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# Controlling the Cell Adhesion Property of Silk Films by Graft Polymerization

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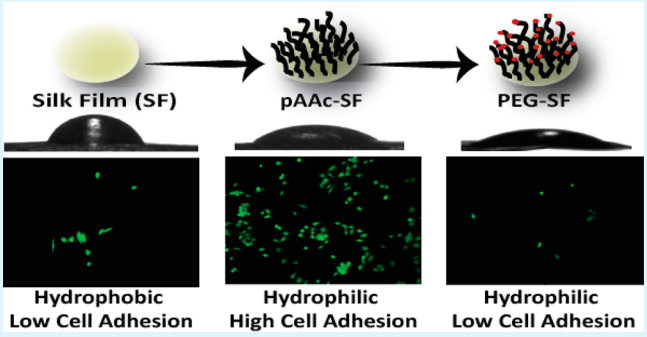
Supporting Information

**ABSTRACT:** We report here a graft polymerization method to improve the cell adhesion property of *Bombyx mori* silk fibroin films. *B. mori* silk has evolved as a promising material for tissue engineering because of its biocompatibility and biodegradability. However, silk's hydrophobic character makes cell adhesion and proliferation difficult. Also, the lack of sufficient reactive amino acid residues makes biofunctionalization via chemical modification challenging. Our study describes a simple method that provides increased chemical handles for tuning of the surface chemistry of regenerated silk films (SFs), thus allowing manipulation of their bioactivity. By grafting pAAc and pHEMA via plasma etching, we have increased carboxylic acid and hydroxyl groups on silk, respectively. These modifications allowed us to tune the hydrophilicity of SFs and provide functional groups for bioconjugation. Our strategy also allowed us to develop silk-based surface coatings, where spatial control over cell adhesion can be achieved. This control over cell adhesion in a particular region of the SFs is difficult to obtain via existing methods of modifying the silk fibroin instead of the SF surface. Thus, our strategy will be a valuable addition to the toolkit of biofunctionalization for enhancing SFs' tissue engineering applications.




**KEYWORDS:** *Bombyx mori, silk films, polymer grafting, cell adhesion*

## INTRODUCTION

In recent years, silk, especially from *Bombyx mori*, has seen revived interest as a biomaterial, primarily as a tissue engineering scaffold, aided by its superior mechanical strength, biocompatibility, and biodegradability.[1−6] Silk fibroins self-assemble into hydrophobic crystalline $\beta$ sheets, which is the origin of silk's mechanical strength and tunable degradability.[5,7−9] However, this comes at the cost of low cell adhesion and proliferation.[1,9] Whereas many strategies for tuning the hydrophilicity resulting in cell adhesion and proliferation properties of silk have been described,[1,3,10,11] biofunctionalization through simple nontoxic chemical modification remains challenging. Modification of reactive amino acid residues of the silk fibroin has been explored for achieving the surface attachment of small molecules, polymers, growth factors, and cell binding ligands.[1,2,6] However, the scarce amount of these amino acids and the nonbiocompatibility of functionalization chemistries are major hindrances. There is an undoubted need for simple methods that can provide chemical handles for tuning of the surface chemistry of silk films (SFs), enabling control of their bioactivity. Moreover, because spatiotemporal patterns of chemical and biophysical cues form the microenvironment of cells in vivo, to realize the full potential of SFs in tissue engineering, it is essential to develop strategies for achieving spatially controlled biofunctionalization to modulate cell adhesion and differentiation.

Common strategies reported for modification of the silk fibroin rely on modifying the reactive amino acid residues using coupling chemistries, amino acid masking, azomodification for click reactions, glycopeptides conjugations, and polymer grafting via atom-transfer radical polymerization and other polymerization techniques.[1,6,9−15] All of these modifications are carried out on silk fibroin in solution, which are later self-assembled in $\beta$ sheets to form water-insoluble SFs or scaffolds. This fibroin modification, after a $\beta$-sheet formation strategy, does not allow control over the presentation of the modifications on the surface of the fibroin films exclusively because the modified amino acids can get buried during the $\beta$-sheet formation. Also, the modifications can interfere in the $\beta$-sheet formation, resulting in altered properties of the SFs.[6]

To present functional groups exclusively on the surface of the film, we carried out surface modification of the SFs after $\beta$-sheet formation. The $\beta$-sheet formation first and modification later strategy, unlike the currently known strategies, will allow us to exclusively control the surface−cell interactions. Surface grafting of polymers such as poly(acrylic acid) (pAAc) or poly(2-hydroxyethyl methacrylate) (pHEMA) possessing chemical functional groups such as carboxylic acid and hydroxyl

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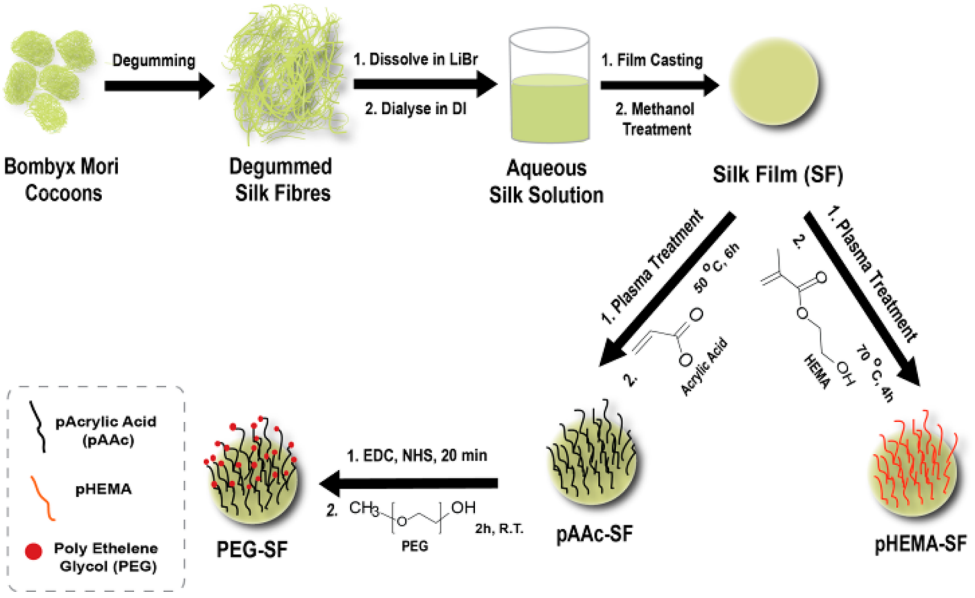
**Scheme 1. Schematic Representation of the Surface Polymer Grafting and Bioconjugation Strategy for Functionalization of Regenerated SFs**




is expected to provide us with a hydrophilic surface, yield more surface functional groups for further manipulation via conjugation, and allow surface-mediated modulation of the bioactivity of SFs.[16] Our surface polymer grafting is based on a plasma-induced graft polymerization technique.[17]

Plasma-induced graft polymerization has been demonstrated on many polymeric substrates, fibers, and fabrics and has also been used to increase the wettability of silk fibroin.[16−21] However, the method has not been explored on regenerated SF surfaces. Also, the effect of surface modification of pristine SFs with pAAc and its bioactivity has not been studied.

Here, we report a simple, scalable, and general strategy to graft functional polymers on regenerated SFs, as illustrated in Scheme 1. A regenerated silk fibroin solution was obtained by degumming *B. mori* cocoons and then dissolving the degummed fibroin fibers in LiBr. Degumming of the silk fibroin is important to remove the immunogenic sericin. The regenerated silk fibroin fiber solution was used for casting SFs, which were further treated with 70% aqueous methanol to induce $\beta$-sheet formation. Methanol treatment along with other methods such as ethanol treatment, water annealing, and mechanical shearing has been used to induce a higher level of crystallinity in silk fibroin by efficient $\beta$-sheet formation.[7,22,23] The water-insoluble SFs obtained after methanol treatment were then subjected to a two-step plasma treatment. First, the SFs were treated for a short time with nitrogen plasma to activate the pristine surface followed by treatment with air to obtain thermally labile groups. The plasma-treated films were then immediately transferred to the polymerization reaction flask where the thermally labile groups decompose and initiate polymerization of the monomers on the SF surface. To introduce reactive functional groups that can be later used for bioconjugation, we grafted pAAc and pHEMA on the SF surface. The grafting was analyzed by X-ray photoelectron spectroscopy (XPS). The XPS spectrum of the unmodified SFs and pAAc−SF and pHEMA−SF films (Figure S1 in the Supporting Information, SI) showed an increase in the intensity of the C−O component at ∼286.5 eV and the intensity of the O−C═O component at ∼289.0 eV in both pAAc−SF and pHEMA−SF. The increase can be attributed to the increased carbonyls and carboxylates after pAAc and pHEMA grafting. For pHEMA, an additional peak at ∼286.7 eV was observed due to C−OH. The carboxylic acid groups on pAAc-SF were subsequently used for conjugating a dye, rhodamine, and a macromolecule, poly(ethylene glycol) (PEG; MW 750). PEG is known to resist protein adsorption and cellular adhesion.[24] We hypothesized that subsequent bioconjugation will allow easy conversion of the cell adhesive (pAAc) surface to the cell nonadhesive (PEG) surface, thus providing control over the cellular adhesion properties. We have also employed our surface technique to obtain spatial control over cellular adhesion on a single SF surface, which is difficult to achieve if the fibroin is first modified chemically and then assembled into $\beta$ sheets.

## EXPERIMENTAL SECTION

**Materials and Method.** Cocoons from *B. mori* were obtained from Central Sericultural Research and Training Institute, Mysore. 2-Hydroxyethyl methacrylate (HEMA), LiBr, poly(ethylene glycol) methyl ether (MW 750), *N*-hydroxysuccinimide (NHS), fluorescein isothiocyanate (FITC), and protease XIV (*Streptomyces griseus*) were obtained from Sigma-Aldrich. Toluidine Blue O was obtained from LOBA Chemicals. 1,3-Dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Spectrochem. All other chemicals were obtained from Merck, India, unless otherwise specified. The cell culture was carried out in Dulbecco's modified Eagle Medium (DMEM; high glucose) obtained from Invitrogen. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for toxicity assessment and Calcein AM dye for fluorescent microscopy were obtained from Invitrogen. The HeLa cell line and L6 (rat skeletal muscle cells) were obtained from the National Centre for Cell Science (NCCS), Pune, India.

**Preparation of an Aqueous Solution of Silk.** The silk fibers were obtained by boiling *B. mori* cocoons for 30 min, two times in an aqueous solution of 0.02 M $Na_2CO_3$. It was then rinsed thoroughly with water to extract the gluelike sericin proteins. The silk fibers were then dissolved in a 9.3 M LiBr solution at 60 °C for 4 h, yielding a 20 wt % solution. This solution was dialyzed against water for 72 h in a cellulose acetate membrane with a 12 kDa molecular weight cutoff followed by centrifugation at 12000 rpm for 20 min at 4 °C to get rid

of any impurities. The final concentration of an aqueous solution of silk fibroins was about 3−4% (w/v), which was refrigerated for bulk storage.

**Preparation of SFs from a Silk Fibroin Solution.** Films were casted by pouring the prepared 3−4% (w/v) silk fibroin solution onto a 35 mm polystyrene Petri dish. The casted films were then dried at room temperature for 24 h and then in vacuum at 60 °C for next 24 h. Films were then immersed into 70% methanol for 30 min to make the films water-insoluble by inducing $\beta$-sheet formation in the casted silk fibroin films.

**Functionalization of the Water-Insoluble SFs by Graft Polymerization.** The methanol-treated SFs were cut into 8-mm-diameter disks using biopsy punches. Polymer was grafted onto the 8 mm SFs via first activation of the surface by plasma treatment followed by free-radical polymerization. Briefly, SFs were first placed in a 18 W radio-frequency $N_2$ plasma connected to a vacuum pump ($7 \times 10^{-1}$ mbar) for 2 min. After $N_2$ treatment, air was introduced into the plasma chamber and maintained at atmospheric pressure for 1 h to generate peroxide and other oxygen-containing functional groups on the SF surface. The films were immediately transferred to a round-bottomed flask containing a $N_2$-purged 20% (v/v) aqueous solution of the monomer acrylic acid (AAc) for pAAc grafting. The grafting reaction was carried out for 6 h at 50 °C, after which the films were washed in water overnight to remove nonsurface-bound polymer. For PEG grafting, pAAc−SFs were immersed in an aqueous solution containing 1:1 equivalent EDC and 5 mol % 4-(dimethylamino)-phenol for 30 min. The films were then incubated with PEG for 6 h.[25] After completion of the reaction, the side products were washed three times with 70% methanol and finally rinsed with deionized (DI) water.

For pHEMA grafting, a similar procedure was followed in which the films were first treated with plasma, as described before, followed by immediate transfer to a round-bottomed flask containing a $N_2$-purged 30% (v/v) pH 2.5 aqueous solution of HEMA. The grafting reaction was carried out for 4 h at 70 °C, after which the films were washed in *N*,*N*-dimethylformamide for 30 min at 70 °C and then washed in water overnight.

**Contact-Angle Measurements for Evaluation of Changes in the Hydrophilicity.** Modified and unmodified SFs were placed on glass slides and dried overnight to obtain a flat film. The water contact angle was measured using a Digidrop contact-angle meter with an 8 $\mu$L water drop size. Three films of each type were analyzed in at least three different regions of each film. The contact angles obtained were plotted, with error bars being the standard deviation between three different measurements of at least three different films.

**Fourier Transform Infrared−Attenuated Total Reflectance (FTIR−ATR) for Evaluation of Changes in the $\beta$-Sheet Formation.** IR absorption spectra of SFs show characteristic bands that can be assigned to the peptide. In accordance with the previously reported literature, untreated soluble SFs have mostly random-coil structures, which are observed at 1650, 1540, and 1230 for amides I−III, whereas after methanol treatment, $\beta$-sheet formation can be confirmed by the shifts in the bands until conformation of the fibroins in SF is clearly indicated by the amide I−III absorption bands at 1625, 1520, and 1270, respectively. To evaluate whether the polymerization resulted in any changes in the SF $\beta$ sheet formed before polymerization, FTIR spectroscopy was used and the bands for amides I−III were compared before and after each polymerization.

**Carboxyl Group Determination.** The amount of pAAc grafting on the PET film surface was characterized by a colorimetric method based on Toluidine Blue O staining. Briefly, the grafted film was placed for 6 h at 30 °C in a 0.5 mM Toluidine Blue O solution prepared at pH 10. The films were then removed and thoroughly washed with NaOH (pH 10) to remove any dye nonspecifically adsorbed to the surface. The bound dye molecules were then desorbed from the films in a 50% acetic acid solution. The final dye content was determined from the optical density (OD) of the solution at 633 nm using a UV−visible spectrophotometer. The increase in carboxyl groups after pAAc grafting was used to calculate the degree of polymerization.

**Degradation Study.** The effect of polymerization on the rate of degradation of SFs was evaluated using protease XIV from *S. griseus* (Sigma-Aldrich) with an activity of 3.5 U/mL. Briefly, 13 ± 5 mg of SF of each type was incubated at 37 °C containing 0.23U/mL of the protease solution at pH 7.4 using a 0.1 M solution of phosphate-buffered saline (PBS). Films were collected at specified time points of 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, 4 days, and 6 days. At least three films were analyzed at all time points. Films were washed with a PBS solution first followed by DI water and then dried for analysis of the mass using an analytical balance. Percent degradation was calculated by calculating the loss in weight.

**Conjugation of a Dye to the Carboxyl Goups Obtained by pAAc Grafting.** pAAc-grafted SFs were incubated with a 2 mM EDC and 5 mM NHS solution in PBS at pH 7.4 for 20 min. After washing with PBS, rhodamine B (1 mg/mL) was added and reacted with the NHS-activated carboxyl groups for 2 h. The films were then washed with ethanol and then DI water. Films were then dissolved in a LiBr solution, and the conjugated dye content was determined from the OD of the solution at 553 nm using a UV−visible spectrophotometer.

**XPS.** XPS measurements were carried out using an ambient-pressure X-ray photoelectron spectrometer from Prevac, Poland, using a monochromatic Al K$\alpha$ X-ray source (1486.6 eV). The detailed core-level spectrum was collected using a pass energy of 50 eV. The samples were made by sticking onto a double-sided C-tape. Due care was taken while making the samples to avoid contribution from the substrate.

**Protein Adsorption Studies.** Protein adsorption was measured on control and treated SFs. Protein adsorption was determined using FITC-labeled bovine serum albumin (BSA) and human immunoglobulin G (IgG). BSA and IgG were first incubated with FITC for 4 h and then dialyzed against DI water at 4 °C for 6 h to remove unreacted FITC. Subsequently, 250 $\mu$L of a 20 $\mu$g/mL solution of FITC-labeled BSA and human IgG in PBS was adsorbed onto the SFs for 2 h at 37 °C. The films were washed with PBS three times, and the fluorescence intensity of the surface was measured using a fluorescence microplate reader (Varioskan Flash Multimode Reader, Thermo Scientific). Excitation/emission for FITC-labeled IgG and BSA was set at 490/520 nm.

**Cell Culture.** The cell adhesion properties of the modified and unmodified SFs were evaluated by culturing the HeLa and L6 cells over the films. First, the HeLa cells were cultured in high-glucose DMEM with 10% fetal bovine serum. At 90% confluency, trypsin was added to the cells for detachment and the cells were centrifuged and redispersed in DMEM culture media. All SF samples were kept in ethanol overnight and then UV-treated for sterilization. Samples were then kept in a 1 mL media solution for 4 h, cut into 7-mm-diameter pieces using a biopsy punch, and placed in a 96-well cell culture plate. The HeLa cells suspended in DMEM culture media were seeded in the wells with a density of 10000 cells/well. Cells were also cultured in the wells without any SFs and were considered to be tissue culture polystyrene (TCPS) controls. The culture plates were then kept in a $CO_2$ incubator at 37 °C with 5% $CO_2$ for 24 h, after which the media were removed and the films were washed with PBS three times before analysis for viability and proliferation. The same protocol was used for the L6 cells. All of the samples were analyzed in triplicate and the experiments repeated at least three times. The data were plotted, with error bars being the standard deviation in the data points of three different experiments with triplicate.

**Cell Viability Assay.** The viability of the cells cultured on modified and unmodified SFs was analyzed by a live/dead staining assay. After 24 h of cell culture, the media were removed and the SFs washed with media and PBS as described in the above section. The assay was done by staining the cultured cells on the SF samples by the addition of 0.1 $\mu$L (2 $\mu$M) of Calcein AM to 100 $\mu$L of DMEM. The samples with staining solutions were incubated at 37 °C for 30 min. The staining solutions were removed and the films washed in PBS three times to remove any excess stain. The stained samples were directly visualized under a fluorescence microscope to observe the viability of the cells. Calcein AM was observed using Alexa Fluor 450 filters.

For cellular patterning of the SFs, half of the pAAc-SF area was conjugated with a 2 M solution of poly(ethylene glycol) methyl ether (MW 750) using carbodiimide coupling, and the rest was left unreacted. The half pAAc and half PEG-grafted SF were then used for

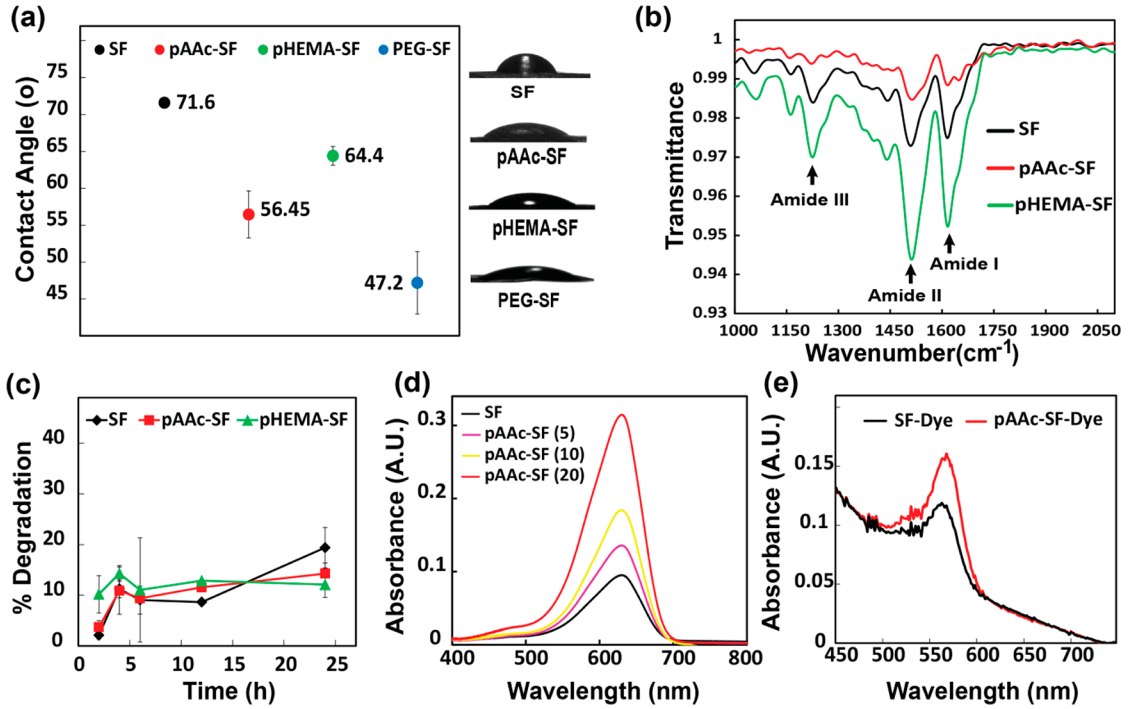



**Figure 1.** (a) Water contact angle for SF and polymer-grafted SFs. (b) FTIR–ATR of pAAc–SF, pHEMA–SF, and unmodified SFs. (c) Enzymatic degradation of SF, pAAc–SF, and pHEMA–SF ($n$ = 3; error bars = SD). (d) UV–visible spectra for toluidine assay for SF (black) and pAAc–SF obtained by varying the AAc monomer concentrations (v/v %). Color code: magenta, 5%; yellow, 10%; red, 20%. (e) UV–visible spectra for rhodamine conjugation for SF and pAAc–SF.

cellular adhesion studies. After 48 h of cell culture on the entire SF, the film was stained for live cells as mentioned above. The final image was reconstructed using an Adobe Illustrator.

**SEM Analysis.** The HeLa cells grown on treated and untreated SFs were analyzed by scanning electron microscopy (SEM). Prior to microscopic observation, the cells attached to the films were fixed with a 2.5% glutaraldehyde phosphate-buffered solution, dehydrated with aqueous ethanol, and then dried in air. Next, a thin layer of gold was deposited on the attached cells using a sputtering technique. The morphology of the HeLa cells attached to the films after incubation for 24 h was observed using environmental SEM (ESEM) with an EDAX EDS system.

**Cell Count by Image Analysis.** Cells cultured on modified and unmodified SFs were quantified using *Image J* software. Images were first processed to obtain binary images, which were used to count the cell numbers. Cells were measured from three different fields of area 1384 × 1036 pixel on a film. This analysis was performed on films from three different experiments.

**MTT Assay.** To quantify the proliferation of the cells cultured over the SF samples, MTT assay was used. After removal of the media and washing of the cells following the 24 h incubation, 20 $\mu$L of MTT (5 mg/mL) was added to the wells in 200 $\mu$L of DMEM culture media. The plate was incubated for 4 h until purple crystals of formazan formed, after which the media were removed and DMSO was added to dissolve the formazan crystals. The concentration of the formazan formed was determined by reading the OD at 550 nm using a plate reader. Statistical analysis was performed using the Student's $t$ test. Results were considered to be statistically significant when $p \leq 0.0001$. All of the data represent mean value ± standard deviation of at least three independent experiments in triplicate in each experiment.

## RESULTS AND DISCUSSION

We first investigated the effect of polymer grafting on the hydrophilicity of SF by measuring the water contact angles. Pristine regenerated SF surfaces are slightly hydrophobic, hindering their ability for better cell attachment.[1,2,4,13] As can be seen from Figure 1a, the water contact angle observed for SF was 71.6 ± 0.70, whereas upon grafting the surface with pAAc and pHEMA, the water contact angle decreased to 56.4 ± 3 and 64.5 ± 1, respectively. Conjugation of PEG via the carboxylic acid groups on pAAc–SF further reduced the contact angle to 47.2 ± 4. The water contact-angle measurement suggests that the surface grafting of these hydrophilic polymers can easily tune the hydrophilicity.

Because the mechanical properties of SF depend on the $\beta$ sheets of silk protein, we next examined whether the surface-grafting technique alters the preformed $\beta$ sheets.[5,8,22,23] Soluble silk fibroin films before methanol treatment have mostly random-coil structures, which change into water-insoluble $\beta$ sheets upon methanol treatment.[8,22,23] The $\beta$-sheet structure can be easily characterized by FTIR by observing the shifts in the amide I–III absorption bands after methanol treatment of SF.[8] The $\beta$-sheet formation was confirmed by comparing the amide absorption peak positions before and after methanol treatment of SF (Figure S2 in the SI). The IR spectra of the SF after methanol treatment and pAAc and pHEMA grafting are shown in Figure 1b. The $\beta$-sheet conformation of the fibroins in SF is clearly indicated by the amide I–III absorption bands. The prevailing $\beta$-sheet conformation in the methanol-treated SF remains unaltered after pAAc and pHEMA grafting, as indicated by their IR spectra.

We next investigated whether polymer grafting alters the degradation kinetics of the SFs by monitoring the weight loss of films. When the SF, pAAc–SF, and pHEMA–SF were incubated with protease XIV enzyme (0.23 U/mL) for 24 h at 37 °C, it was observed that the degradation profile remained similar (Figure 1c) with about ∼15–20% weight loss (degradation) of the films in 24 h and about 45–55% weight loss after 6 days (Figure S3 in the SI). This slow degradation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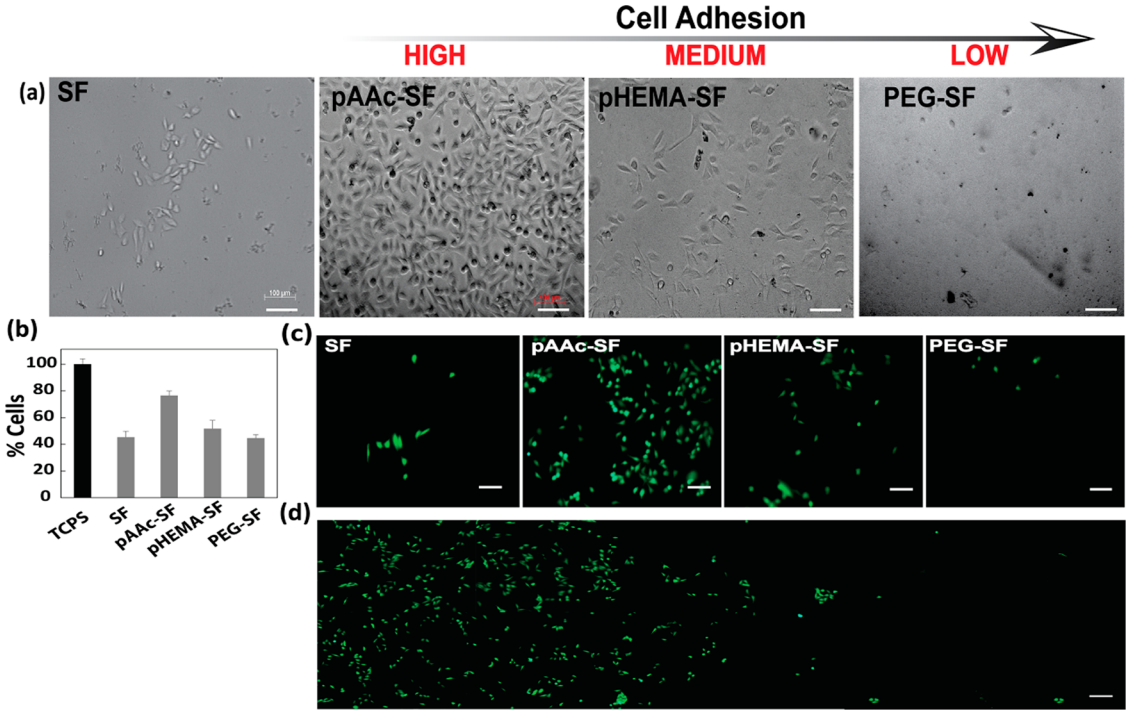


**Figure 2.** (a) Optical images of the HeLa cells on SF and polymer-grafted SFs after 24 h of culture. (b) Percent viable and proliferated HeLa cells after 24 h of culture on SF and polymer-grafted SFs. ($n$ = 3; error bars = SD). TCPS was used as a positive control, and all values were normalized accordingly. (c) Calcein AM staining of HeLa cells proliferating on SF and polymer-grafted SFs after 24 h of culture (all images are at 10× magnification; scale bar = 100 $\mu$m). (d) Reconstructed image of HeLa cell proliferation on pAAc−SF, where half of the film was conjugated with PEG, suggesting our ability to spatially control cell adhesion. The image was taken after 48 h of culture and staining with Calcein AM (10× magnification; scale bar = 200 $\mu$m).

rate is in accordance with the literature[22,26] and suggests that the degradability of SFs after pAAc and pHEMA grafting remains unaltered.

In silk fibroin, a meager ∼3% of the total amino acid content is aspartic and glutamic acid residues, which provides the carboxylic acid group for functionalization via the carbodiimide chemistry.[1] Carboxylic acid groups not only provide easy access to further modifications and increase in the hydrophilicity but also have been shown to promote differentiation of the cells.[1,2,15,27] We quantified the increase in carboxylic acid groups on the SF surface after pAAc grafting by Toluidine Blue O assay. Toluidine Blue O dye complexes in a 1:1 ratio with carboxylic acid groups and the amount of carboxylic acid groups can be easily quantified by the absorbance of the complex at 633 nm.[27,28] On the basis of the assay, the pAAc grafting on SF resulted in a ∼3-fold increase in the amount of carboxylic acid groups, as evidenced by the UV−visible spectra of SF and pAAc−SF in Figure 1d. As can be seen from the spectra, the amount of carboxylic acid groups increased by increasing the monomer concentration from 5% to 20% (v/v) aqueous AAc, thus providing control over the amount of carboxylic acid groups on the surface of SFs. The staining with the dye also suggested relatively uniform grafting across samples (Figure S4 in the SI). We further confirmed the availability of the increased carboxylic acid functional groups for bioconjugation, by conjugation of rhodamine via the carbodiimide coupling reaction. As is evident from the absorbance of the rhodamine-conjugated SF and pAAc−SF (Figure 1e), more conjugation handles (more conjugated rhodamine) were available for pAAc−SF compared to SF.

Finally, we examined the ability to modulate cellular adhesion by the polymer-modified SFs. We cultured HeLa cells over unmodified SF, pAAc−SF, pHEMA−SF, and PEG−SF for 24 h and then analyzed the films for cell adhesion and proliferation. SF without any modifications showed the lowest cell adhesion. pAAc−SF showed a much higher amount of cells adhering and proliferating compared to pHEMA, whereas PEG−SF showed almost no cell adhesion, similar to unmodified SF (Figure 2a). The viability and proliferation of the cells was quantified by the MTT assay. A significant increase in the amount of cells was observed for pAAc−SF compared to unmodified SF (Figure 2b). The higher cell adhesion in pAAc-SF compared to that of pHEMA-SF, PEG−SF, and unmodified SF might be due to the ability of pAAc−SF to adsorb more proteins, as indicated by the adsorption of BSA and IgG on the SFs (Figure S5a,b in the SI). Interestingly, PEG conjugation on pAAc−SF increased the hydrophilicity, but the activity of the film reverted back to low cell adhesion and protein adsorption properties. The adherent cells were alive, as confirmed by the calcein AM staining of the cells after 24 h of culture on modified films (Figure 2c). Thus, carboxylic acid groups appeared to significantly ($p \leq 0.0001$) promote attachment, spreading, and growth of the cells, at a level comparable to that of the tissue culture polystyrene. The cell count was also obtained by image analysis (Figure S6 in the SI). There was no apparent difference in the cellular morphology of the cells grown on SFs before and after treatment, as indicated by the SEM images and high-resolution optical micrographs (20× and 40×; Figures S7 and S8 in the SI). The modulation of cell adhesion on the SF and modified SFs was not cell-line-specific because similar adhesion profiles (low for PEG−SF,


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high for pAAc−SF, and medium for pHEMA−SF) were observed when rat skeletal muscle cells (L6) were cultured on silk and modified silk (Figure S9 in the SI). These results clearly indicate our ability to tune the cellular adhesive property of SFs from low to medium to high while also tuning the hydrophilicity. It also reiterates the importance of surface groups for modulating the cellular response. It is suggested that charged groups such as carboxyl can facilitate cell adhesion.[6,15,27−29] Also, the influence of surface functional groups on the differentiation of human mesenchymal stem cells has earlier been reported.[6,12] Our strategy can be easily employed to develop a patterned surface with multiple functionalities for controlling cellular proliferation. For tissue engineering applications, precise spatial control of cell adhesion is essential and is usually achieved via adhesive ligands. While our technique allows the conjugation of adhesive ligands to create complex topographies and chemical surfaces, we explored whether, by conjugating PEG on part of pAAc−SF (high cell adhesion), we can achieve precise spatial control over cell adhesion on the films. As can be seen from Figure 2d, in a preliminary cellular adhesion experiment, half of pAAc−SF had high cell adhesion, whereas the other half, where PEG was conjugated to pAAc−SF, had no cells. This suggests our ability to use this technique for obtaining a patterned silk substrate without the use of lithographic techniques. Such control over cell adhesion in a particular region of the SFs is difficult to obtain via the postfibroin chemical modification, $\beta$-sheet formation strategies.

## CONCLUSION

In conclusion, we have developed a simple surface technique involving plasma etching for grafting pAAc and pHEMA on regenerated SFs. The polymer grafting allowed us to tune the water contact angles with ease. The carboxyl functional groups on pAAc-grafted SFs were further used to conjugate other polymers or dyes like rhodamine. By this strategy, we were able to tune the property of SFs from low cell adhesion (unmodified SF) to increased cell adhesion (pAAc−SF) and back to low cell adhesion (PEG−SF). Also, we were able to achieve spatial control over the cellular adhesion property of the SFs via our surface modification technique. Such tunability of the surface chemistry and bioactivity of SF substrates is an advantage, which will enable many complex bioactive SF surfaces, with much utility in tissue engineering.

## ASSOCIATED CONTENT

### Supporting Information

Detailed characterization of the surface modification, enzymatic degradation, protein adsorption, and cell adhesion on modified and unmodified SFs. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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