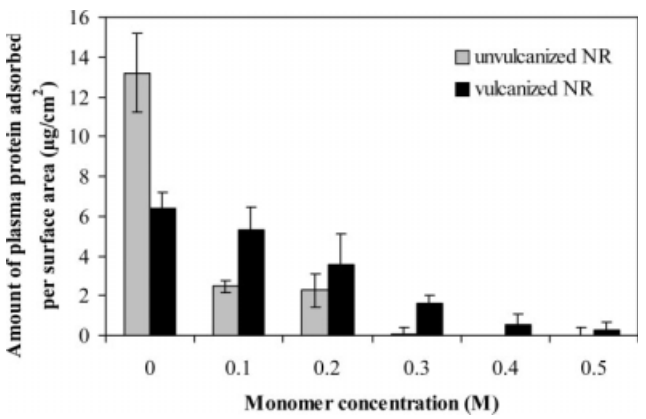


Figure 8 Amount of plasma protein adsorbed per surface area of NR latex films before and after graft polymerization for 150 min as a function of PEGMA concentration.

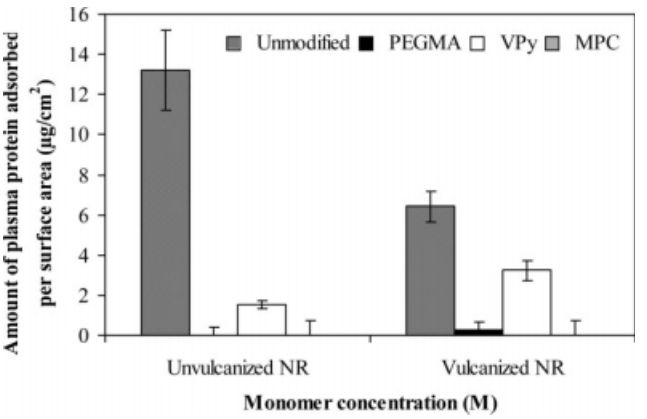


Figure 9 Amount of plasma protein adsorbed per surface area of NR latex films before and after graft polymerization with 0.5*M* PEGMA, VPy, and MPC for 150 min.

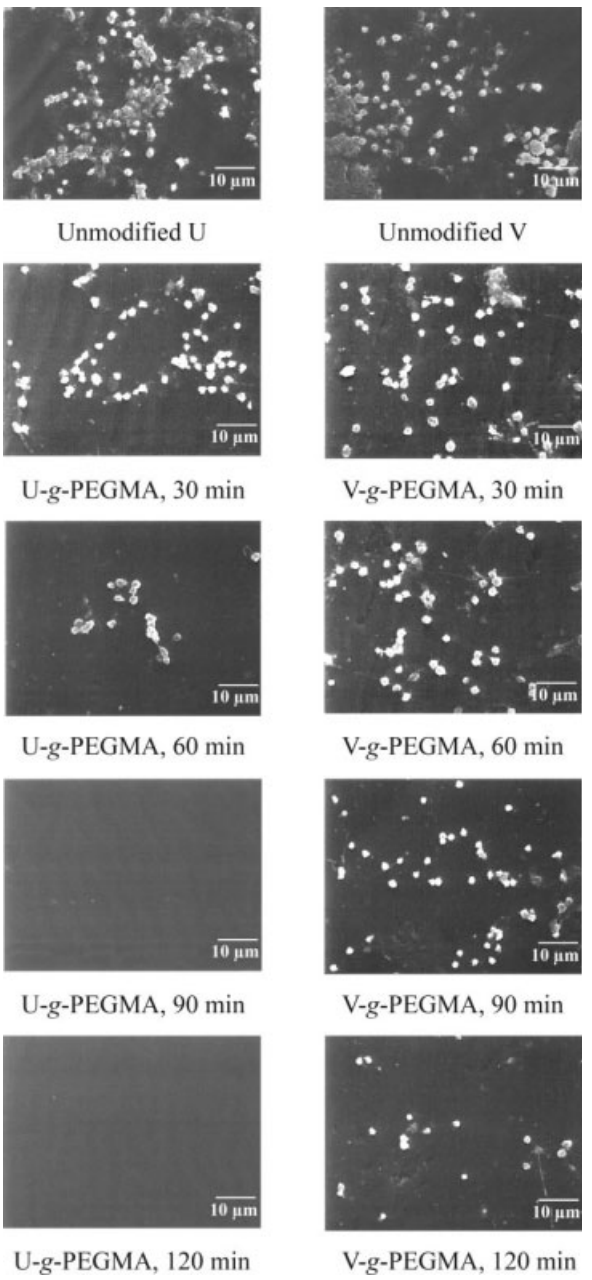


Figure 10 SEM micrographs of unvulcanized NR (U) and vulcanized NR (V) latex films before and after graft polymerization with 0.5*M* PEGMA as a function of grafting time after contacting with PRP.

vulcanized NR latex film at the same grafting condition. (2) It is also possible that the native water-soluble proteins belonging to the virgin NR latex, which cannot be completely removed by the rinsing process, diffuse out, particularly from the unvulcanized NR latex film. If that is the case, the diffusion should be somewhat inhibited by the crosslinked layer of the vulcanized NR latex film.

For comparison, plasma protein adsorption of NR latex films graft polymerized by VPy and MPC was also investigated. The monomer concentration of 0.5*M* and the grafting time of 150 min, which resulted in the highest grafting yield, were chosen as the conditions for graft polymerization. Grafting yields and contact angles of the surface-modified NR latex films are listed in Table I. Plasma protein adsorption data are displayed in Figure 9. As expected, NR-*g*-VPy, having a relatively low grafting yield (<0.5 wt %) in comparison with NR-*g*-PEGMA and NR-*g*-MPC, was not able to entirely prevent the plasma protein adsorption. This supports the previous speculation that a certain percentage of grafting

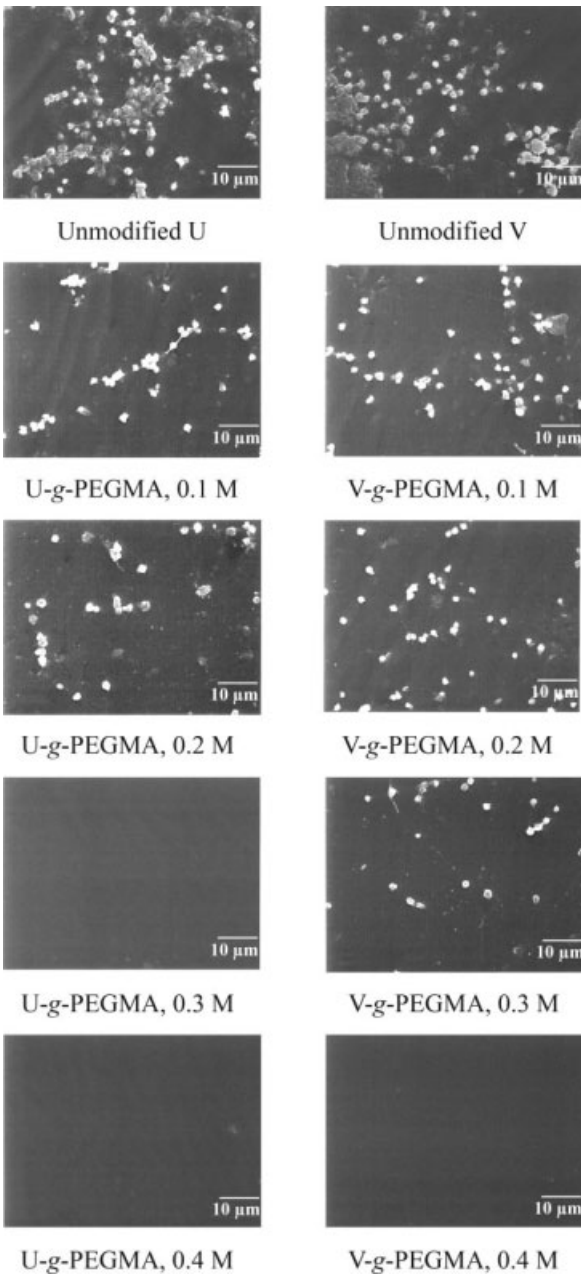


Figure 11 SEM micrographs of unvulcanized NR (U) and vulcanized NR (V) latex films before and after graft polymerization for 150 min as a function of PEGMA concentration after contacting with PRP.

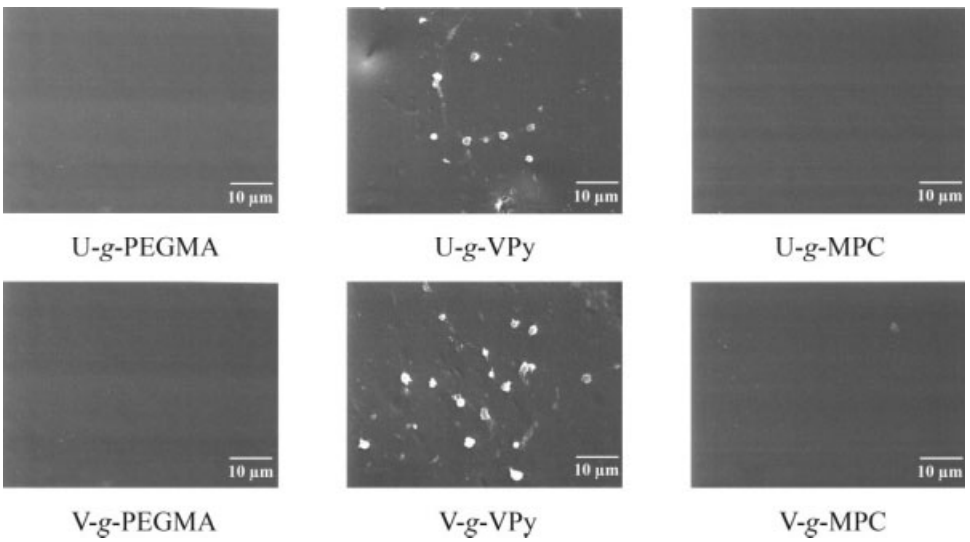


Figure 12 SEM micrographs of unvulcanized NR (U) and vulcanized NR (V) latex films before and after graft polymerization with 0.5*M* PEGMA, VPy, and MPC for 150 min after contacting with PRP.

yield (at least 1 wt %) is indispensable for achieving absolute antithrombogenicity.

It is well known that platelets also contribute to the thrombus formation. In general, a foreign substrate induces adhesion and activation of platelets with the adsorbed protein layer serving as a controlling factor of the platelet response. The extent of platelet adhesion on the modified NR latex films was monitored by scanning electron microscopy. Figures 10 and 11 show SEM micrographs displaying the responses to platelet adhesion on unvulcanized and vulcanized NR latex films, respectively, after exposure to PRP. There are a large number of platelets attached to the unmodified NR latex films indicating their poor blood compatibility. Fewer platelets were able to adhere to the surface of the modified NR latex films as the grafting yield increased, as a function of both grafting time and monomer concentration. The decreasing platelet adhesion truly reflects the improvement in blood compatibility of NR latex films. Figure 12 shows the comparative results of platelet adhesion on NR latex films after graft polymerization with 0.5*M* PEGMA, VPy and MPC for 150 min. PEGMA and MPC are undoubtedly more suitable monomers to be utilized for graft polymerization to improve the blood compatibility of NR latex films than VPy. In particular, MPC seems to be the most effective choice, considering that the highest grafting yield and enhanced hydrophilicity were obtained under the same grafting conditions. The results well complement the protein adsorption data described in the preceding section. With regard to the work formerly reported by Yoda[22] and Razzak et al.,[23–25] this present work has demonstrated that the high grafting yield is unnecessary and as low as 1 wt % is sufficient to sustain remarkable improvement in hemocompatibility of NR latex films.

Mechanical properties

To examine the feasibility of the graft polymerization process for practical purposes, the mechanical

TABLE II
Tensile Strength, Elongation at Break, and Modulus of Vulcanized NR Latex Films Before and After Graft Polymerization with 0.5*M* PEGMA

Sample	Grafting yield (wt %)	Tensile strength (MPa)	Elongation at break (%)	Tensile modulus (M300) (MPa)
Virgin NR	–	19.84 ± 4.00	1049.60 ± 91.23	0.79 ± 0.17
Controlled NR[a]	–	19.88 ± 2.33	1057.70 ± 60.87	0.77 ± 0.11
NR-*g*-PEGMA, 60 min grafting	0.16 ± 0.07	25.31 ± 2.25	1123.60 ± 91.04	0.77 ± 0.06
NR-*g*-PEGMA, 120 min grafting	0.79 ± 0.30	32.68 ± 3.42	1134.58 ± 62.67	1.13 ± 0.21
NR-*g*-PEGMA, 150 min grafting	0.85 ± 0.10	37.30 ± 1.39	1146.80 ± 41.87	1.34 ± 0.04

[a] NR latex films were subjected to benzophenone soaking and UV irradiation for 150 min.

properties of a selected series of modified NR latex films, PEGMA-*g*-vulcanized NR latex films, were investigated. As outlined in Table II, it can be realized that the graft polymerization unexpectedly caused a significant increase of vulcanized NR tensile strength, which increased proportionally with increasing grafting time and yield. Because UV exposure alone did not affect the tensile strength of the vulcanized NR latex film (see controlled NR latex film), the elevation of tensile strength should originate from the existence of the surface modified layer, not by the extra crosslinking within the NR latex film possibly induced during UV exposure. Slight increases of elongation at break and modulus were also evidenced as a consequence of surface grafting. Nonetheless, the overall tensile properties suggest that UV exposure and grafting do not cause adverse effects on mechanical properties of NR latex films. UV-induced graft polymerization is therefore a feasible method that can be used to improve the blood compatibility of NR latex films.

CONCLUSIONS

The efficiency of graft polymerization of the hydrophilic monomers PEGMA, VPy, and MPC on NR latex films was influenced by grafting time and monomer concentration. In general, a higher concentration and a longer grafting time led to a higher grafting yield and surface hydrophilicity. Under the same conditions, the grafting yields of vulcanized NR latex films were lower than those of the unvulcanized NR latex films, mainly due to the crosslinked network generated after vulcanization blocking the permeation of the photosensitizer as well as the monomer. The success of surface grafting was also confirmed by ATR-FTIR analysis. Among the three monomers used in this study, VPy is inferior to PEGMA and MPC. This is ascribed to its lower reactivity towards graft polymerization that led to a small extent of surface grafting and only a slight improvement of hemocompatibility. The complete absence of plasma protein adsorption and platelet adhesion on the surface-grafted NR latex films having more than 1 wt % grafting yield strongly suggested that blood compatibility of NR latex films can be significantly improved. Although additional tensile strength was introduced to the NR latex films after graft polymerization, the overall mechanical properties were not adversely affected, implying that the grafting is truly confined to the surface. This study has demonstrated the potential of UV-induced graft polymerization of hydrophilic monomers as an effective and versatile method for improving the blood compatibility of NR.

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