Collagen-Based New Biomedicial Films: Synthesis, Property, and Cell Adhesion

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Received 28 July 2000; accepted 6 October 2000

ABSTRACT: Grafted-collagen films with poly(methyl methacrylate), poly(butyl methacrylate), and poly(styrene) were prepared by photoirradiation of the collagen films in the presence of respective monomers. Cell adhesion onto the films was investigated, and the amount of cells adhered onto every grafted films was almost the same as that onto the collagen film crosslinked with glutaraldehyde. Because the swollen grafted films are stronger than the collagen film, they are expected to be good biomedical materials.
© 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2433–2438, 2001

Key words: collagen; graft polymerization; biomedical films; cell adhesion; high wet-strength

INTRODUCTION

Collagen, which is a fibrillar protein, has received considerabe interest because of its abundant existence in animals and has many important biological functions, such as tissue formation and cell adhesion. So collagen is an expected biocompatible material, but has problems in its mechanical strength. However, synthetic polymers have enough mechanical strength, but have poor biocompatibility.

Hence, material obtained by the combination of collagen and synthetic polymer (bioartificial polymeric material) should be useful for the biomedical field, because it is expected to have biocompatibility and enough mechanical strength.

Some bioartificial polymeric materials have been proposed.[1–4] These are based on blends of biological and synthetic polymers,[1,2] adsorbed collagen to materials,[3] or plasma-induced graft polymerization onto collagen films.[4]

We also have investigated the collagen-based material and found a good method as shown in the following. By photoirradiation of a collagen film in the presence of several vinyl monomers (except vinyl acetate), polymerization took place efficiently in the film to produce poly(vinyl monomer) grafted-collagen film. One of the surfaces (collagen side in Fig. 1) has no vinyl polymer up to 4 μm.[5]

In this study, cell adhesion tests were performed on graft films thus obtained to provide the biocompatible films.

EXPERIMENTAL

Materials

Collagen used was provided by Koken Co. (Tokyo, Japan). Collagen was dissolved in 0.1*N* acetic acid to a 0.5% (w/w) solution. The solution was casted on a Teflon plate, and the solvent was evaporated

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Journal of Applied Polymer Science, Vol. 81, 2433–2438 (2001)
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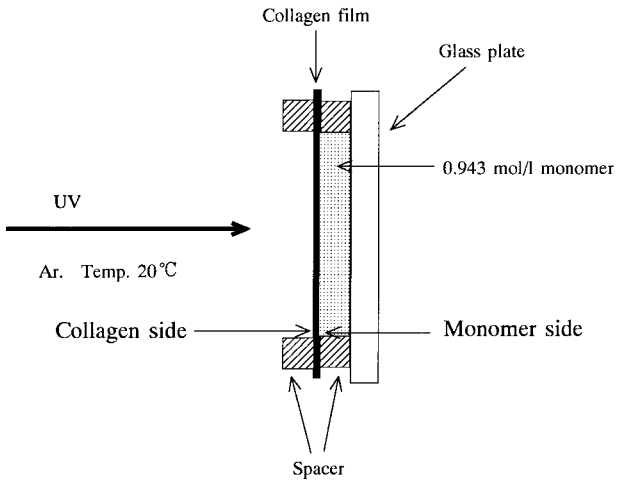

Figure 1 Graft polymerization apparatus.

at 20°C for more than 7 days. After vacuum drying overnight at 20°C, films with a thickness of about 0.02 mm were obtained. Methyl methacrylate (MMA), butyl methacrylate (BMA), and styrene (St) were purified to remove the inhibitor by common method.

Graft polymerizations of MMA, BMA, and St onto collagen films were performed by using the apparatus in Figure 1 as described in a previous article.[5] The 0.943 mol/L monomers in the apparatus were irradiated with ultraviolet light through the collagen film. We refer to the surface faced to monomer as the monomer side and the other side as the collagen side. The unreacted monomers and homopolymers were removed from the grafted-collagen films by washing with acetone for poly(methyl methacrylate) (PMMA) and poly(butyl methacrylate) (PBMA), and toluene for poly(styrene) (PSt) at room temperature overnight, followed by drying under vacuum overnight. The amount of polymer grafted onto the collagen film was determined by the increase in the film weight.

Characterization of the Grafted Films

Contact Angle Measurement

Static contact angles were measured using the sessile drop method.[5] The water drops were monitored by means of a video camera and the contact angle was evaluated from computer printouts of the video screen and evaluated as an average value of three measurements.

Swelling Ratio and Tensile Strength of Swollen Grafted-Collagen Films

Grafted-collagen films were placed in distilled water for 24 h. These samples were removed from the water and weighed after removing excess surface water by blotting with a laboratory tissue paper. The swelling ratios were determined by using the following equation:

$$\text{Swelling ratio} = (\text{Swollen weight} - \text{Dry weight})/\text{Dry weight}$$

Tensile strength measurements of swollen grafted film were performed on 10×20 mm with Strograph M100 (Toyoseiki Co., Tokyo, Japan).

Cell Adhesion Assays

Human dermal fibroblasts and a fibroblast growth medium kit, which contains a 500-mL bottle of fibroblast basal medium and four supplements that are 1 μg/mL human recombinant fibroblast growth factor, 5 mg/mL insulin, 50 mg/mL gentamicin, and 50 mg/mL amphotericin, were purchased from Iwaki Co.(Tokyo, Japan). Glutaraldehyde crosslinked and grafted-collagen films were immersed in fibroblast growth medium for 2 h before seeding the cells. Human dermal fibroblasts were suspended in fibroblast basal medium (1×10^5 cells/mL) and seeded onto the samples in an atmosphere of 5% CO_2 at 37°C. After 16 h, the assay was stopped by washing off the unadhered cells with phosphate buffered saline. Adhered cells were fixed with 2.5% glutaraldehyde in phosphate buffered saline (1 h).

Scanning Electron Microscopy

Fixed fibroblasts were processed for scanning electron microscopy. The films were further dehydrated through ethyl alcohol/water (V/V) series from 50 to 100%. After a final immersion, the specimen were placed in a Critical Point Dryer (HCP2; Hitachi Co., Tokyo, Japan). The coverslips were mounted to scanning electron microscope stubs with silver conductive paint, and after platinum coating, the specimen were observed with a scanning electron microscope (S-4000; Hitachi Co.) at 15 kV.

RESULTS AND DISCUSSION

Photo-Graft Polymerization onto Collagen Films

The photo-graft polymerization of MMA, BMA, and St onto the collagen films were performed at

Table I Photo-Graft Polymerization of Various Monomers onto the Collagen Films

Monomer	Irradiation Time (h)	Concentration (mol/L)	Grafting (wt %)
BMA	2	0.943	334
	3	0.943	388
	4	0.943	653
MMA	2	0.943	291
	3	0.943	442
	4	0.943	602
St	2	0.943	80
	3	0.943	307
	4	0.943	350

Solvent, MeOH. Collagen films, 40–70 mg. Solution, 5.5 mL. Temperature, 20°C.

20°C as shown in Figure 1, and various grafted films were obtained as listed in Table I.[6] As shown in Table I, photo-graft polymerization of three monomers took place in the film efficiently. We found the structure of the thus obtained grafted-collagen film from attenuated total reflection spectra, scanning electron microscope (SEM) observation, and the viscoelasticity as follows. From the result of attenuated total reflection spectra using the crystal of Ge and KRS-5, although the film contained a large amount of synthetic polymer, one of the surfaces contained no synthetic polymer up to 0.4 mm.[5] The dynamic modulus and dynamic loss modulus of the PMMA-grafted-collagen films were measured, and showed that PMMA was packed closely. Collagen could be removed from the PMMA-grafted-collagen films by decomposition with acid, and PMMA films were obtained. The surfaces and section of the PMMA

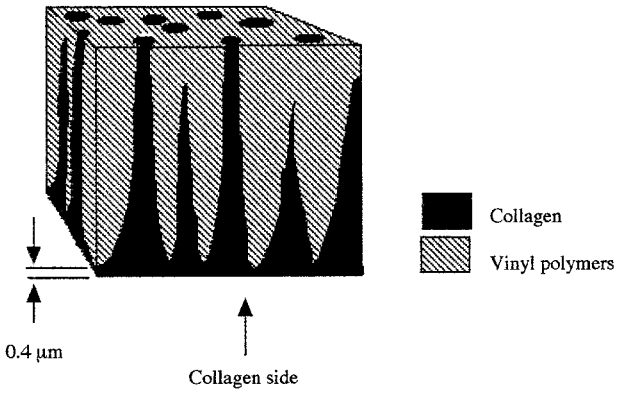

Figure 2 Schematic representations of synthetic polymers grafted-collagen films.

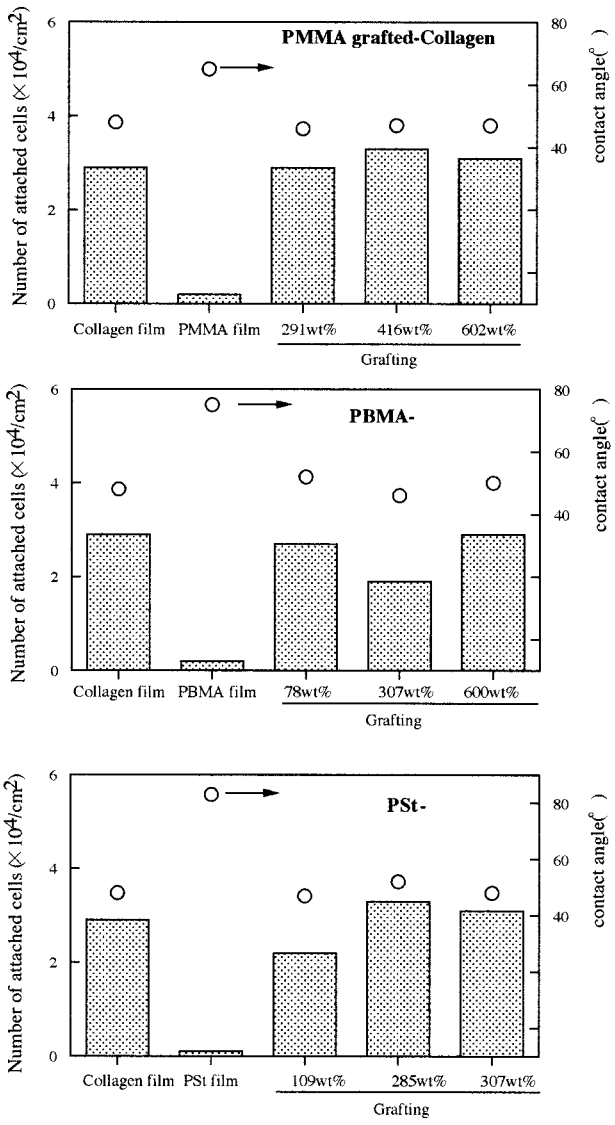

Figure 3 Contact angles and the number of adhered cells for/onto the surface of the collagen side of grafted-collagen films.

films were observed with SEM, and a porous structure was found. On the basis of these results, the structure of grafted-collagen film was proposed as follows: the synthetic polymer are driven into the collagen matrix like piles as shown in Figure 2.[7] Cell adhesion tests on this collagen side of the grafted-collagen film were performed in this study.

Therefore, these grafted-collagen films should have a hydrophilic surface like collagen, and contact angles of collagen film; grafted films and collagen film crosslinked with glutaraldehyde were measured, and are shown in Figure 3. These val-

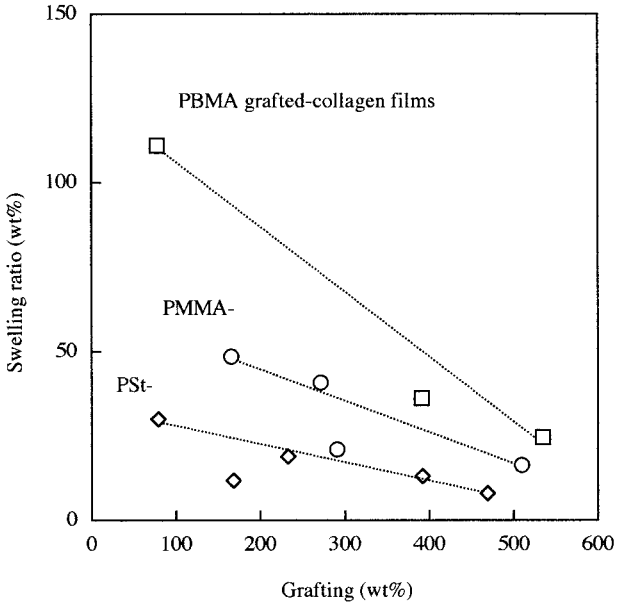

Figure 4 Swelling ratio of grafted-collagen films. Swelling ratio = (wet weight − dry weight)/dry weight.

ues support the idea that one of the surfaces contained no PMMA, PBMA, or PSt up to 0.4 mm and the biomedical use is expected.

Tensile Strength of the Swollen Grafted-Collagen Films

Swelling ratio of the grafted-collagen films and the tensile strength of the swollen grafted-collagen films are shown in Figures 4 and 5, respectively.

These films were insoluble in water but swelled well, and the swelling ratio of the grafted-collagen films decreased with increased grafting. However, the swelling ratio, even for high grafting films, showed over 10% and all swollen films were very flexible. The dry grafted-collagen films of high grafting are hard and fragile, but as these films are considered to be in contact with blood, they should be used wet and they should be flexible. Therefore, the mechanical property of the swollen state is important.

The tensile strength of the swollen grafted-collagen film increased with increased grafting, especially in the case of PSt grafted-collagen films, and at even 100 wt % grafting, the tensile strength of the swollen grafted-collagen film is higher than 1 MPa which is the same as swollen collagen film crosslinked with glutaraldehyde.

Cell Adhesion to the Grafted Films

Figure 6 shows the SEM photographs of the films of grafted-collagen, PMMA, PBMA, PSt, and collagen adhered with human fibroblast cells. Cell adhesion onto PMMA, PBMA, and PSt film surfaces is scarcely observed, but cells adhered as well onto grafted-collagen films as onto collagen films crosslinked with glutaraldehyde. The effect of the grafted polymers on the cell adhesion was not observed until 600 wt % of grafting, as shown in Figure 3, because of the collagen layer existing. Hence, these grafted films are expected to be good biomedical films.

CONCLUSION

PMMA, PBMA, and PSt grafted-collagen films were easily obtained by photoirradiation and one of the surfaces contained no synthetic polymer up to 0.4 mm. Although the dry grafted-collagen films of high grafting are hard and fragile, all films swollen were very flexible and the tensile strength of swollen grafted-collagen films was higher than that of collagen film crosslinked with glutaraldehyde. As a result of cell adhesion onto the grafted-collagen surface, cells adhered as well onto grafted-collagen films as onto collagen films crosslinked with glutaraldehyde.

From the characteristics described above, the grafted-collagen films are a new type of biomate-

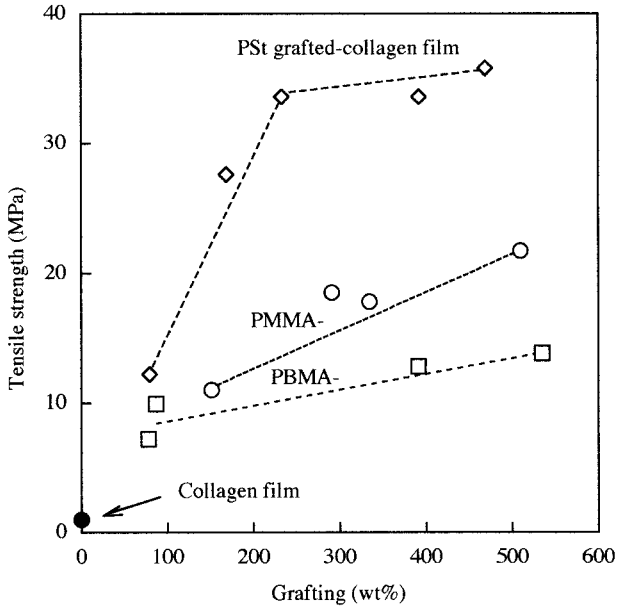

Figure 5 Tensile strength of swollen grafted films.

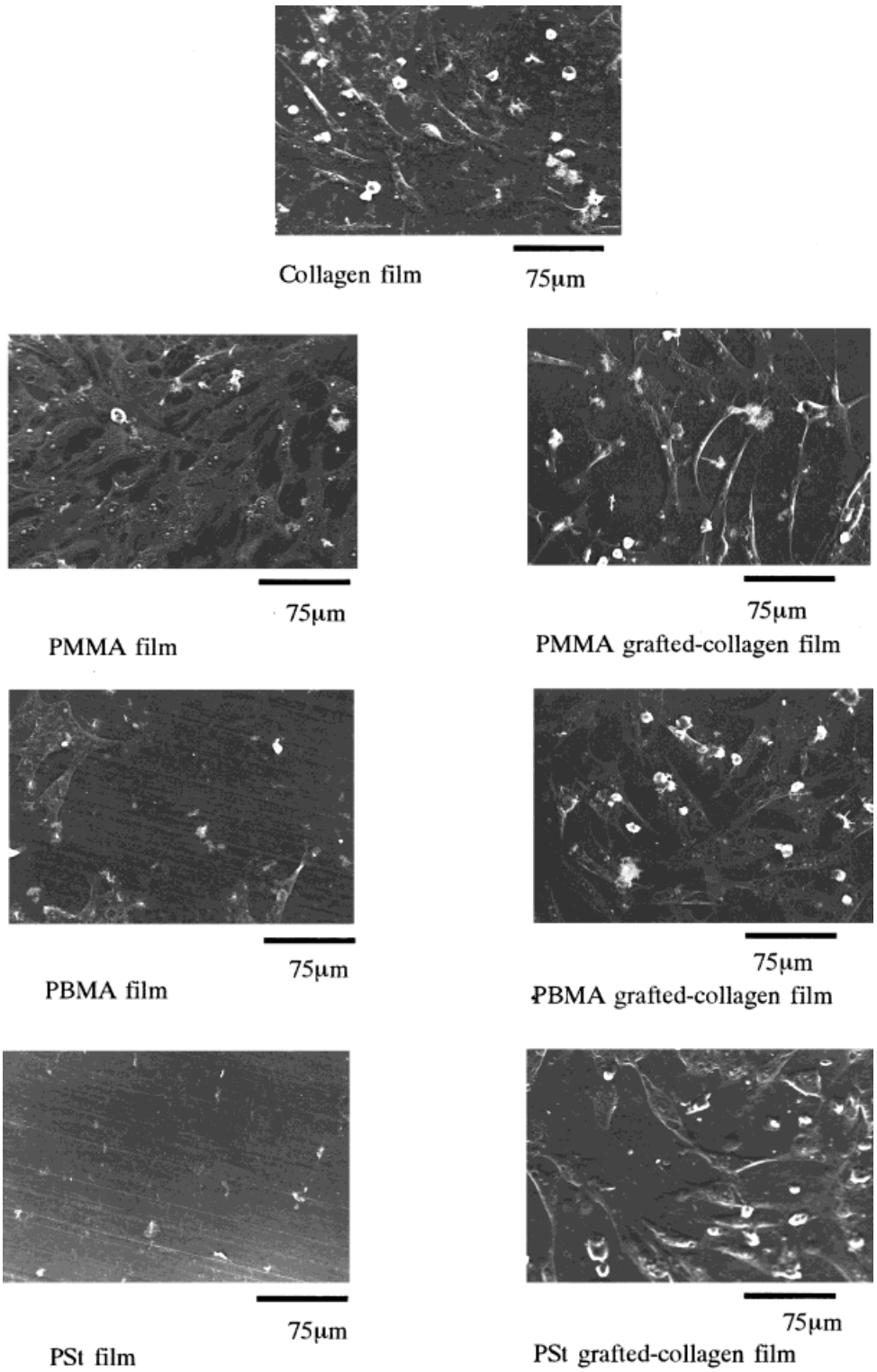

Figure 6 SEM photographs of the films of grafted-collagen-PMMA, PBMA, and PSt, and collagen adhered with human fibroblast cells.

rial and are expected to be useful for constructing a variety of tissue or organ substitutes. These films allow fibroblasts to cover the surface (especially on the collagen side) and then the collagen is substituted with self-tissues.

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