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Grafting of *N*-vinyl caprolactam and methacrylic acid onto silicone rubber films for drug-eluting products

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ABSTRACT: Silicone rubber (SR), a material widely used in the biomedical field, was modified with stimuli-responsive poly(*N*-vinyl caprolactam) (PVCL) and poly(methacrylic acid) (PMAA) with the aim of improving its ability to host drug molecules. The grafting of PVCL and PMAA onto SR was carried out by means of a γ-ray preirradiation method, and the dependence of the grafting yield on the comonomer concentration, preirradiation dose, temperature, and reaction time was evaluated. Modified SR films were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry, thermogravimetric analysis, and swelling studies to confirm the grafting of the copolymer. The SR-*g*-[vinyl caprolactam (VCL)/methacrylic acid (MAA)] copolymers showed a sensitivity to the temperature and pH, high hemocompatibility, and low affinity to bovine serum albumin and fibrinogen proteins. Moreover, the SR-*g*-(VCL/MAA) copolymers were able to host some nonsteroidal anti-inflammatory drugs, such as diclofenac and ibuprofen, and the antifungal agent nystatin. The graft copolymer was shown to be useful for providing sustained release for several hours; this indicates that the modified SR is a promising material for drug-eluting medical devices. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41855.

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INTRODUCTION

Recent years have witnessed a remarkable growth in the development of stimuli-responsive materials for innovative applications in tissue engineering and drug delivery and, more recently, in the preparation of drug–device combination products.[1–5] Drug-eluting medical devices, also called drug–device combo products, notably improve the efficacy and safety of the treatments as each of these devices can deliver the active substance at the needed place for the right time. The drug may synergistically act on the performance and extend the lifespan of the implantable/insertable device, preventing adverse foreign-body reactions (e.g., an inflammatory response, which may result in encapsulation by fibrosis) and other side effects derived from the adherence of host proteins and cells or the proliferation of microorganisms.[5–8] Thus, drug-eluting medical devices may help to overcome device-related complications that are refractory to conventional systemic drug administration.[4]

Most current drug-eluting devices passively control the release; that is, the rate is governed by the dissolution or diffusion through the device itself when the drug is incorporated in the bulk of the material during its fabrication (compounding), in a later step (presoaking), or through inert or erodible polymer coatings to which the drug has been previously added.[9–12] Nevertheless, the active control of both the loading and release kinetics is attracting growing attention, and responsive brushes and networks grafted onto devices seem to be suitable tools for such a purpose.[13–18] Among other suitable techniques, graft polymerization induced by γ-ray irradiation is advantageous as it does not require chemical initiators or catalysts and can start from a variety of monomers or prepolymers with different functionalities to fulfill specific requirements to cover a large surface in a short time.[1,19–21] This technique is based on the generation of free radicals onto a polymeric matrix by the action of ionizing radiation; this is followed by the graft polymerization of various monomers (acrylamides, acrylates, vinyl pyridines, etc.). Micrometer-sized brushes or networks formed on the surface

Additional Supporting Information may be found in the online version of this article.

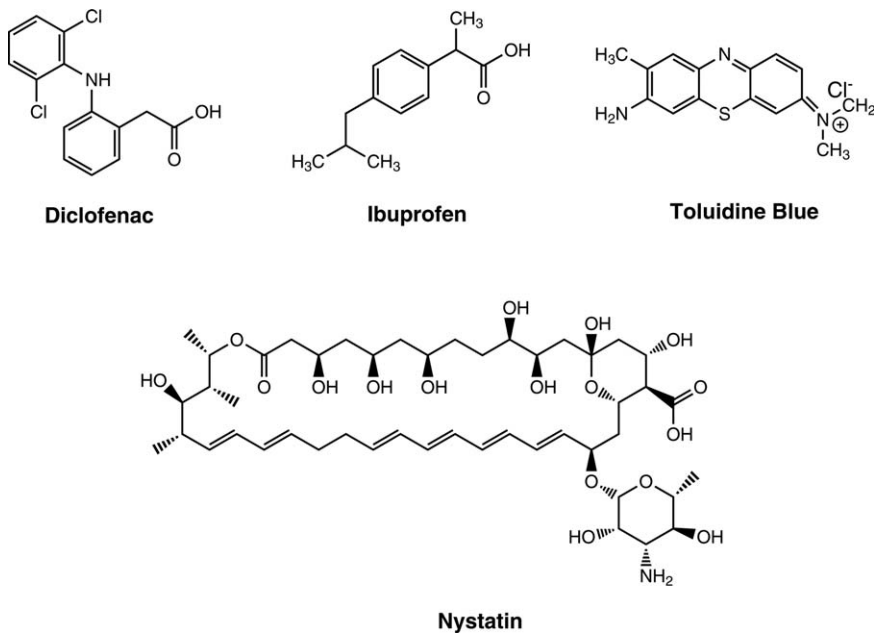

Figure 1. Molecular structures of the drugs (diclofenac, ibuprofen, and nystatin) and dye (TBO) used in this study. Diclofenac interacted with the carbonyl groups of SR-*g*-(VCL/MAA) through the C—Cl bonds and the amino group, whereas ibuprofen interacted through its carboxylic group with the amide group of VCL. The conjugated double bonds and the hydroxyl groups of nystatin may have interacted with the VCL ring and the carboxylic and amide groups in the graft copolymer, respectively. TBO is known to react with the carboxylic group of MAA through its amine group in a 1 : 1 molar ratio.

and, in some cases, also in the bulk of the substrate may have adequate mesh size and chemical groups for the diffusion and binding of a variety of drugs. For example, polypropylene was grafted with *N*-isopropyl acrylamide and *N*-(3-aminopropyl) methacrylamide hydrochloride for loading with nalidixic acid and the prevention of *Escherichia coli* colonization.[15] Also, polypropylene and polyethylene were functionalized at their surfaces with previous cyclodextrin grafting of glycidyl methacrylate by means of γ radiation to host miconazole and prevent biofilm formation by *Candida albicans*.[19]

The aim of this study was to graft poly(*N*-vinyl caprolactam) (PVCL) and poly(methacrylic acid) (PMAA) onto the inert alkyl siloxane moieties of silicone rubber (SR) films to generate materials that were able to load therapeutic doses of nonsteroidal anti-inflammatory drugs (NSAIDs) and antifungal agents. SR is widely used to prepare medical devices, including orthopedic, ophthalmic, and aesthetic devices, catheters, drains, and shunts,[22–24] but its capability to take up drugs by soaking or impregnation is very limited. On the other hand, PVCL is attracting growing attention as a temperature-responsive polymer because of its higher biocompatibility compared with poly(*N*-isopropyl acrylamide).[25–27] Among pH-responsive polymers, PMAA is particularly suitable for biomedical applications as its pK_a (5–6) is in the physiological range.[28] Although few have been explored, combinations of PVCL and PMAA have been shown useful in the preparation of temperature- and pH-responsive microgels for drug delivery.[29–31] An *N*-vinyl caprolactam (VCL)/methacrylic acid (MAA) copolymer network grafted onto SR, providing hydrophobic and ionic binding points, is expected to provide an adequate environment for the hosting and controlled release of amphiphilic drugs, such as diclofenac and ibuprofen (NSAIDs) or nystatin (antifungal agent; these structures are depicted in Figure 1). Diclofenac is expected to strongly interact with the carbonyl groups of SR-*g*-(VCL/MAA) through C—Cl bonds and amino groups, whereas ibuprofen can interact through its carboxylic group with the amide group of VCL. The hydrophobic (conjugated double bonds) and the hydrophilic (hydroxyl groups) regions of nystatin may interact with the VCL ring and the carboxylic and amide groups in the graft copolymer, respectively. For biomedical applications, it is also important to evaluate the affinity of the developed materials for common proteins, such as albumin and fibrinogen, which are known to prevent and promote, respectively, biofouling phenomena. The grafted copolymers could expand the biomedical applications of SR materials and provide a new option to the growing list of biocompatible smart materials suitable as components of medical devices.

EXPERIMENTAL

Materials

SR was obtained from Goodfellow (Huntingdon, United Kingdom), washed with ethanol (J. T. Baker, Mexico) for 24 h, and then dried under reduced pressure. VCL, methacrylic acid (MAA), bovine serum albumin (BSA), fibrinogen, sodium ibuprofen, citrate phosphate dextrose, citric acid, disodium hydrogen phosphate (Na_2HPO_4), sodium hydroxide (NaOH), and sodium chloride (NaCl) were obtained from Sigma Aldrich (St. Louis, MO). The monomers were distilled under reduced pressure before use. Toluene was obtained from J. T. Baker (Mexico), toluidine blue (TBO; Figure 1) was obtained from Panreac

Table I. Variables Tested for the Synthesis of the SR-*g*-(VCL/MAA) Copolymer

Parameter	Dose (kGy)	Temperature (°C)	Reaction time (h)	[VCL/MAA] (vol %)
Dose	5–100	80	12	50
Temperature	60	Range of 60–80	12	50
Reaction time	60	80	3–24	50
[VCL/MAA]	100	80	12	6–95

Quimica S.A.U. (Castellar del Vallés, Barcelona, Spain), sodium diclofenac was obtained from Vorquimica S.L. (Spain), and nystatin was obtained from Alfa Aesar. Purified water (resistivity > 18 MΩ·cm, MilliQ, Millipore Iberica, Madrid, Spain) was used in all of the experiments.

Synthesis of SR-*g*-MAA and SR-*g*-(VCL/MAA)

SR-*g*-MAA was synthesized by the placement of MAA (70% v/v) in a glass ampule containing a preirradiated pristine SR (100 kGy). Then, this solution was deaerated with argon and incubated at 70°C for 1–10 h.[5,17] The simultaneous grafting of VCL and MAA onto SR to obtain the SR-*g*-(VCL/MAA) copolymer was carried out as follows. The SR films (previously weighed) were placed in glass ampules and then irradiated in the presence of air with a ^{60}Co γ source (Gammabeam 651 PT, Nordion International) at preirradiation doses from 10 to 100 kGy and a dose rate of 10.4 kGy/h. After that, a mixture of the VCL and MAA monomers (1 : 1 v/v ratio) in toluene (50% v/v) was added, and the ampule was degassed by repeated freeze–thaw cycles and then sealed. The ampules were heated at various temperatures (from 60 to 80°C) to carry out the grafting process. After grafting, the copolymer films were soaked twice in ethanol and water under magnetic stirring for periods of 12 h to remove the nongrafted copolymer and residual monomers (ultraviolet–visible spectra scans of the washing solutions indicated no residual monomers). Then, the films were dried under reduced pressure, and the grafting percentage was calculated as follows:

$$\text{Grafting } (\%) = 100\left[\left(W_g - W_0\right)/W_0\right] \quad (1)$$

where W_g and W_0 are the weights of the grafted and initial films, respectively. Table I summarizes the synthesis experiments.

Characterization of SR-*g*-(VCL/MAA)

Fourier transform infrared (FTIR)–attenuated total reflectance spectra were obtained with a PerkinElmer Spectrum 100 spectrometer (PerkinElmer Cetus Instruments, Norwalk, CT). To determine the equilibrium water uptake, the graft copolymers were immersed in distilled water for various periods of time. The equilibrium water uptake was achieved after 3 h.

The lower critical solution temperature was determined by the measurement of the degree of swelling[32] of the SR-*g*-(VCL/MAA) films in water from 25 to 50°C for 3 h. At each temperature, the surface of the copolymer films was wiped with filter paper to remove free water, and then, the swollen samples were weighed. The swelling percentages were determined from the weights of the swollen (W_s) and dried (W_d) films as follows:

$$\text{Swelling } (\%) = 100[(W_s - W_d)/W_d] \quad (2)$$

The critical pH point was determined from the swelling of films placed for 3 h in citric acid/Na_2HPO_4 buffer solutions with pH's ranging from 2.6 to 8.9.

The decomposition temperature was determined under a nitrogen atmosphere with a TGA Q50 (TA Instruments, New Castle, DE). Differential scanning calorimetry (DSC) runs were recorded with a DSC 2010 calorimeter (TA Instruments, New Castle, DE) at heating rate of 10°C/min with sample weights of about 5 mg.

Quantification of the Surface Carboxylic Acid Groups

The quantification of carboxylic acid groups was carried out according to Sano *et al.*[33] Briefly, pristine SR and SR-*g*-(VCL/MMA) 10–76% graft film pieces with dimensions of 0.5 × 0.5 cm^2 were incubated for 1 h at 40°C in 5 mL of TBO solution (15 mg/L) in fresh 1 m*M* NaOH. Then, the pieces were rinsed with a 1 m*M* NaOH solution to remove free dye. Finally, the bound TBO was desorbed by the incubation of SR-*g*-(VCL/MAA) in 5 mL of acetic acid (50% v/v) for 30 min at 40°C. The amount of bound TBO was quantified from absorbance measurements at 630 nm. The number of COOH groups was determined with the following equation:[34]

$$[\text{—COOH}]\ \left(\text{nmol/cm}^2\right) = AV/Sd\varepsilon \quad (3)$$

—where A is the absorbance, ε is the extinction coefficient of TBO (0.031 cm^3 nmol^{-1} cm^{-1}), d is the light path length (cm), V is the volume of the desorption solution (cm^3), and S is the area of the sample surface (cm^2). The experiments were carried out in triplicate.

Hemolysis Assay[35]

SR-*g*-(VCL/MAA) films with graft percentages of 28, 52, and 99% were cut into small pieces of 1 × 1 cm^2 and equilibrated in 4 mL of 0.9% NaCl solution for 30 min at 37°C. Then, human blood (0.2 mL pretreated with 14% v/v citrate phosphate dextrose anticoagulant solution and obtained from healthy volunteers, Centro de Transfusión de Galicia, Spain) was added to each sample and incubated for 60 min at 37°C. Positive or negative controls were obtained according to Qu *et al.*[35] by the addition of 0.2 mL of human blood to 4 mL of MilliQ water or saline solution, respectively. All of the solutions were centrifuged at 900*g* for 10 min. The absorbance of the supernatant was measured at 542 nm (Agilent 8453, Germany). The percentage of hemolysis was calculated, in triplicate, as follows:

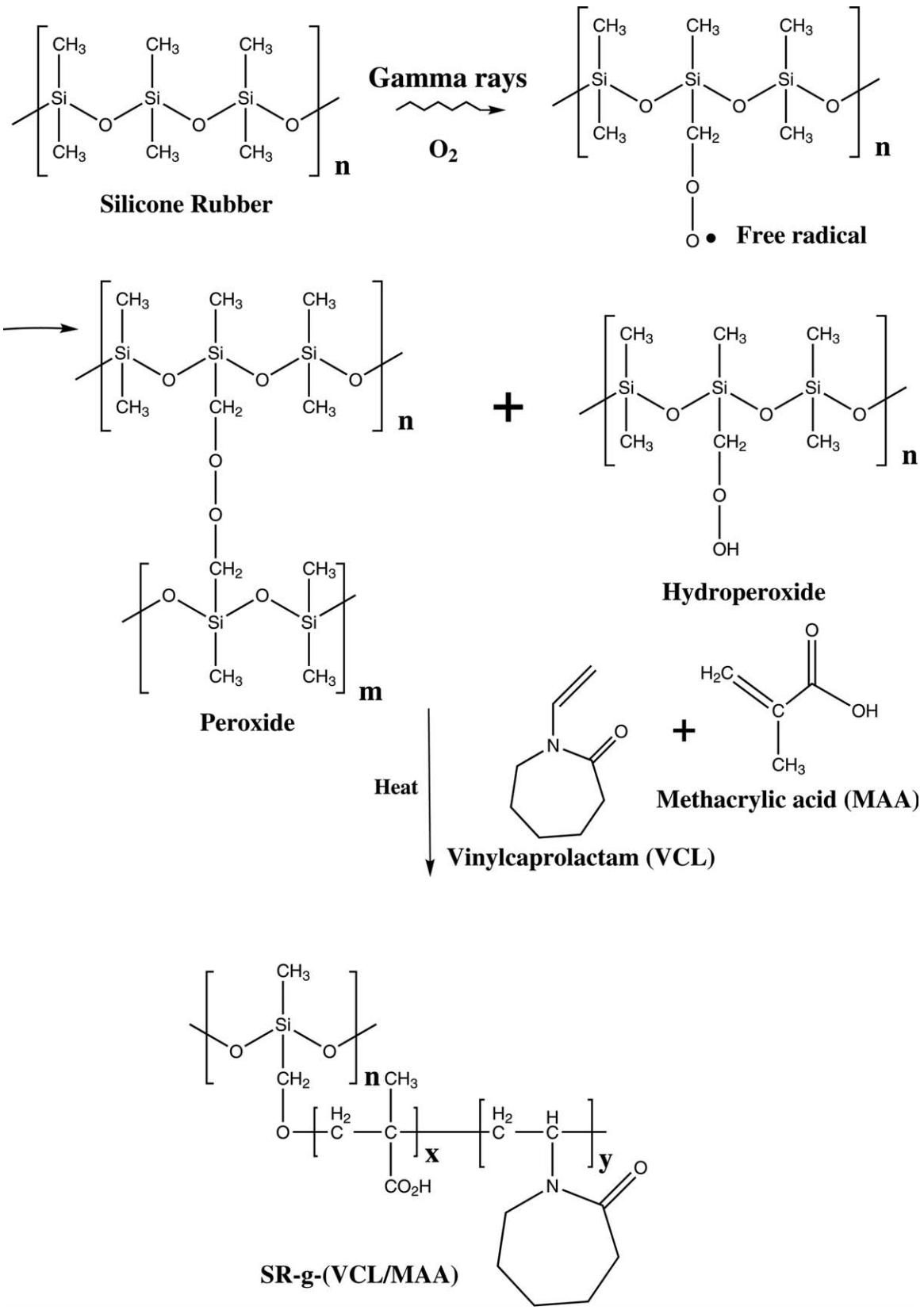

Figure 2. Graft process of VCL and MAA onto SR.

$$\text{Hemolysis}(\%) = \frac{A_{\text{sample}} - A_{C-}}{A_{C+} - A_{C-}} \quad (4)$$

where A_{sample} is the absorbance of the sample and A_{C-} and A_{C+} represent the absorbances of the negative and positive controls, respectively. All experiments were carried out in triplicate.

Protein Adsorption Studies

We determined the protein adsorption by the SR-*g*-(VCL/MAA) samples by following the methodology described by Contreras-García *et al.*,[16] which was slightly modified. Copolymer films with 28, 52, and 99% graft cut as pieces of 0.5 × 0.5 cm^2 (previously soaked in phosphate buffer at pH 7.4) were immersed in freshly prepared aqueous solution of BSA (30 mg/mL) or fibrinogen (1 mg/mL) contained in Eppendorf Lobind tubes of 2 mL and incubated at 37°C for 24 h. Then, the films were removed, and the amount of protein in the solution was determined by the measurement of the absorbance (Agilent 8453, Germany) at 278 nm for BSA and 280 nm for fibrinogen. The experiments were carried out in triplicate. The adsorbed amount of protein was calculated from the difference between the initial (C_{pi}) and final (C_{pf}) concentrations (mg/mL) with the following equation:

$$\text{Protein loaded}\left(\text{mg/cm}^2\right) = \left[\left(C_{pi} - C_{pf}\right) \times V_p\right] / S \quad (5)$$

where V_p is the volume of the protein solution (mL).

Loading and Release of Diclofenac and Ibuprofen

Pristine SR, SR-*g*-MAA, and SR-*g*-(VCL/MAA) 26–86% graft films (1 × 1 cm^2, previously dried) were immersed in 5 mL of sodium diclofenac or sodium ibuprofen (0.04 mg/mL) aqueous solution at room temperature and kept in the dark. The concentrations of diclofenac and ibuprofen were spectrophotometrically monitored (Agilent 8453, Germany) as a function of the time at 276 and 223 nm, respectively, for 10 days. The amount of drug loaded was estimated with the following equation:

$$\text{Drug loaded}\left(\text{mg/cm}^2\right) = \left[\left(C_{di} - C_{df}\right) \times V_d\right] / S \quad (6)$$

where C_{di} and C_{df} are the initial and final drug concentrations (mg/mL), respectively; V_d is the volume of the drug solution (mL); and S the surface area of the film (cm^2).

For the release tests, the drug-loaded copolymer films were dried at room temperature (for a more convenient storage) and then placed in vials with 5 mL of phosphate buffer pH 7.4 at 37°C. At specific time intervals, 2 mL was withdrawn from the release medium (after being gently hand-shaken to ensure a homogeneous concentration in the medium), the absorbance was measured at the respective wavelength of each drug, and the sample returned to the vial. All experiments were done in triplicate without stirring under sink conditions. The drug release was calculated as follows:

$$\text{Drug released } (\%) = (M_t / M_l) \times 100 \quad (7)$$

where M_l is the total amount of drug loaded (mg) and M_t is the amount of drug released (mg) at the corresponding time.

Loading and Release of Nystatin

Pristine SR and SR-*g*-(VCL/MAA) 27–80% graft films (1 × 1 cm^2, previously dried) were placed in vials containing 20 mL of nystatin aqueous solution (20 mg/L) and then kept in the dark at room temperature. The nystatin concentration was monitored over 10 days by the measurement of the absorbance at 305 nm. The loading experiments were carried out in triplicate, and the amount loaded was calculated as indicated previously. The nystatin-loaded films were rinsed with water and then immersed in 5 mL of phosphate buffer at pH 7.4 and 37°C. Two milliliters of release medium was withdrawn at specific time intervals and returned to the vial immediately after the measurement of the absorbance at 305 nm. All of the experiments were made in triplicate under gentle oscillatory movement and sink conditions.

RESULTS AND DISCUSSION

Synthesis of SR-*g*-(VCL/MAA)

The polymerization reaction leading to the formation of the copolymer SR-*g*-(VCL/MAA) is given in Figure 2. First, SR was irradiated in air, and peroxides and hydroperoxides were formed on the polymeric backbone. These peroxy intermediates were then decomposed under heating to yield macroradicals; these reacted with the double bonds of VCL and MAA to trigger the grafting onto SR. The grafting was carried out with a fixed VCL/MAA ratio (1 : 1 v/v) to modify the total monomer concentration in toluene, preirradiation dose, reaction time, and temperature. The 1 : 1 v/v VCL/MAA ratio was identified in preliminary studies as the one that provided the highest grafting percentage because sole VCL solutions did not lead to significant grafting. The VCL monomer is a large molecule containing a seven-membered caprolactam ring in the chair conformation;[36] such a large size may have hindered the diffusion of monomer molecules to the radiation grafting active sites. Thus, a mixture with MAA was mandatory for the grafting of VCL in the form of a copolymer via free-radical polymerization.

The effect of the total monomer concentration on the grafting of VCL and MAA onto SR was evaluated, with the preirradiation dose of 100 kGy at 80°C kept constant for 12 h [Figure 3(a)]. The amount of copolymer grafted increased as the total monomer concentration was raised; it reached a plateau at 80% v/v. A further increase in the monomer concentration did not modify the grafting percentage, and this can be explained as follows. As the monomer concentration increased, the diffusion into the bulk of SR became favored, and thus, more monomers had access to the reactive points for the propagation of growing chains.[37] Under the tested conditions, grafting occurred both at the surface and in the bulk. When the monomer concentration was too large, the increase in the viscosity hindered the monomer diffusion toward the polymeric matrix, and consequently, the amount of copolymer grafted did not increase further.

With regard to the effect of the preirradiation dose, the grafting yield of VCL/MAA onto the SR films at 80°C (reaction time = 12 h) increased as the preirradiation dose did [Figure 3(b)]. This was because the creation of more grafting sites (peroxy and hydroperoxy groups) onto SR films (see Figure 2).

The dependence of the grafting yields on the reaction time and temperature is shown in Figure 3(c,d), respectively. Hydroperoxides are more active than peroxides, which require a higher temperature for decomposition.[38] In other words, if the

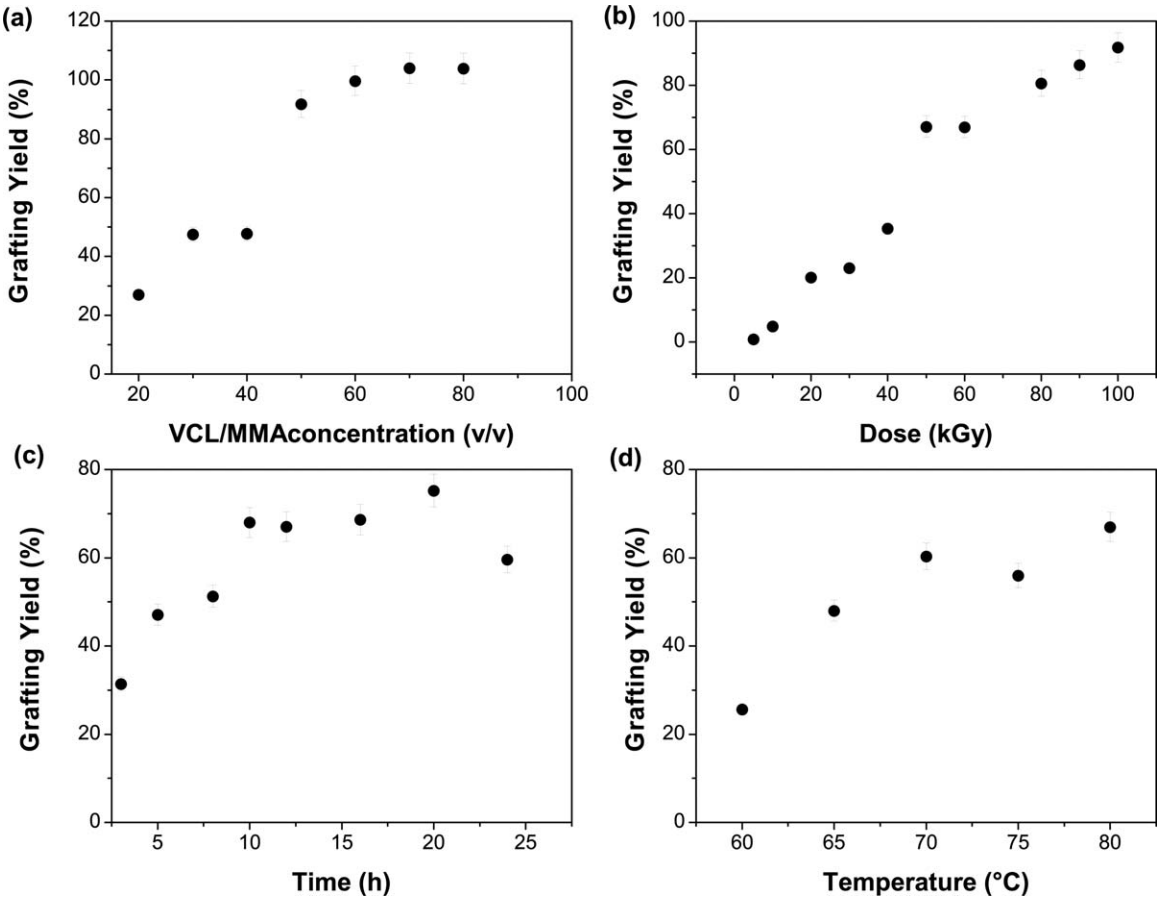

Figure 3. Effect of the (a) monomer concentration (100 kGy, 80°C, and 12 h), (b) preirradiation dose (80°C, 12 h, and VCL/MAA concentration = 50%), (c) reaction time (60 kGy, 80°C, and VCL/MAA concentration = 50%), and (d) temperature (60 kGy, 12 h, and VCL/MAA concentration = 50%) on the grafting yield of VCL/MAA onto SR.

temperature increased, more grafting sites were generated onto SR; also, the higher the temperature was, the better the comonomer diffusion in the film was, and therefore, the higher the grafting percentage was. For further experiments, the reaction time was set at 12 h as this time provided a high grafting yield in a reasonable operation time. Batches of copolymers having distinct grafting percentages were prepared to evaluate the effect of this parameter on the copolymer features. Taking into account the exact percentage of grafting, trends on the dependence of certain features on the grafting percentage were evaluated.

Characterization of SR-*g*-(VCL/MAA)

The FTIR spectra of SR, SR-*g*-(VCL/MAA) with 68% graft, and the VCL-*co*-MAA copolymer formed during the grafting reaction are shown in Figure S1 (Supporting Information). The spectrum of the pristine SR film showed a band at 1005 cm^{-1} that was due to the stretching vibrations of the Si—O—C bond and signals at 2963 and 1258 cm^{-1} that corresponded to C—H bonds in CH_3 and Si—CH_3 groups, respectively.[39] The copolymer VCL-*co*-MAA showed a peak at 1714 cm^{-1} that was due to the C=O carboxylic acid group of MAA and a band at 1614 cm^{-1} that corresponded to the amide group of VCL. This copolymer also showed peaks at 1260 and 1160 cm^{-1} that belonged to the C—N stretching vibrations from VCL, and two bands at 2914 and 2848 cm^{-1} that corresponded to the C—H stretching vibrations. These characteristic peaks were observed in the spectrum of the graft copolymer SR-*g*-(VCL/MAA) and confirmed the presence of both monomers in the grafting. Additional peaks of the VCL lactam ring were observed in the 1420–1486-cm^{-1} region.[40]

DSC runs of the VCL-*co*-MAA copolymer exhibited two endothermic transitions; one at 145°C that was due to the glass transition of PVCL[41] and another at 220°C, which was attributed to the melting of the copolymer[30] (Figure S2; Supporting Information). No transitions were observed for SR because its glass-transition temperature (−129°C)[42] was not in the temperature interval evaluated. SR-*g*-(VCL/MAA) with 68 and 103% grafting showed the same transitions, and this corroborated the presence of both PVCL and PMAA in the copolymer.

Thermogravimetric analysis was used to investigate the thermal stability of the copolymer SR-*g*-(VCL/MAA) in the temperature range 0–800°C under an inert nitrogen atmosphere. SR was stable up to 500°C (10 wt % loss; Figure 4), whereas the VCL-*co*-MAA copolymer showed three weight losses occurring in the regions 180–260, 350–450, and 510–640°C. The first stage of weight loss corresponded to PMAA decarboxylation. The

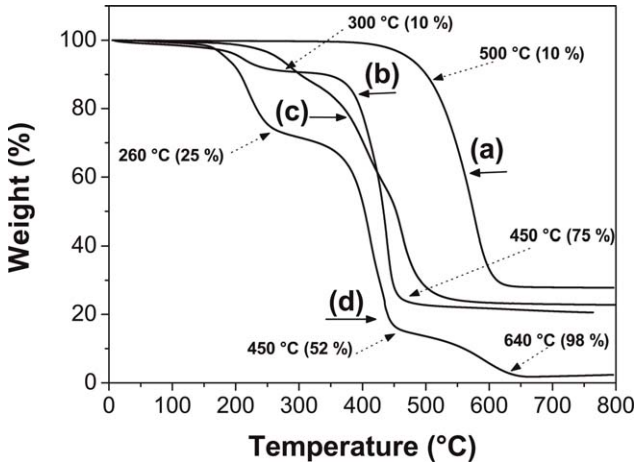

Figure 4. Thermogravimetric curves of the (a) SR, (b) SR-*g*-(VCL/MAA) with 68% grafting, (c) SR-*g*-(VCL/MAA) with 103% grafting, and (d) VCL-*co*-MAA copolymer. The values in parentheses correspond to the weight loss percentage up to the given temperature.

maximum weight loss (52%) occurred in the second stage. The thermal degradation of the SR-*g*-(VCL/MAA) 68% graft was shown by the two weight losses occurring in the 200–300°C region (due to the decarboxylation of PMAA) and 350–450°C region (due to the scission of the main chain of PMAA and PVCL).[43,44]

Temperature- and pH-Responsive Swelling

The pristine SR films did not swell in water in the pH and temperature ranges evaluated. The SR-*g*-VCL/MAA films (26 to 68% graft) swelled in water more as the temperature was raised [Figure 5(a)]. The PMAA and PVCL chains formed complexes via hydrogen bonding between the carboxylic acid groups and the amide groups.[45] This interaction was favored at low temperatures, and therefore, the copolymer showed hydrophobic characteristics. However, the complex was disentangled as the temperature rose and the network started to swell. This behavior led to an upper critical solution temperature for SR-*g*-(VCL/MAAA) of around 35°C. On the other hand, when swelling studies were carried out at pH 3.1, the graft copolymer showed a lower critical solution temperature around 33 °C [Figure 5(b)]. This different behavior was due to the fact that at acid pH, the PMAA chains collapsed (globular conformation) and could not form complexes with the PVCL chains. Therefore, swelling was governed by PVCL, which shrank as the temperature increased. At both 25 and 45°C, the SR-*g*-(VCL/MAA) copolymers exhibited a critical pH around 5.2 [Figure 5(c,d)]; this resembled the behavior previously reported for PMAA.[28] This corroborated the fact

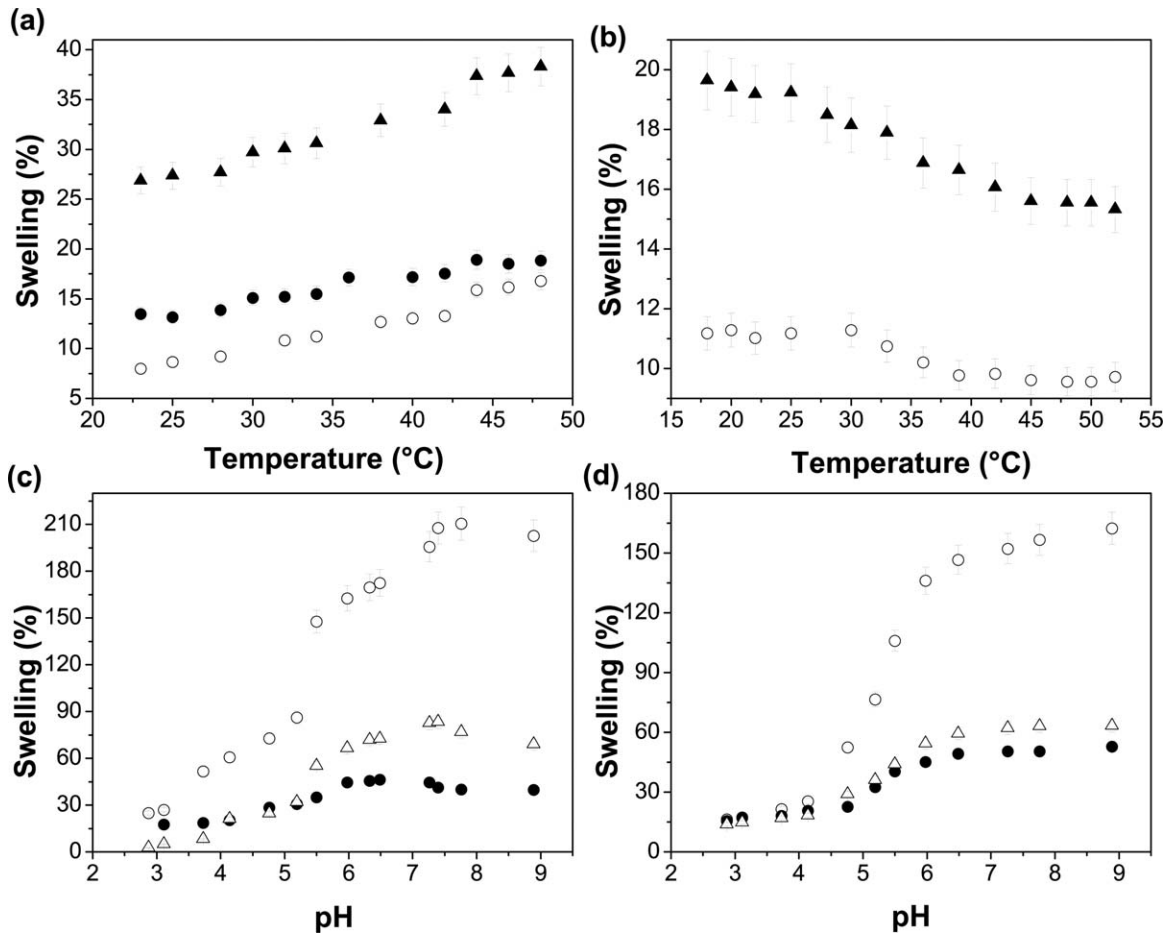

Figure 5. Effect of the temperature on the swelling of SR-*g*-(VCL/MAA) in (a) water and (b) a buffer solution (pH 3) at different graft yields: (○) 26, (•) 35, and (▲) 68%. Effect of pH at (c) 25 and (d) 45°C for grafting yields of (•) 22, (△) 48, and (○) 67%.

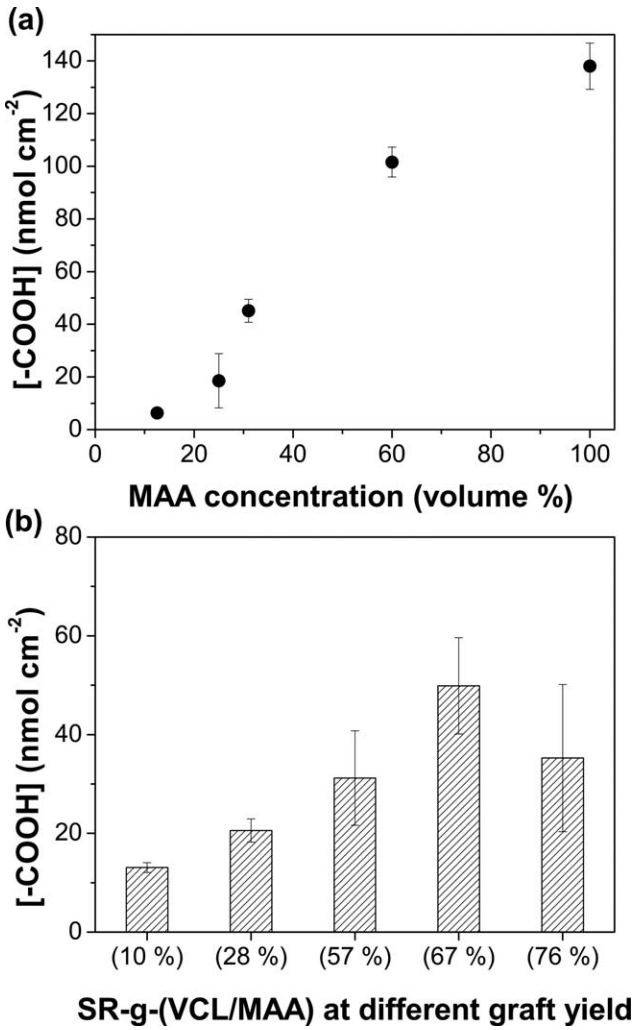

Figure 6. Dependence of the degree of carboxylation with respect to the (a) MAA concentration in the initial VCL/MAA ratio for SR-*g*-(VCL/MAA) with 3, 10, 23, 66, and 112% grafting and (b) grafting yield of VCL/MAA onto SR for the films prepared with an initial VCL/MAA ratio of 1 : 1. Error bars indicate standard deviations ($n = 3$).

that this component was responsible for the pH responsiveness of the grafted copolymer brushes. Overall, the swelling results indicated that a given copolymer was similarly swollen in aqueous media with the pH ranging from 6 to 8 (thus including the physiological pH 7.4) and was slightly more swollen at 37°C than at room temperature. When the grafting percentage increased, the swelling became larger, and at acid pH (~3), the collapse of the copolymer was favored by the increase in the temperature.

Quantification of the Surface Carboxylic Acid Groups

Complex formation with quaternary ammonium groups of TBO was used to quantify carboxylic acid groups on the surface of SR-*g*-(VCL/MAA). Initial tests with films grafted with monomer solutions prepared with different VCL/MAA feed ratios revealed that the number of carboxylic acid groups progressively increased with the MAA proportion in the monomer solution [Figure 6(a)]. The films were homogeneously dyed when the TBO solution was applied; this suggested a homogeneous distribution of the carboxylic acid on the film surface. Experiments carried out with grafted copolymers synthesized with a 1 : 1 v/v VCL/MAA ratio revealed that the number of COOH groups increased proportionally to the grafting percentage; this indicated that the VCL/MAA proportion was kept almost constant in the grafted copolymer despite the grafting yield [Figure 6(b)]. The large deviations recorded for the samples with the highest grafting percentages could be explained by the growth of the chains inside the SR bulk, and this may have made TBO diffusion difficult.

Hemolysis and Protein Adsorption

Hemocompatibility and protein adsorption were evaluated for a first screening of the biocompatibility of the grafted materials. The results of the hemolysis tests for SR and SR-*g*-VCL/MAA with different graft percentages are shown in Table II. The grafting did not cause any deleterious effects on the hemocompatibility and showed hemolysis values below 1%.

The *in vivo* performance of a material can be anticipated, to a certain extent, from the *in vitro* adsorption of albumin and fibrinogen.[19] Albumin is the preponderant blood protein in plasma, and because of its thromboresistant ability, the covalent attachment of albumin has a profound influence on the subsequent events in the blood coagulation cascade, such as reduced platelet adhesion and aggregation. This, thereby, prevents subsequent thrombus formation. Also, albumin adsorption prevents the binding of microorganisms, whereas fibrinogen enhances microbial and platelet adhesion and thrombus formation.[46] For the adsorption study, the concentrations of albumin and fibrinogen were chosen to mimic the levels in human blood. SR-*g*-(VCL/MAA) films did not adsorb relevant amounts of these proteins even after 24 h of incubation (Table II). Only the film with 28% grafting adsorbed fibrinogen after 24 h (0.152 mg/cm^2). Overall, these results indicate that the SR-*g*-(VCL/MAA) films were not prone to biofouling.

Loading of Diclofenac, Ibuprofen, and Nystatin

Functionalization with stimuli-responsive polymers is being evaluated for the preparation of drug-eluting medical devices

Table II. Hemolysis Percentage and Amount of BSA and Fibrinogen Adsorbed on the Pristine SR (0% grafting) and SR-*g*-(VCL/MAA) Films Prepared with Different Grafting Percentages

Grafting yield (%)	Hemolysis (%)	BSA adsorbed (mg/cm^2)		Fibrinogen adsorbed (mg/cm^2)	
		1 h	24 h	1 h	24 h
0	1.05 (0.19)	nd	0.30 (0.05)	nd	nd
28	0.80 (0.50)	nd	nd	nd	0.15 (0.04)
52	0.59 (0.56)	nd	nd	nd	nd
99	0.70 (0.44)	n.d	nd	nd	nd

Values between parentheses are the standard deviations.
nd, not detected.

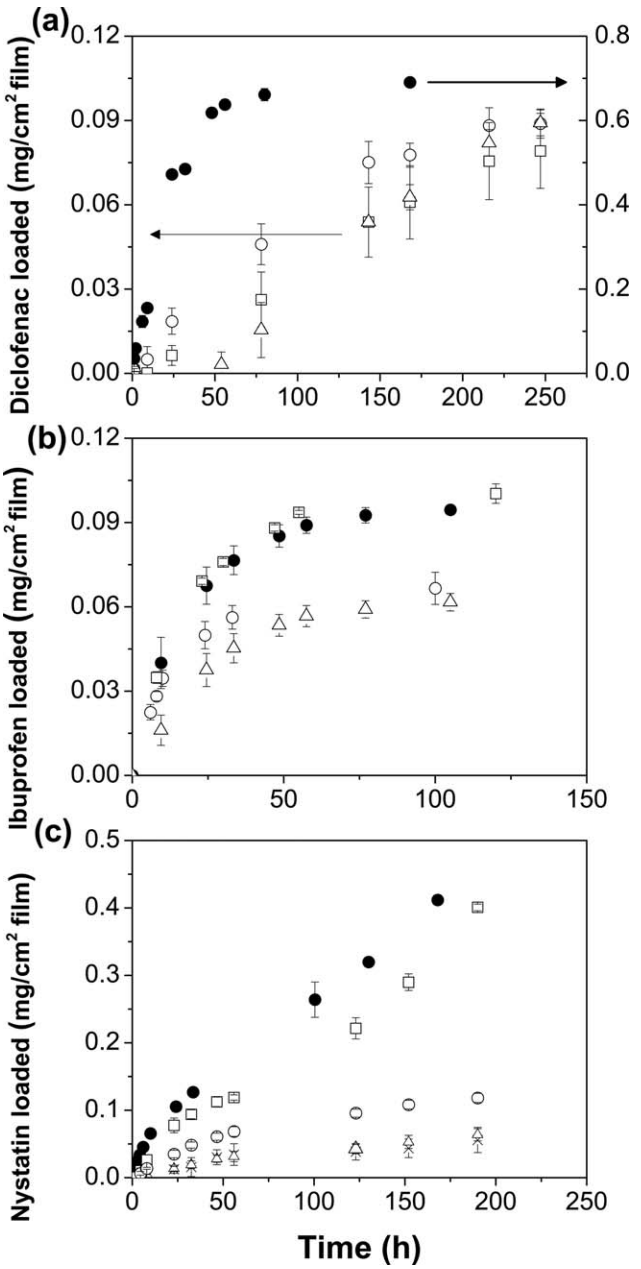

Figure 7. (a) Diclofenac loaded by (•) SR-*g*-MAA with 56% grafting and SR-*g*-(VCL/MAA) with (□) 86, (○) 51, and (△) 26% grafting. (b) Ibuprofen loaded by (•) SR-*g*-MAA with 29% grafting and SR-*g*-(VCL/MAA) with (□) 60, (○) 47, and (△) 26% grafting. (c) Nystatin loaded by (•) SR-*g*-MAA with 29% grafting, (×) pristine SR, and SR-*g*-(VCL/MAA) with (□) 80, (○) 54, and (△) 27% grafting. The loading conditions included soaking in aqueous drug solutions, room temperature, and protection from light.

with promising results.[5,15,20,47] The bioactive molecules can chemically interact through reversible bonds with the modified materials to become trapped in a three-dimensional polymer network from which they can be released in a controlled way. Responsiveness to, for example, the temperature and/or pH enables the tuning of the network mesh size to facilitate drug diffusion during loading and to regulate it after the insertion/implantation of the medical device. In this study, the loading conditions (aqueous medium at 20°C, in the dark, pH ≈ 7) were chosen to obtain swollen grafted networks and to ensure drug stability.[48–50] As expected, the unmodified SR films could not take up significant amounts of any of the two NSAIDs tested. By contrast, the SR-*g*-(VCL/MAA) films loaded relevant amounts of diclofenac and ibuprofen; the loading equilibria were reached in about 150 and 50 h, respectively [Figure 7(a,b)]. Interestingly, the uptake of ibuprofen [Figure 7(b)] occurred more quickly, and the amount loaded at the equilibrium by the SR-*g*-(VCL/MAA) films with 60% grafting was the same as that loaded by a film grafted with a similar amount of MAA alone (i.e., SR-*g*-MAA with 29% grafting), probably because the ibuprofen molecular size was smaller (206.29 g/mol) than that of diclofenac (296.15 g/mol) and the ibuprofen was adsorbed through nonspecific hydrophobic interactions. In addition, the carboxylic groups of ibuprofen can interact with the amide groups of PVCL.[51] SR-*g*-MAA exhibited a notably greater affinity for diclofenac [Figure 7(a)] compared to ibuprofen; this could be explained by the stronger interactions the C—Cl bonds, and the amino groups of diclofenac (absent in ibuprofen) could be established with the carbonyl groups of the grafted polymer.[52] However, such an affinity seemed to not be enough to completely break the complexes between the PVCL and PMAA in the SR-*g*-(VCL/MAA) films, and as a consequence, all of the SR-*g*-(VCL/MAA) films were able to host similar amounts of diclofenac with no regard to their grafting yield.

Because the therapeutic concentration for NSAIDs has been reported to be 10^{-5} M,[53] the amounts of diclofenac and ibuprofen loaded by SR-*g*-(VCL/MAA) could be sufficient to prevent inflammatory events in the area surrounding an implanted device. In fact, a 1-cm^2 piece of material containing 0.050 mg of drug and immersed in 16 mL of fluid could provide a therapeutic concentration. This is a large volume compared with that available in the implantation site of most medical devices.[18]

The grafting of PVCL and PMAA also provided SR with the ability to take up nystatin [Figure 7(c)]. Nystatin is a large (926.1 Da) amphiphilic polyene antifungal agent that can establish hydrophobic interactions through its conjugated double bonds,[54] and it can form hydrogen bonds with amide and carboxylic groups from grafted copolymers through its hydroxyl groups.[55] This resulted in higher loading amounts compared to the NSAIDs.

The loading by the SR-*g*-(VCL/MAA) films increased progressively with the grafting percentage. A remarkably high affinity for MAA was observed when the loading of SR-*g*-MAA was evaluated because of the better access to the binding points [Figure 7(c)]. The minimum inhibitory concentration of nystatin against *C. albicans* has been reported to be 1.56 mg/L.[56] This means that a 1-g piece (ca. 4 cm^2) of SR-*g*-(VCL/MAA) with 80% grafting could incorporate enough nystatin to prevent the growth of *C. albicans* in 1 L of aqueous medium.

Release of Diclofenac, Ibuprofen, and Nystatin

The release experiments were performed at 37°C and pH 7.4 to mimic physiological conditions; these conditions may be less favorable ones for the SR-*g*-(VCL/MAA) films to regulate drug

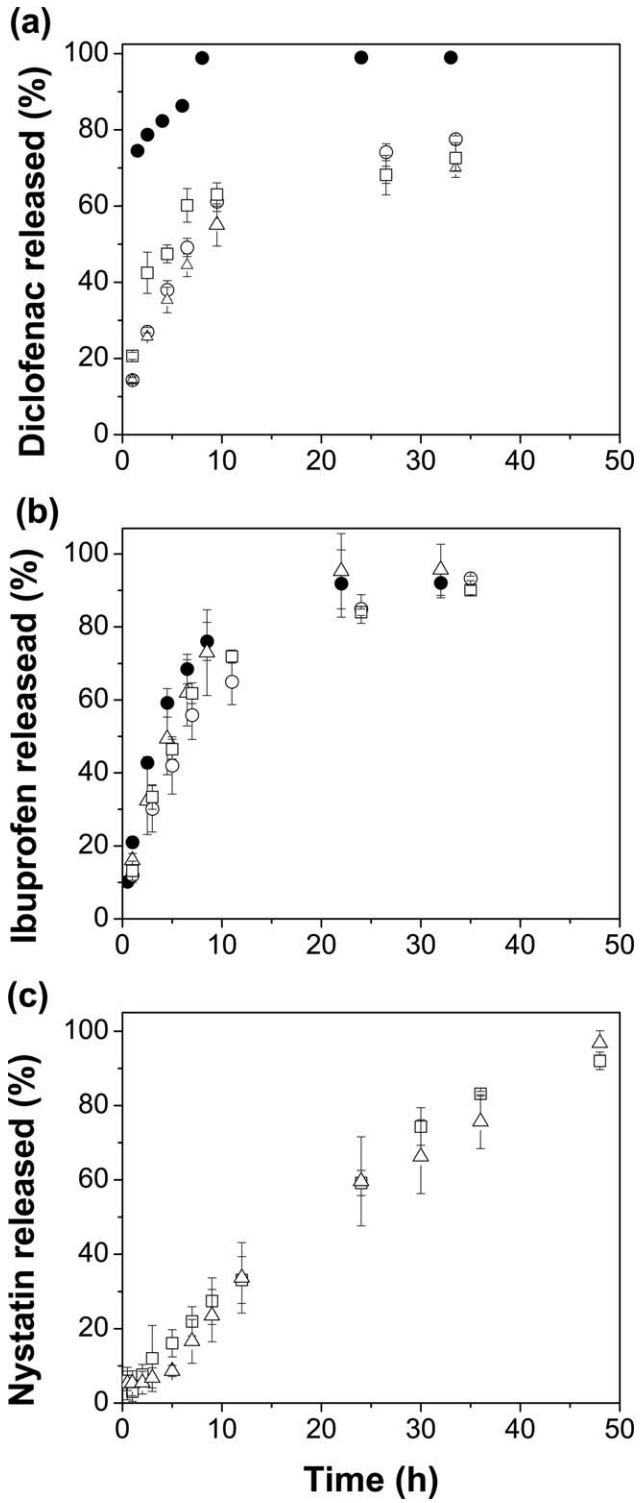

Figure 8. (a) Diclofenac percentage released from (•) SR-*g*-MAA with 56% grafting and SR-*g*-(VCL/MAA) with (□) 86, (○) 51, and (△) 26% grafting. (b) Ibuprofen percentage released from (•)SR-*g*-MAA with 29% grafting and SR-*g*-(VCL/MAA) with (□) 60, (○) 47%, and (△) 26% grafting. (c) Nystatin released (%) from SR-*g*-(VCL/MAA) with (□) 80 and (△) 27% grafting.

release because of the swelling of the grafted copolymers. Importantly, the SR-*g*-(VCL/MAA) films showed sustained release of both diclofenac and ibuprofen for 24 h, despite the swelling of the network and the fact that the drug molecules could easily diffuse through the grafted brushes. Moreover, the solubility of these drugs at pH 7.4 was high (Figure 8). Thus, controlled release could occur from the SR-*g*-(VCL/MAA) films because the drug molecules were hosted inside the complex grafted brushes and interacted with them at a relatively high intensity. By contrast, the SR grafted with MAA alone (i.e., SR-*g*-MAA) showed an important burst of diclofenac, and this release was complete in less than 10 h. Namely, as the brushes expanded, the drug was rapidly released. The controlled release of nystatin was prolonged for 48 h [Figure 8(c)]; this could have been related to the marked hydrophobic character of this drug and, consequently, its stronger affinity for the SR-*g*-(VCL/MAA) films (as observed in the loading). Thus, despite the fact that the experiments were carried out under sink conditions, nystatin release required a longer time for the breaking of the interactions with the grafted films. As observed with diclofenac and ibuprofen, no significant differences in the release rate were noticed when the SR-*g*-(VCL/MAA) films with different grafting percentages were observed.

CONCLUSIONS

SR was modified with a VCL-*co*-MAA copolymer by the application of a preirradiation method that involved γ-ray irradiation of SR and subsequent immersion in the comonomer solution. Various reaction conditions enabled us to tune the grafting percentages. Compared with the pristine SR, the grafted copolymer led to superior features for use as components of hemocompatible medical devices, particularly with regard to the possibility of hosting drugs and controlling their release under physiological conditions. The implemented grafting approach is quite versatile and could be applied to other polymer substrates and a variety of drugs able to interact with carboxylic acid groups. In particular, the grafting of PVCL and PMAA onto SR may be suitable for the development of drug-eluting devices (combo products) with potential anti-inflammatory or antifungal performance.

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