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ORIGINAL ARTICLE

Preparation of temperature-responsive, cationized, poly (ε-caprolactone)-based, cross-linked materials by a macromonomer design and positive charge control on the surface

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Abstract

In this study, a convenient method to synthesize cationic macromonomers containing branched poly(ε-caprolactone) (PCL) was developed, and stable materials were derived by photo-cross-linking reactions. In fact, a bromomethyl-terminated modification was carried out at the hydroxyl end groups of the starting PCL; then, the terminal groups reacted with 2,2′-dimethylaminoethyl methacrylate to afford the objective macromonomers, which had N,N′-dimethylmethacrylamino groups at the chain ends. The resulting PCL-based materials were cross-linked by UV light irradiation and were stable against exposure to organic solvents and heating above the softening points. The surface properties of the cationic, PCL, cross-linked membrane were evaluated by measuring the zeta potentials and performing anionic dye adsorption tests using Acid Red 87. As expected, the cationic, PCL, cross-linked membrane surfaces showed a positive charge and greater dye adsorption than the naked PCL, which depended on the cationic contents and temperature. Over the softening point, the positive charge steeply increased. The morphologies of adhered human mesenchymal stem cells on the PCL materials with lower cationic contents were preliminarily observed and shown to be well dispersed. The PCL-based materials in this study could enhance cell interaction and be useful for scaffold or mechanobiology studies.

Introduction

Polycaprolactone (PCL) is well known as a semi-crystalline polymer, and some studies on temperature-responsive materials based on crystal melting have been reported [1, 2]. In addition, such features are suitable for the design of shape memory materials and specifically designed polymers that contain PCL as a backbone or a side chain can demonstrate shape memory properties [3–6]. Our group has also successfully prepared surface shape memory materials derived from PCL that could contribute to the progress of mechano-biological research [7–9]. Using a well-designed silicon wafer mold to create surface patterns, the effects of the surface nano-topography on cell functions have been discussed [10]. These PCL-based materials were exclusively composed of PCL by cross-linking a mixture of 4-branched and 2-branched PCL macromonomers. By controlling the mixing ratio, the softening temperature could be modulated. Furthermore, the photoreactive groups at each chain end allowed stable cross-linked materials to be achieved using only UV light irradiation.

Many studies dealing with PCL-based materials as scaffolds in regenerative medicine have been reported due to their biodegradability and biocompatibility. Recently, electrospun PCLs have been utilized as scaffolds mimicking the extracellular matrix [11]. Ideally, cell interactions with such artificial scaffolds should be enhanced; therefore, functional material design is an exciting subject. Modification of PCL via the addition of functional groups is a highly promising approach for designing bio-conjugates or

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controlling the interactions of the material with living cells or biological components. For example, functionalized PCL was prepared by a ring-opening copolymerization using CL and carboxyl-protected CL [12]. The carboxy groups could be useful for bioconjugation. A simple method to enhance cell interactions with scaffolds is cationization. For example, graft or block copolymer systems have been used to introduce cationic groups in polyesters, such as polyethylene glycol-block-(PCL-graft-poly(2-(dimethylamino) ethyl methacrylate)) [13]. Materials using other cationic segments and chitosan-graft-PCL/PCL nanofibers for scaffold materials have also been reported [14]. To prepare PCL-based networks for shape memory materials, end functional groups have been effectively used [15, 16].

Thus, the development of PCL-based, cross-linked materials with cationic moieties is interesting because they could be used as shape memory materials that can also interact with bio-components by electrostatic interactions. To achieve this, haloalkyl and amino groups were used for the cationization of PCL. The hydroxyl end groups in the PCL starting material were converted to bromomethyl groups by a reaction with bromoacetyl bromide. Subsequently, the bromomethyl-terminated PCL was reacted with N,N-dimethylaminoethyl methacrylate to afford the desired cationic macromonomers. This preparation was very convenient for the simultaneous introduction of cationic groups and cross-linkable moieties. Designing the preparation of cationic, cross-linked, PC-based materials allows the new design of copolymerization with branched, cationic, PCL macromonomers. Additionally, preliminary cell interaction tests were carried out using human mesenchymal stem cells to test the potential enhancement of cell interactions for mechanobiology research.

Materials and methods

Materials

ε-Caprolactone, pentaerythritol, tetramethylene glycol, stannous octanoate, bromoacetyl bromide, a trimethylamine THF solution, 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone as a photosensitizer, and tetrabromofluorescein sodium salt (Acid Red 87) as an anionic dye were purchased from Tokyo Chemical Industry (Tokyo, Japan) and used as received. Triethylamine was dried over KOH. The THF, chloroform and ethyl acetate used in the reaction and purification were of reagent grade and used as received. For gel permeation chromatography (GPC) measurements, analytical grade THF was used. Human mesenchymal stem cells (hMSCs) and MSC growth medium supplemented with mesenchymal cell growth supplement, L-glutamine, and GA-1000 (gentamicin sulfate/amphotericin-B) were purchased from Lonza. Dulbecco's phosphate buffered saline (D-PBS) and ultra-pure distilled water (DNAse and RNAse, free) were purchased from Nacalai Tesque (Kyoto, Japan) and Invitrogen, respectively. Triton-X100, 4,6-diamidino-2-phenylindole (DAPI), and phalloidin conjugated with rhodamine were purchased from Sigma.

Characterizations

^{1}H-NMR spectra were recorded using a JEOL RESONANCE spectrometer (JNM-ECP500) (Tokyo, Japan) operating at 400 MHz to confirm that the reaction occurred and to calculate the conversion. Deuterated chloroform was used as the solvent, and chemical shifts were expressed in ppm with respect to tetramethylsilane (TMS). The molecular weights of the PCL starting material and bromomethyl-terminated PCL were estimated using gel permeation chromatography (Tosoh HLC-8220 GPC). A calibration was performed using a polystyrene standard. The thermal properties of the sample were investigated at each reaction step using a differential scanning calorimeter (DSC, DSC6100, Seiko Instruments Inc., Tokyo, Japan). The zeta potentials of the PCL films were measured using a zeta potential analyzer (ELS-2000ZS, Otsuka Electronics, Osaka, Japan).

Preparation of bromomethyl-terminated 4-branched PCL (4b10PCL-Br)

Branched PCL was prepared by a conventional ring-opening polymerization of CL initiated by pentaerythritol (PE) or tetramethylene glycol (TMG)[7]. For 4-branched PCL, a few drops of stannous octanoate were added to a flask containing 1. of dried PE and 54.0 g of CL, and the reaction mixture was stirred under a dry nitrogen atmosphere at 140 °C for 6 h. After being allowed to cool, the resulting viscous liquid was diluted with THF and poured into a mixture of ethyl acetate and hexane. The desired 4-branched PCL was obtained as a white powder (yield 94.3%). ^{1}H-NMR δ ($CDCl_3$, p.p.m.): 1.40 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 1.65 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$-), 2.31 (t, –CO–CH_2 $CH_2CH_2CH_2CH_2O$–), 3.62 (m, –CO–$CH_2CH_2CH_2CH_2$ CH_2OH (chain end)) and 4.04 (–$CH_2CH_2CH_2CH_2CH_2O$–). GPC measurements indicated that the number-average molecular weight and its distribution were 6670 and 1.17, respectively.

Next, 6.0 mL of bromoacetyl bromide was added to a THF solution containing 10.0 g of the 4-branched PCL prepared by the above procedure and stirred for 24 h. The reaction mixture was subsequently poured into a mixture of ethyl acetate and hexane to afford and purify

the 4b10PCL-Br (yield 83.9%). ^{1}H-NMR δ ($CDCl_3$, p.p.m.): 1.40 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 1.65 (m, –-CO–$CH_2CH_2CH_2CH_2CH_2O$–), 2.31 (t, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 3.88 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–CO–CH_2Br), 4.08 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–CO–CH_2Br) and 4.04 (–$CH_2CH_2CH_2CH_2CH_2O$–). The same procedure was adopted to prepare 2-branched bromomethyl-terminated PCL (2b20PCL-Br) using 2-branched PCL with TMG as an initiator.

Preparation of 4-branched PCL with cationic and cross-linkable moieties (4b10PCL-DMAEMA)

A total of 5.0 g of the 4b10PCL-Br prepared by the above procedure and 1.0 mL of dimethyl aminoethylmethacrylate were mixed in THF and stirred for 24 h. The resulting solution was poured into a large mixture of ethyl acetate and hexane to afford 4b10PCL-DMAEMA as a white powder (yield 42.4%). ^{1}H-NMR δ ($CDCl_3$, p.p.m.): 1.40 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 1.65 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 1.96 (s, –CO–C(CH_3)=CH_2), 2.31 (t, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 3.72 (m, –O–CO–$CH_2N^+(CH_3)_2CH_2CH_2$–CO–C(CH_3)=CH_2) 4.04 (–$CH_2CH_2CH_2CH_2CH_2O$–), 4.35 (m, –O–CO–$CH_2N^+(CH_3)_2CH_2CH_2$–CO–C(CH_3)=CH_2), 4.67 (m, –O–CO–$CH_2N^+(CH_3)_2CH_2CH_2$–CO–C(CH_3)=CH_2) 5.03 (m, –O–CO–$CH_2N^+(CH_3)_2CH_2CH_2$–CO–C(CH_3)=CH_2), 5.64, 6.08 (m, –O–CO–$CH_2N^+(CH_3)_2CH_2CH_2$–CO–C(CH_3)=CH_2). 2-Branched compounds (2b20PCL-DMAEMA) were prepared by the same procedure using 2b20PCL-Br. Separately, non-cationic, methacryloyl-terminated, branched PCL (2b20PCL-MA and 4b10PCL-MA) were prepared using the starting branched PCL and methacryloyl chloride. ^{1}H-NMR δ ($CDCl_3$, p.p.m.): 1.40 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 1.65 (m, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 1.96 (s, –CO–C(CH_3)=CH_2), 2.31 (t, –CO–$CH_2CH_2CH_2CH_2CH_2O$–), 4.04 (–$CH_2CH_2CH_2CH_2CH_2O$–), 5.56 and 6.07 (m, –CO–C(CH_3)=CH_2).

UV-light irradiation cross-linking reaction for membrane-type materials

Then, 1.0 g of a mixture of the cationic PCL macromonomers (2b20PCL-DMAEMA and 4b10PCL-DMAEMA) and non-cationic, methacryloyl-terminated, branched PCL (2b20PCL-MA and 4b10PCL-MA) was dissolved in 1.5 mL of THF containing an adequate amount of the photosensitizer. This solution was poured into a 5.0 × 5.0 × 0.1 cm mold and quickly sandwiched between a PET sheet and a glass plate. A light-triggered, cross-linking reaction was carried out by irradiating each side of the plates for 10 min using a high-pressure mercury lamp. The obtained PCL film was swollen in acetone for 1 d and dried in a vacuum oven. Eventually, light yellow-colored films were obtained.

Surface and bulk characteristics of cross-liked, cationic PCL

The surface zeta-potentials of the prepared films were measured using a zeta-potential analyzer (Otsuka Electronics, Japan). Each value was obtained under temperature-controlled conditions. Additionally, anionic dye adsorption experiments were carried out using Acid Red 87. Each film was immersed in the aqueous dye solution (0.33 mg/mL) for 3 min, after which the film was carefully removed and rinsed with distilled water for 30 s. The change in the luminance due to dye adsorption was evaluated using a color histogram analysis with Adobe Photoshop® software. Preliminarily, the shape memory property of the prepared, cross-linked, PCL-based materials was examined. The materials were expanded at 60 °C and cooled to 25 °C. Then, they were heated to 60 °C and cooled to 25 °C, and the shape recovery was confirmed.

Cell interaction experiments using human mesenchymal stem cells

Before starting the cell culture, the cross-linked, cationic PCL substrates were sterilized using ethylene oxide gas. Human mesenchymal stem cells were seeded at a density of 3.5×10^3 cells cm^{-2} on a sterilized PCL surface with varied cationic contents and cultured in hMSC growth medium at 37 °C for 24 h. The hMSCs on the PCL surfaces were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 5 min at room temperature. To visualize F-actin and the nucleus, the cells were treated with rhodamine phalloidin for 30 min and DAPI for 5 min in the dark at room temperature. The cell morphology was then imaged using a fluorescence microscope (Nikon ECLIPSE TE2000-U).

Results and discussion

Control of the cationic content of the PCL, cross-linked materials with a fixed ratio of the 2-branched and 4-branched macromonomers

To prepare the cross-linked, stable, PCL materials with cationic groups, cationization and polymerizable group introduction were performed simultaneously, as seen in Fig. 1. Table 1 summarizes the results of the precursor preparations and the desired macromonomers. The ^{1}H-NMR spectra proved that each reaction proceeded successfully with high yields and good reactivity, as shown in

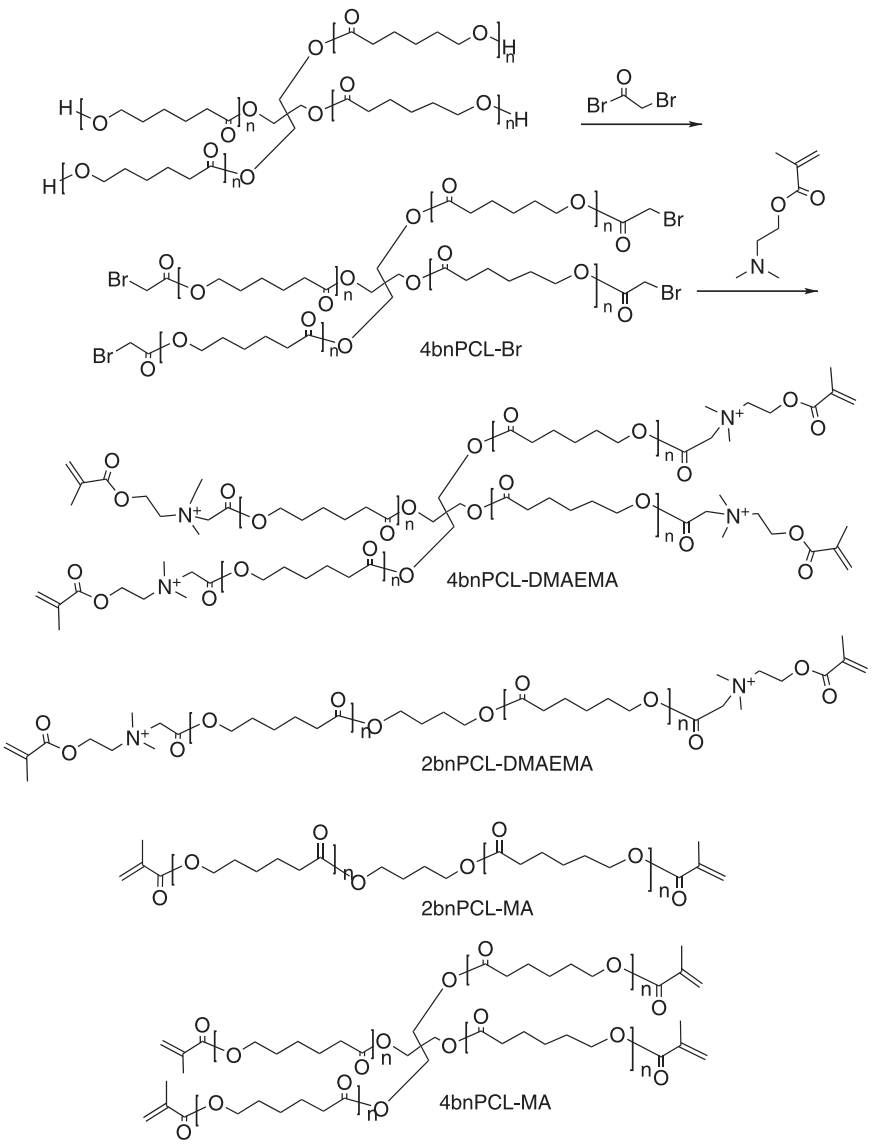

Fig. 1 Preparation schemes for the 2-branched and 4-branched PCL macromonomers with cationic moieties

Table 1 Preparation of cationic PCL macromonomers

Sample	Introduction (%)	Yield (%)
4b10PCL	–	94.3
4b10PCL-Br	92.3[a]	83.3
4b10PCL-DMAEMA	72.8	41.1
4b10PCL-MA	78.1[b]	43.2
2b20PCL	–	96.1
2b20PCL-Br	95.1[a]	86.4
2b20PCL-DMAEMA	87.1	100
2b20PCL-MA	78.7[b]	70.1

[a] The values mean the bromomethyl group introduction

[b] The values mean introduction of methacryloyl groups

Fig. 2. A characteristic peak was observed at approximately 3.8 p.p.m. Additionally, peaks corresponding to the introduction of dimethylmethacrylammonium groups were observed at approximately 6.1, 5.6, 4.6, 4.4, and 1.9 p.p.m. By using an excess amount of the reagents, almost all the hydroxyl groups reacted with bromoacetyl bromide, and the corresponding bromomethyl groups then reacted with N,N-dimethylaminoethylmethacrylate. Branched PCLs without cationic moieties were prepared separately by the reaction using hydroxy-terminated PCL starting material and methacryloyl chloride.

Previously, we reported that the softening points of PCL-based materials can be modulated by changing the mixing ratios of the 2-branched and 4-branched macromonomers [7–9]. In this study, we investigated the effect of varying cationic contents with a fixed ratio of 2-branched and 4-branched macromonomers. As shown in Fig. 1, the 2-branched and 4-branched macromonomers with and without cationic moieties are referred to as 2b20PCL-DMAEMA, 2b20PCL-MA, 4b10PCL-DMAEMA, and 4b10PCL-MA.

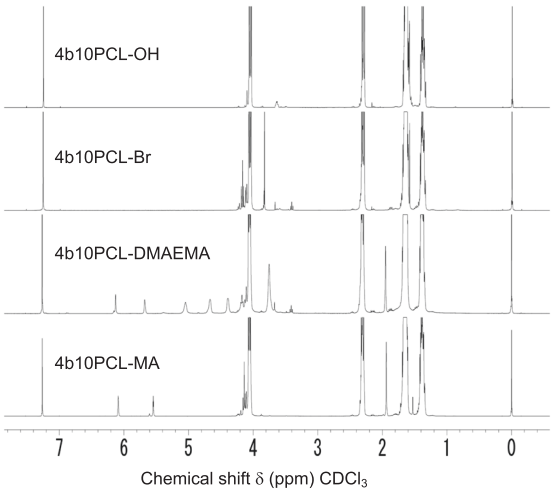

Fig. 2 ^{1}H-NMR spectra of the starting, bromomethyl, and dimethylmethacrylammonium PCLs with a 4-branched structure

Table 2 Softening points of the cross-linked, PCL materials

Cation (%)	0	30	50	70	100
Softening point (°C)	46.7	45.6	46.0	39.3	41.2

Here, "20" and "10" refer to the repeating unit of PCL. The ratio of the 2-branched to the 4-branched macromonomers is written in the form A:B. The percentage x represents the cationic content of the PCL for both "A" and "B", that is, x % of 2b20-DMAEMA and (100-x)% of 2b20-MA and x% of 4b10-DMAEMA and (100−x)% of 4b10-MA. In this study, we fixed the ratio to 7:3 for the first trial. The observed feeding contents of the macromonomers and softening points of the corresponding cross-linked materials are listed in Table 2. The tendency of the softening point of the higher cation content samples shifting to lower temperatures was observed. The variation in the softening points was not slight, and cationic groups prevent crystallization of PCL chains by possible electrostatic repulsion. After the cross-linking reaction, a polymer content was not observed in the solvent used for purification. This means that almost all the macromonomers participate in the reaction. Moreover, the cationic materials showed shape memory properties similar to those of the PCL, cross-linked materials without cationic moieties, as shown in Fig. 3. The results suggested that these materials could be useful as shape memory materials with controllable positive charges.

Zeta-potential measurements of the cationized, cross-linked, PCL materials

To confirm the effect of cationization on the surface properties of the materials, their zeta potentials were measured. Figure 4 shows the zeta potentials of the cross-linked, PCL materials with different cation contents. At 25 °C, which is below the softening points of the cross-linked materials, the zeta-potentials increased with the increasing cation content (Fig. 4a). However, at a cation content of 50% or higher, the zeta-potential was constant. The reason for this is not clear, but the preparation procedure could explain the results. As described in the experimental section, the cross-linking reaction was carried out between two PET sheets. Because the PET surface is hydrophobic, the hydrophilic cationic moieties in the PCL chains are less likely to be located near the PET above a certain cationic content, and the cationic sites would be locally immobilized inside the cross-linked materials.

To investigate the effect of the temperature on the surface zeta potentials, the zeta-potential values of the samples with a cation content of 100% were measured at different temperatures (Fig. 4b). Interestingly, the zeta potential increased steeply near the softening temperature (42 °C). The zeta potential values of the sample surfaces are closely related to the mobility of the PCL chains in the materials, which is linked to temperature-responsive properties. Original PCL is a semi-crystalline polymer, and cross-linked PCL also shows similar crystallization-melting behavior. The number of cationic moieties surely reflects the zeta potential value; furthermore, the value would be influenced by the chain movement. The cationic moieties could move to the interface of the materials and the aqueous medium easily due to chain mobility over the softening point. After a sufficiently long cooling time, the zeta potential returned to its original value. This indicates that the polymer chains created a network and formed stable materials. Therefore, these materials are expected to be useful for the adsorption of anionic compounds. To test this hypothesis, we carried out an anionic dye adsorption experiment as described in the following section.

Dye adsorption onto the cationized, cross-linked, PCL materials

To study the electrostatic interactions of cationic PCL, we tested the adsorption of anionic dyes above and below the softening temperature using samples with different cationic contents. Figure 5a shows the adsorption of Acid Red 87 onto the samples at 0 and 60 °C. These temperatures were significantly below and above the softening points. Obviously, more dye molecules were able to interact with the cationic PCL with higher cationic contents, and a larger adsorption was observed for samples above their softening points. To quantify these results, the luminance of the red in each sample was calculated using Adobe Photoshop® software. The luminance values were plotted against the cationic contents shown in Figs. 5b, c. The proportional relationship indicated that the feed ratios of the cationic PCL macromonomer reflected the actual cationic charge

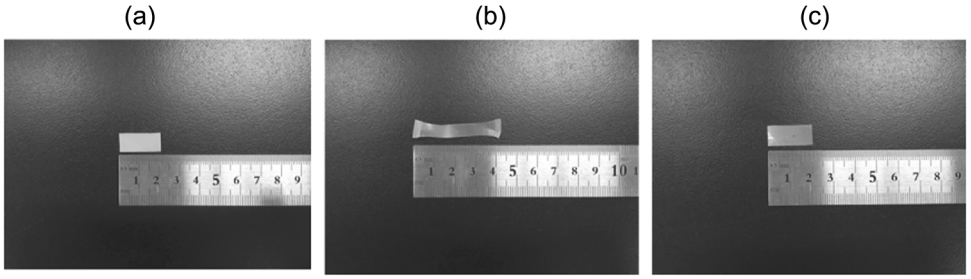

Fig. 3 Shape memory of ross-linked PCL with 30%cationic moieties. **a** Original shape at 25 °C, (**b**) Expanded at 60 °C and cooled to 25 °C, (**c**) Heated to 60 °C and cooled to 25 °C

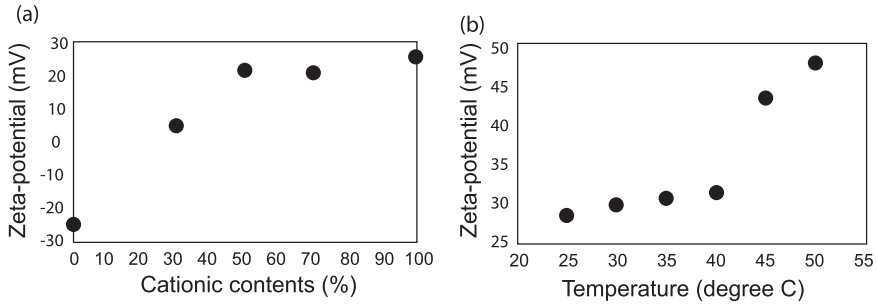

Fig. 4 Zeta potential measurements of PCL, cross-linked materials with cationic moieties. **a** Effect of the cationic contents, (**b**) effect of the temperature on a sample with 100% cationic content

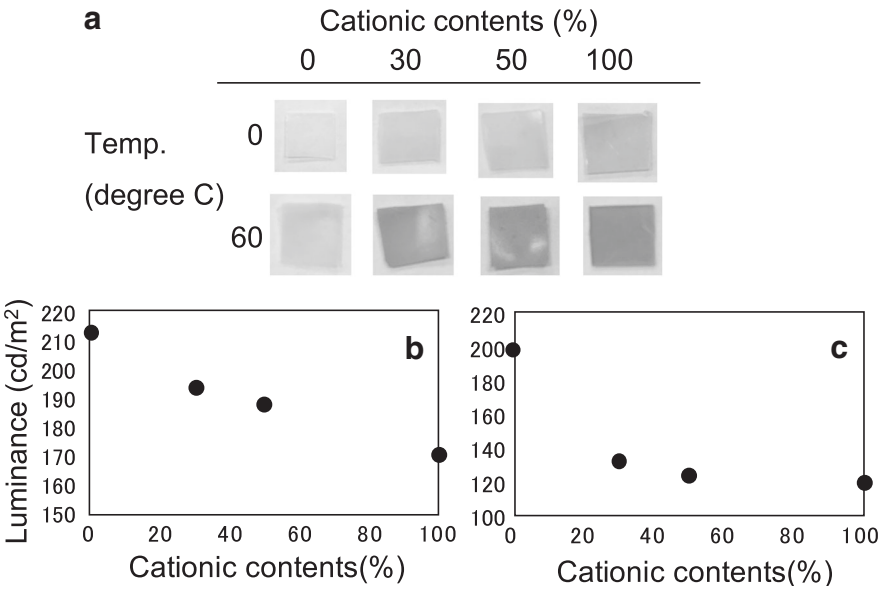

Fig. 5 Anionic dye adsorption experiments. **a** Photos of adsorbed dye on the cross-linked, PCL materials with cationic moieties. **b** Luminance change at 0 °C, **c** Luminance change at 60 °C

intensities. More dye was adsorbed on the surfaces at 60 °C, which was due to the larger charge density, as shown in Fig. 4b.

Stem cell adhesion on the PCL-based materials with varied cationic contents

Previous studies have reported that stem cell behaviors are regulated by substrate properties, such as biochemical, physicochemical, and mechano-structural factors, individually or synergistically [17, 18]. The surface charge and functionality of substrates are known to be important factors that have a strong impact on cellular behavior [19, 20]. Li et al. reported that positive charges on the surface of materials can strongly promote cell spreading during attachment and improve the differentiation of rat bone marrow mesenchymal stem cells for osteogenesis more efficiently than negative and neutral charges, even though they did not create a surface coating with adhesive proteins such as collagen or fibronectin [21]. Additionally, Luca et al. also demonstrated that positively charged polymers affect the fate of stem cells through modulation of interactions with potent signaling proteins [22]. These examples clearly suggest the importance of understanding the

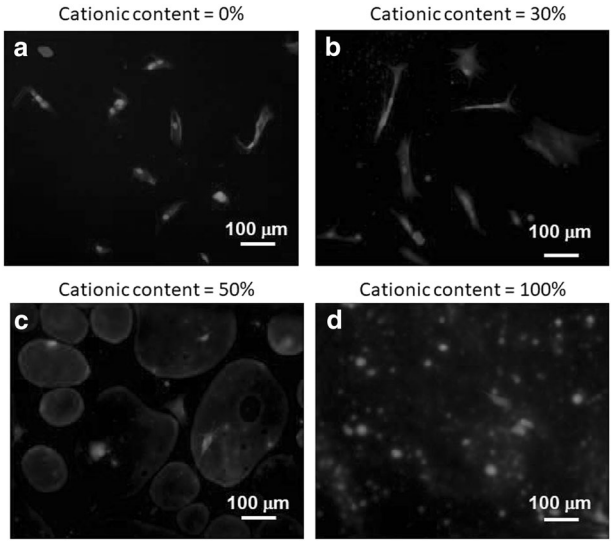

Fig. 6 Cell interactions using human mesenchymal stem cells. Cationic contents are (**a**) 0%, (**b**) 30%, (**c**) 50% and (**d**) 100%

interactions between surface charge and stem cell behavior, especially for our future work involving stem cell differentiation.

Based on the results described above, we performed a preliminary investigation of cell adhesion on cationized, PCL surfaces using hMSC with the aim of using these materials in biomedical applications. The materials used were stable against ethylene oxide sterilization. Immunostained images of hMSCs on the PCL samples are shown in Fig. 6. Here, no collagen or fibronectin was coated onto the materials to clarify the role of the cationic moiety introduction. Thus, the adsorbed proteins on the surfaces were due to serum exposure in the MSC growth medium. As seen in the figure, well-spread cells were observed on the PCL with a cationic content of 30% compared with that on the 0% cationic content control sample. This indicated that a 30% cationic content PCL surface would be preferable for hMSC adhesion. However, on highly positive surfaces with cationic contents of 50 or 100%, almost no cells were alive after 24 h. Higher contents of cationic moieties are toxic and may cause serious damage to cells by membrane disruption as a result of the stronger interactions between the surface and cell. We observed clear differences in the hMSC adhesion and the morphology of the adhered cells depending on the cationic content of the PCL substrates. Therefore, we will explore stem cell differentiation induced by surface charge without the use of any biochemical differentiation factors in our future work.

As discussed above, the developed system has potential as a novel, dynamic culture platform because the surface charge intensity can be controlled by changing the temperature. Therefore, cationic PCLs with different cationic contents represent a promising platform for investigating the effect of dynamic surface charge on the fate of stem cells. However, as strong electrostatic interactions can occur depending on the cationic content, the charge intensity should be carefully modulated with regards to its values above and below the softening temperatures. This process could involve dynamic stiffness changes due to the crystal-amorphous phase transition of PCL, suggesting that these materials are a promising platform for mechanobiology studies.

Conclusion

This study demonstrated convenient preparation methods for the introduction of cationic and cross-linkable moieties into 2-branched and 4-branched PCLs and their corresponding stable materials. The cationic content and ratio of 2-branched and 4-branched monomers could be simultaneously controlled by incorporating non-cationic macromonomers. Zeta potential measurements proved that the cationic charge could be controlled by changing the temperatures; this fact was also supported by the anionic dye adsorption tests. Human MSC adhesion was observed on the PCL materials with different cationic contents. Lower contents were preferable because the samples with higher contents caused severe damage to the cells. Consequently, such materials are promising for biomaterial research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Cai T, Li M, Zhang B, Neoh K-G, Kang E-T. Hyperbranched polycaprolactone-click-poly(N-vinylcaprolactam) amphiphilic copolymers and their applications as temperature-responsive membranes. J Mater Chem B. 2014;2:814–25.
2. Hu Y, Zhao NN, Li JS, Yang WT, Xu FJ. Temperature-responsive porous polycaprolactone-based films via surface-initiated ATRP for protein delivery. J Mater Chem. 2012;2:21257–64.
3. Sisson AL, Ekinci D, Lendlein A. The contemporary role of epsilon-caprolactone chemistry to create advanced polymer architectures. Polymer. 2013;54:4333–50.
4. Saatchi M, Behl M, Nochel U, Lendlein A. Copolymer networks from oligo(epsilon-caprolactone) and n-butyl acrylate enable a reversible bidirectional shape-memory effect at human body temperature. Macromol Rapid Commun. 2015;36:880–4.
5. Yang P, Zhu G, Shen X, Yan X, Nie J. Poly(epsilon-caprolactone)-based shape memory polymers crosslinked by polyhedral oligomeric silsesquioxane. RSC Adv. 2016;6:90212–9.

6. Chan BQY, Liow SS, Loh XJ. Organic-inorganic shape memory thermoplastic polyurethane based on polycaprolactone and polydimethylsiloxane. RSC Adv. 2016;6:34946–54.
7. Ebara M, Uto K, Idota N, Hoffman JM, Aoyagi T. Shape-memory surface with dynamically tunable nano-geometry activated by body heat. Adv Mater. 2012;24:273–8.
8. Uto K, Ebara M, Aoyagi T. Temperature-responsive poly(ε-caprolactone) cell culture platform with reversibly tunable nanoroughness and elasticity for control of myoblast morphology. Int J Mol Sci. 2014;15:1511–24.
9. Uto K, Mano SS, Aoyagi T, Ebara M. Substrate fluidity regulates cell adhesion and morphology on poly(ε-caprolactone)-based materials. ACS Biomater Sci Eng. 2016;2:446–53.
10. Ebara M, Akimoto M, Uto K, Shiba K, Yoshikawa G, Aoyagi T. Focus on the interlude between topographic transition and cell response on shape-memory surfaces. Polymer. 2014;55:5961–8.
11. Suwantong O. Biomedical applications of electrospun polycaprolactone fiber mats. Polym Adv Technol. 2016;27:1264–73.
12. Mahm A, Xiong X-B, Lavasanifar A. Novel self-associating poly (ethylene oxide)-block-poly(ε-caprolactone) block copolymers with functional side groups on the polyester block for drug delivery. Macromolecules. 2006;39:9419–28.
13. Guo ST, Huang YY, Wei T, Zhang WD, Wang WW, Lin D, Zhang X, Kumar A, Du QA, Xing JF. Amphiphilic and biodegradable methoxy polyethylene glycol-block-(polycaprolactone-graft-poly(2-(dimethylamino)ethyl methacrylate)) as an effective gene carrier. Biomaterials. 2010;32:879–89.
14. Chen HL, Huang J, Yu JH, Liu SY, Gu P. Electrospun chitosan-graft-poly (epsilon-caprolactone)/poly (epsilon-caprolactone) cationic nanofibrous mats as potential scaffolds for skin tissue engineering. Int J Biol Macromol. 2011;48:13–19.
15. Defize T, Riva R, Raquez J–M, Dubois P, Jerome C, Alexandre M. Thermoreversibly crosslinked poly(ε-caprolactone) as recyclable shape-memory polymer network. Macromol Rapid Commun. 2011;32:1264–9.
16. van Beek DJM, Spiering AJH, Peters GWM, Nijenhuis K, Sijbesma RP. Unidirectional dimerization and stacking of ureidopyrimidinone end groups in polycaprolactone supramolecular polymers. Macromolecules. 2007;40:8464–75.
17. Uto K, Tsui JH, DeForest CA, Kim DH-O. Dynamically tunable cell culture platforms for tissue engineering and mechanobiology. Prog Polym Sci. 2017;65:53–82.
18. Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. Nature. 2009;462:433–41.
19. Arima Y, Iwata H. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. Biomaterials. 2007;28:3074–82.
20. Schneider GB, English A, Abraham M, Zaharias R, Stanford C, Keller J. The effect of hydrogel charge density on cell attachment. Biomaterials. 2004;25:3023–8.
21. Li J, Mou X, Qiu J, Wang S, Wang D, Sun D, Guo W, Li D, Kumar A, Yang X, Li A, Liu H. Surface charge regulation of osteogenic differentiation of mesenchymal stem cell on polarized ferroelectric crystal substrate. Adv Health Mater. 2015;4:998–1003.
22. Luca ID, Salle AD, Alessio N, Margarucci S, Simeone M, Galderisi U, Calarco A, Peluso G. Positively charged polymers modulate the fate of human mesenchymal stromal cells via ephrinB2/EphB4 signaling. Stem Cell Res. 2016;17:248–55.