Synthesis of Photocrosslinkable Copolymers of Cinnamoyl Group-modified Methacrylate and 2-Hydroxyethyl Methacrylate, and Fibroblast Cell Growth on Their Thin Films

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Cinnamoyl-modified methacrylate, 2-cinnamoyloxyethyl methacrylate (CEMA), was synthesized from 2-hydroxyethyl methacrylate (HEMA) and cinnamoyl chloride. Copolymers with various cinnamoyl contents were synthesized by random copolymerization of HEMA and CEMA at different feed ratios. Thus three copolymers with different CEMA contents were synthesized, and the monomer composition ([HEMA]:[CEMA]) in the copolymers ranged from 75:25 to 0:100. The coating films of copolymers on glass plates were prepared by the dip-coating method. Photo-induced dimerization of the cinnamoyl groups in the copolymers was confirmed by the ultraviolet-visible (UV-Vis) spectral changes. The cell proliferation on the photocrosslinked copolymers was tested using 3T3 Swiss albino mouse embryo fibroblasts. The results of the cell proliferation assay revealed that the photocrosslinked copolymers carrying more cinnamoyl groups promoted the cell proliferation compared with the photocrosslinked copolymers carrying less cinnamoyl groups. According to the results in the present study and our previous studies on cinnamoyl-modified trehalose and cinnamoyl-modified hydroxypropyl cellulose, it is presumable that the photodimerized cinnamoyl groups existing on the surface of materials at high density have some preferable effect on promoting fibroblast cell adhesion.

Keywords: Cinnamoyl group, 2-Hydroxyethyl methacrylate, Copolymer, Photocrosslinking, Cell compatibility, Fibroblast

1. Introduction

Recently, controllability and biocompatibility of the outermost surface layer has been investigated, since physical properties of the outermost surface layer of the material are known to be important for biomaterials [1-4]. In this context, materials having photoreactive or photoresponsive groups have attracted attention due to its high potential for surface controllability and pattern-formation ability [5-7]. In the present study, we focused on a cinnamoyl group as a photoreactive group for fabrication of surface coating materials. Compounds having cinnamoyl groups are often found in nature, especially in plant bodies. Two cinnamoyl groups are known to undergo photo-dimerization by ultraviolet (UV) irradiation to yield a cyclobutane ring structure [8] (Scheme 1). This reaction has been used to fabricate photocrosslinked polymer materials [9-14] and photocrosslinked hydrogels [15-17], as well as materials for photoinduced alignment of liquid crystals [18-22]. Since cinnamoyl groups have proved to be low toxic [23,24], it is expected to apply the cinnamoyl-modified
polymers to safety cell-contacting materials. Some research groups have studied the antioxidant activity of cinnamoyl derivatives [25, 26]. Except for our original reports [27,28], however, there are only few reports regarding the cell proliferation assays carried out on photocrosslinked materials carrying cinnamoyl groups at high density. Wu et al. [29] carried out the in vitro cell adhesion and growth assay of butyl rubber derivatives that were prepared from hydroxyl-functionalized butyl rubber and cinnamoyl chloride, cured by UV-light exposure. They showed that no toxic compound leaches from these cinnamoyl-functionalized butyl rubber derivatives, but myoblast cell adhesion was low on the crosslinked films. Zhu et al. [30] carried out the in vitro cell adhesion and growth assay of biodegradable polyester photo-crosslinked films prepared from poly(glycerol-co-sebacate) and cinnamoyl chloride. These polymer films showed biodegradability and cell adherent properties. In these two studies, the degree of substitution (DS) of cinnamoyl groups was not so high (up to 45%). Though dead cells were scarcely observed, cell proliferation was lower than that on the control tissue culture polystyrene (TCPS). Some research groups have studied cinnamoyl groups as crosslinking agents to bind cinnamoyl groups and form gels by light irradiation in the formation of hydrogels [15-17,31-33], but most of them did not report cell proliferation assays of photocrosslinked materials. Miyamoto et al. [32] evaluated the in vivo biocompatibility of cinnamoyl-modified hyaluronate, and found that the photo-crosslinked hyaluronate gel has preferable biocompatibility. In this study, cinnamoyl groups was used as a crosslinking agent, and the DS was also not so high (~20%).

From the results of our previous study, photocrosslinked TC with higher DS promoted cell proliferation compared to TC with lower DS. The contact angle of photocrosslinked TC with a higher DS was 101.0 ± 1.6° which was much higher than that of TCPS, and it is out of the range known to be suitable for cell adhesion [35, 36]. Nevertheless, cells proliferated most unexpectedly on TC with higher DS. We considered that these results were due to the specific properties of photodimerized cinnamoyl groups or its ester structure of trehalose, though there are many unclear points. Since trehalose is used for preservation of proteins, organs and cells [37], the effect of trehalose was also considered as a factor. In order to investigate the necessity of trehalose, we synthesized hydroxypropyl cellulose (HPC) cinnamate (HPC-C) with different DS and carried out the cell growth assay. The assay revealed that the number of adherent cells slightly increased on the photocrosslinked HPC-C with highest DS compared to that with lower DS [38]. Therefore, as the next step, we subsequently planned to investigate the effect of cinnamoyl groups on cell adhesion and proliferation using polymers without carbohydrate backbone.

Poly(2-hydroxyethyl methacrylate) (PHEMA) have been known to be non-toxic to cells and organisms and are widely used as biomaterials [39,40]. Horák et al. [41] reported that PHEMA hydrogel spherical particles prepared by radical suspension polymerization was found to be non-toxic by the in vitro tests using human larynx epidermoid carcinoma cells. Therefore we chose PHEMA as a non-toxic backbone polymer for cinnamoyl modification.

In this study, 2-cinnamoyloxyethyl methacrylate (CEMA) was synthesized by reacting 2-hydroxyethyl methacrylate (HEMA) and cinnamoyl chloride. Then one homopolymer (PCEMA) and two copolymers (poly(HEMA-co-CEMA)) with different monomer composition were synthesized from HEMA and CEMA changing the feed ratio of monomers (Scheme 2). The obtained polymers were coated on a cover glass to prepare a photocrosslinked thin film. The thin films were evaluated for fibroblast cell adhesion and proliferation.

2. Experimental

2.1. Materials
Cinnamoyl chloride was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). 2-Hydroxyethyl methacrylate (HEMA), 2,2'-azobis(isobutyronitrile) (AIBN), triethylamine and organic solvents were obtained from Kanto Chemical Co. (Tokyo, Japan). These reagents and solvents were used without further purification. We were afforded 3T3 Swiss albino mouse embryo fibroblast cells (RCB1642) by Riken BRC Cell Bank (Ibaraki, Japan). Dulbecco’s modified Eagle’s medium (D-MEM), penicillin–streptomycin solution, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Wako Pure Chemical Ind., Ltd (Osaka, Japan). Fetal bovine serum (FBS), 25% trypsin–ethylenediamine-tetraacetic acid (EDTA) solution, and 0.4% trypan blue solution were from Gibco (Carlsbad, CA, USA).

2.2. Synthesis of CEMA

CEMA was synthesized according to the method previously reported by Kim et al. [42]. HEMA (66 mmol) was dissolved in anhydrous tetrahydrofuran (THF) (100 mL), and triethylamine (66 mmol) were added to the solution. A solution of cinnamoyl chloride (79 mmol) in THF (50 mL) was added dropwise to the mixture of HEMA and triethylamine. After stirring at 0 °C for 2 h, triethylamine hydrochloride salt was removed by filtration as a side product. Then the solution was evaporated to a small volume (~20 mL), and the desired product was separated from the reaction solution by pouring the reaction solution into hexane (500 mL). Pale yellow liquid product of CEMA was obtained after filtration and drying in vacuo (yield: 53%).

2.3. Synthesis of poly(HEMA-co-CEMA)

Polymers carrying cinnamoyl groups in the side chain (poly(HEMA-co-CEMA)) was prepared by reacting the feeding molar ratio of HEMA and CEMA at 55:45, 10:90 and 0:100. HEMA and CEMA was dissolved in N\textsubscript{3},N\textsubscript{3}-dimethylformamide (DMF) (15 mL), and AIBN (1 wt%) was added to the solution. After stirring at 70 °C for 24 h in a nitrogen atmosphere, the reaction product was purified by precipitation in water and washing with methanol. White powder was obtained respectively after drying in vacuo for 2 days.

2.4. Photocuring of polymers carrying cinnamoyl groups in the side chain

Polymer thin films on glass substrate were prepared by the dip-coating method with 20 mg/mL solution of polymers in chloroform using an Aiden DC4100 dip coater (Aiden Co., Ltd., Kobe, Japan). The dipping time was 1 min and the raising speed was 0.5 mm/s. Quartz glass plates with 10 mm width and 1 mm thickness were used for the UV–Vis spectroscopy. For cell growth, round-shape cover glasses with a diameter of 15 mm (Matsunami Glass Ind., Ltd, Osaka, Japan) were used as substrates. These glass substrates were cleaned with the mixed acid solution of concentrated nitric acid, concentrated sulfuric acid, and water (1:3:6) before coating with polymers. UV light irradiation was carried...
out by using an Ushio SP-7 spot UV curing system with a 250-W deep UV lamp (Ushio Inc., Tokyo, Japan) through a poly(methyl methacrylate) (PMMA) plate to cut the shorter-wavelength light. The samples were placed at a distance of 17 cm from the light source, and the light intensity was 60 mW/cm².

2.5. Characterization  
To analyze the chemical structure and monomer composition of polymer products, proton nuclear magnetic resonance (¹H NMR) measurement was carried out using a Bruker AV-400 spectrometer (Bruker, Billerica, MA) with samples dissolved in CDCl₃. The monomer composition of copolymers was calculated from the peak integral values of ¹H NMR spectra. Molecular weight measurement was carried out using a gel permeation chromatography (GPC) system equipped with two Shodex SB-806M HQ columns (diameter: 8 mm, length: 300 mm) (Showa Denko K.K., Tokyo, Japan), a Shodex DS-4 pump, and a Shodex RI-101 detector. DMF was used as an eluent, and polystyrene samples with low polydispersity (molecular weight ranged from 580 to 377,400) were used as a molecular standard. UV-Vis spectra were recorded on a JASCO V-650 spectrophotometer (JASCO Corp., Tokyo, Japan) with an attachment for the measurement of plates and films. The surface morphologies of photo-cured polymers were observed by a Hitachi S-4700 field emission scanning electron microscope (FE-SEM) (Hitachi High-Technologies Corp., Tokyo, Japan). Each sample was coated with gold prior to the observation using a Hitachi E-1030 ion sputter (Hitachi High-Technologies Corp.). The wettability of the copolymer surface was evaluated by the water contact angle measurement. A 4-μL droplet of ultrapure water was placed at five positions on each thin film followed by taking a digital picture to determine the static contact angle by an Excimer SImage mini contact angle meter (Excimer Inc., Yokohama, Japan). The five values were averaged.

To investigate the thermal degradation behaviors, a thermogravimetric analysis (TGA) were carried out using a PerkinElmer Pyris 1 differential scanning analyzer (PerkinElmer Inc.). Ten milligrams of each sample were sealed in an aluminum sample pan and the temperature was raised from -10 °C to 200 °C at the rate of 10 °C/min, then lowered to -10 °C at the rate of 10 °C/min, and raised to 200 °C at the rate of 10 °C/min. Glass transition temperature (Tₐ) was determined from the DSC thermogram obtained in the second heating step.

2.6. Cell proliferation assay  
Round-shape cover slips (15 mm diameter) coated with photocrosslinked poly(HEMA-co-CEMA) were used for cell proliferation assay. The coated slips were placed in a 24-well TCPS plate, and sterilized with ethylene oxide gas. After washing with D-MEM, 3T3 Swiss albino mouse embryo fibroblast cells were seeded at the density of 3 × 10³ cells/mL. 300 μL of D-MEM containing 10% FBS and 1% penicillin–streptomycin solution was added to the cells as a medium, and the cells were incubated at 37 °C in a CO₂ incubator with 5% CO₂. Cell growth and morphology were observed using a Carl-Zeiss Axio Vert.A1 phase-contrast microscope (Carl Zeiss AG, Oberkochen, Germany). After predetermined time, cells adhered onto each sample were treated with 0.25% trypsin–EDTA solution and trypan blue solution, and the number of cells was calculated using a hemocytometer. Cell proliferation was also assayed by MTT assay, which is based on the mitochondrial activity of viable cells. To the samples, a 300 μL of MTT solution (1.0 mg/mL) was added and incubated for 90 min at 37 °C under 5% CO₂. The solution was mixed with a 300 μL of cell lysing solution containing 10% polyoxyethylene octylphenyl ether (NP-40). The absorbance of the resulting solution at 570 nm was measured using a Bio-Rad iMark microplate reader (Bio-Rad Laboratories Inc., Hercules, CA, USA) (n = 4 for each assay). Cell staining experiment was carried out using fluorescent-labeled phalloidin (Phalloidin-iFluor 488 Reagent, Abcam plc., Cambridge, MA) for staining of actin fibers and 4',6-diamidino-2-phenylindole (DAPI, for staining of nuclei). The cells of 2-day culture were fixed and stained. The stained cell images were observed by a Keyence BZ-X710 fluorescent microscope (Keyence Corp., Osaka, Japan).
3. Results and discussion

3.1. Synthesis of poly(HEMA-co-CEMA)

HEMA and CEMA were copolymerized by the free-radical copolymerization using AIBN as a radical initiator. The homopolymer of CEMA and random copolymers of HEMA and CEMA were analyzed by $^1$H NMR spectroscopy. Figure 1 shows the $^1$H NMR spectra of HEMA, CEMA and polymerization products. The signals corresponding to the methyldiene group of methacrylate at 5.6 ppm and 6.2 ppm disappeared, and the signal corresponding to the methylene groups emerged around 1.9 ppm after the polymerization. According to the calculation from the integral values of $^1$H NMR spectra, the monomer composition of poly(HEMA-co-CEMA) was 60:40 and 24:76 (HEMA:CEMA), corresponding to the polymerization feed ratio of 55:45 and 10:90 (HEMA:CEMA), respectively (Table 1). Therefore, the reaction products with monomer composition of 60:40 and 24:76 was defined as P(H60/C40) and P(H25/C75), respectively. The homopolymer of CEMA was defined as

![Fig. 1. $^1$H NMR spectra of (a) HEMA, (b) CEMA, (c) P(H60/C40), (d) P(H25/C75) and (e) P(H0/C100); CDCl$_3$ was used as a solvent for the measurement.](image)

| Sample        | Feed ratio | Monomer composition | Molecular weight | PDI* |
|---------------|------------|---------------------|------------------|------|
|               | HEMA      | CEMA | HEMA | CEMA | $M_n$ | $M_w$ |      |
| P(H60/C40)    | 55        | 45    | 60   | 40   | 15,000 | 91,000 | 6.1  |
| P(H25/C75)    | 10        | 90    | 24   | 76   | 17,000 | 65,000 | 3.8  |
| P(H0/C100)    | 0         | 100   | 0    | 100  | 17,000 | 92,000 | 5.4  |

* Polydispersity index ($M_w/M_n$)
P(H0/C100). The number-average molecular weight ($M_n$) and the weight-average molecular weight ($M_w$) of poly(HEMA-co-CEMA) were shown in Table 1. Copolymers with any monomer composition were soluble in dimethylsulfoxide (DMSO), DMF, acetone, tetrahydrofuran, chloroform, acetonitrile and ethyl acetate; and they were insoluble in water, methanol, ethanol, diethyl ether, toluene and hexane.

3.2. Thermal properties of poly(HEMA-co-CEMA)

TGA and DSC analyses were carried out to investigate thermal properties. Figure 2 shows the results of TGA measurements for poly(HEMA-co-CEMA). We observed one-step degradation behavior for all samples at around 200-400 °C. P(H60/C40) had lower decomposition temperature than P(H25/C75) and P(H0/C100). This decrease of thermal stability of P(H60/C40) was considered to be caused by the existence of the hydroxy groups of HEMA components which can be involved in the degradation process of PHEMA [43].

![TGA curves of P(H60/C40), P(H25/C75) and P(H0/C100).](image)

Figure 3 shows DSC thermograms of poly(HEMA-co-CEMA) with various monomer compositions. Each thermogram of poly(HEMA-co-CEMA) shows one glass transition temperature ($T_g$). The $T_g$ of poly(HEMA-co-CEMA) decreased with an increase of the contents of CEMA components in the copolymer. As we previously reported for HPC [38], $T_g$ is considered to be influenced by the intermolecular interaction between hydroxyl groups. A decrease of hydroxy groups leads to a decrease of intermolecular interaction and subsequently to a decrease of $T_g$. Ali et al. [44] reported the DSC measurement of hydrated poly(HEMA-co-CEMA). In the literature, they briefly reported that the $T_g$ of hydrated poly(HEMA-co-CEMA) increased with an increase of the contents of CEMA components in the copolymer, contrary to our results. This difference is due to the presence or absence of water, which is generally known to weaken hydrogen bonds, and implying that the intermolecular hydrogen bonds should influence the micro-Brownian motion of poly(HEMA-co-CEMA) chains.

![DSC curves of P(H60/C40), P(H25/C75) and P(H0/C100). Arrows point to glass transition temperatures.](image)

3.3. Photocrosslinking of poly(HEMA-co-CEMA)

Homogeneous and transparent thin films of uncured poly(HEMA-co-CEMA) were obtained by the dip-coating method. Photocrosslinking of poly(HEMA-co-CEMA) occurred by UV irradiation because the polymer has a number of cinnamoyl groups in one molecule, and the course of crosslinking was detected through the UV-Vis spectral changes. The UV-Vis spectra of poly(HEMA-co-CEMA) are shown in Fig. 4 with variation of UV irradiation time. The unirradiated poly(HEMA-co-CEMA) had a main absorption peak at 283 nm attributed to the ethenylbenzene unit of cinnamoyl groups. This absorption decreased in the time course of UV irradiation, suggesting the occurrence of photo-dimerization [13-15,17,29,30,33,34,38,44]. The rate of this absorption decline of P(H60/C40) was higher than that of P(H25/C75) and that of P(H0/C100). This phenomenon was considered to depend on the density of cinnamoyl groups. For poly(HEMA-co-CEMA) with high density of cinnamoyl groups, the reaction became slower by the limitation of mobility of side chains in course
of crosslinking. Nonetheless, homogeneous transparent thin films of uncured poly(HEMA-co-CEMA) on cover glass were obtained by the dip-coating method, and all films were apparently homogeneous and transparent after crosslinking. The homogeneity was kept also in micro scale (Fig. 5) when observed by FE-SEM.

3.4. Surface wettability of poly(HEMA-co-CEMA)

The surface wettability is also an important factor for the use of materials for biomaterials [45]. The contact angles of uncured and photocrosslinked poly(HEMA-co-CEMA) are shown in Fig. 6. The contact angles of uncured P(H60/C40), P(H25/C75) and P(H0/C100) were 61.7° ± 2.8°, 68.1° ± 3.1° and 75.8° ± 2.4°, respectively. On the other hand, the contact angles of photocrosslinked films, P(H60/C40)-UV, P(H25/C75)-UV and P(H0/C100)-UV, were 69.6° ± 2.6°, 74.9° ± 2.6° and 78.4° ± 2.4°, respectively. An increase of a contact angle means a decrease of wettability. Therefore, the surface wettability of poly(HEMA-co-CEMA) decreased with an increase of CEMA component and with
photocrosslinking. This tendency was previously observed as we reported in our previous study [27, 38].

3.5. Cell culture assays on photocrosslinked polymers carrying cinnamoyl groups in the side chain

3T3 Swiss albino fibroblast cells were cultured on P(H60/C40)-UV, P(H25/C75)-UV and P(H0/C100)-UV thin films in comparison with an acid-washed cover glass and TCPS. The polymer samples were coated on cover glasses by the dip-coating method. The number of cells attached on each film was counted using a hemocytometer. Figure 7 shows the shape and morphology of cells grown on each sample after a 5-day culture, and Fig. 8 shows the number of cells grown on each sample measured by the cell count method after detachment of cells on each sample. There observed many spread cells on the photocrosslinked polymers carrying cinnamoyl groups in the side chain, especially compared to an acid-washed cover glass. Cell viability was also determined by trypan blue staining for poly(HEMA-co-CEMA). We found very few cells dead on each sample, suggesting nontoxicity of the photocrosslinked polymers carrying cinnamoyl groups in the side chain. Fibroblasts adhering to TCPS and photocrosslinked poly(HEMA-co-CEMA) spread to the entire surface of each sample, although a few number of cells adhered to acid-washed cover glass which is too hydrophilic for cell adhesion. The morphology of cells adhered on P(H0/C100)-UV was that of a cell moderately spreading to obtain larger area for adhesion. Figure 9 shows the results of the MTT assay. The MTT assay reflects cell proliferation measured from the aspect of mitochondrial activity. As a result, the cell
proliferation on P(H60/C40)-UV and P(H25/C75)-UV was no less than TCPS, and that on P(H0/C100)-UV was found to be promoted more than TCPS until 5-day culture. The result was consistent with the result of the cell count method above.

We observed the development of cytoskeleton of cells adhered on P(H0/C100)-UV by an immunofluorescence microscopy method using fluorescent probe-modified phalloidin which can bind to actin filaments. Figure 10 shows the microscopic images of cytoskeleton and nuclei of 3T3 Swiss albino cells on P(H0/C100)-UV. This result indicates that the cytoskeleton of cells developed well on the substrate, suggesting the adhesion was supported by the cytoskeleton.

These results are similar to the cell proliferation results on trehalose cinnamate and cinnamoyl-modified HPC, which we previously reported [27,38]. These studies revealed that the fibroblast cell adhesion and proliferation on crosslinked polymers were promoted most on thin films of photocrosslinked polymers carrying most cinnamoyl groups. In our early discussion [27], we had postulated that the molecular structure of crosslinked cinnamoyl trehalose might have been important for the cell proliferation. More properly, though we described the phenomenon as if the cells recognize the molecular structure, this should be applied to the recognition of proteins that may firstly contact with the polymer surface. Indeed, amount of protein adsorbed on UV-irradiated poly(HEMA-co-CEMA) increased with an increase of hydrophobicity (2.2 μg, 2.3 μg, and 2.8 μg, for P(H60/40)-UV, P(H25/C75)-UV, and P(H0/C100)-UV, respectively) when bicinchoninic acid (BCA) assay was carried out on each sample impregnated in the medium containing 10% FBS. Generally, protein adsorption is known to increase on the hydrophobic surface, but it does not always promote cell adhesion because the protein structure on the surface is important as well as amount of adsorbed protein. Therefore, in the
previous study in which we observed good cell proliferation on cinnamoyl-modified HPC, we presented an alternative discussion from the viewpoint of the surface modulus (hardness) of the sample. Fibroblast cells are known to adhere stiffer surface than softer surface as the research group of Wang advocated [46,47]. However, we had not been able to deny the possibility of the combinatorial structure of dimerized cinnamoyl (truxillyl or truxinyl) and glucose yet. Therefore, in the present study, we tried to investigate the cell growth on the photocrosslinked polymers without the structure of glucose units. In this context, the fact that fibroblast cells preferred the crosslinked methacrylate polymer surface with more cinnamoyl groups proved that the cells do not discriminate the combinatorial structure of dimerized cinnamoyl and glucose units. Our research results support the consideration that the fibroblast cells recognize stiffness of material surface and prefer stiffer substrate. Yeung et al. [48] controlled stiffness of the polyacrylamide gel moduli by crosslinking and investigated cell morphology, adhesion and α5 integrin production. Their results showed that the stiffness of the material was mainly important for the adhesion of fibroblasts on substrate, and that the expression of α5 integrin in fibroblasts increased with an increase of substrate stiffness. We think that this situation can be applied to the fibroblasts behavior on our crosslinked polymer substrates.

4. Conclusion

Three types of copolymers with a poly(methacrylate) main chain carrying cinnamoyl groups in the side chain (poly(HEMA-co-CEMA)) were synthesized by copolymerization of HEMA and CEMA at different ratios. The thin films of poly(HEMA-co-CEMA) on cover glass substrate were prepared by the dip-coating method, and UV irradiation on the thin films was carried out. The UV-Vis spectra of the copolymers changed by the UV irradiation; a decrease of the absorbance around 280 nm was observed, implying the dimerization of cinnamoyl groups. The homogeneity and transparency of the thin film was kept during the UV irradiation. Cell compatibility was investigated through culturing of fibroblast cells on each copolymer. The fibroblast cells adhered and grew on thin films of each photocrosslinked polymer. Furthermore, the highest proliferation of cells was observed on the crosslinked polymer with the highest cinnamoyl content. We considered this highest proliferation was due to the stiffness of the substrate. Currently, we are investigating the effect on cell adhesion and proliferation using photoreactive groups other than cinnamoyl groups to prove that cinnamoyl groups would not be involved with the promotion of cell proliferation.

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