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Shape Memory RGD-Containing Networks: Synthesis, Characterization, and Application in Cell Culture

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Summary: Hydrogels have found wide application in tissue engineering and cell mechanobiology research due to their tunable, and often biomimetic, biochemical and biomechanical properties. Although it has been known for more than a decade that hydrogels can be designed to exhibit shape memory functionality—the ability to change from one defined shape to another when triggered by a defined stimulus—shape memory hydrogels have not previously been exploited in tissue engineering or mechanobiology research. Here we report the development of a biodegradable and biocompatible hydrogel with tailored shape memory as well as desirable mechanical property for soft-tissue applications. A shape memory hydrogel was synthesized by photopolymerization of PCL3k diacry-lates together with acrylate-PEG2k-GRGDS in the presence of a crosslinker, tetrathiol, and photoinitiator, DMPA, through thiol-ene chemistry. Differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA) were carried out to examine the thermal, mechanical and shape memory properties of the hydrogel in dry and wet states. Cell culture studies were performed to characterize material cytocompatibility. We found that the PCL phase was crystalline in the hydrogel, providing an excellent, reproducible shape memory effect. The transition temperature (shape memory "trigger") was tuned to fall between room and body temperature. Both cell attachment and proliferation studies revealed that the presentation of GRGDS molecules in the hydrogel facilitated fibroblasts adhesion and spreading on the hydrogel surface. This hydrogel, tailored to exhibit shape memory behavior in the cell culture compatible temperature range, should provide new opportunities for "smart" shape-changing scaffolds and substrates for application in tissue engineering and investigation of cell mechanobiology.

Keywords: cell culture; GRGDS peptide; hydrogel; PCL; poly(ethylene glycol) (PEG); shape memory

Introduction

Hydrogels have been widely explored as biomaterial scaffolds for diverse biomedical applications, including the repair or replacement of tissues and organs through tissue engineering and regenerative medicine. The potential for hydrogels to exhibit shape memory functionality—the ability to change from one defined shape to another when triggered by a defined stimulus—has been recognized for more than a decade.[1–4] However, shape memory hydrogels have not previously been exploited in tissue engineering or regenerative medicine, shape-memory having only recently been applied successfully with attached and viable cells.[5,6]

Hydrogels employed as implantable biomedical devices must possess appropriate biocompatibility and bioactivity to

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facilitate cell-polymer interactions and avoid adverse physiological reactions between implants and surrounding host tissues. However, most synthetic hydrogels typically exhibit minimal or no intrinsic biological activity.[2,7–9] Consequently, much work has been done to incorporate bioactive factors and peptides into these scaffolds, both physically and chemically, in order to provide them with signaling domains that have specific interactions with surrounding cells by molecular recognition.[7,10–17] Arg-Gly-Asp (RGD) peptide sequences derived from ECM proteins are the most widely studied cell-binding domains for the bioactive modification of scaffolds.

The goal of the present study was to develop a biodegradable and biocompatible hydrogel scaffold with tailored shape memory effect and good bioactivity as well as desirable mechanical property for soft-tissue application. To this end, elastic, biodegradable aliphatic polyester poly(ε-caprolactone) (PCL) macromers were co-photocured with monoacrylated Gly-Arg-Gly-Asp-Ser (GRGDS) peptide sequences with PEG spacer arms. This yielded a PCL/PEG-GRGDS network with water-swelling capacity. The swelling, thermal characterization, mechanical property, and one-way shape memory effects of the hydrogel were examined. Further, the attachment of fibroblasts on the smart bioactive hydrogel was investigated, illustrating the potential of the shape memory hydrogels for biomedical applications.

Materials and Methods

Materials Synthesis and Preparation

Hydroxyl end-functionalized poly(ε-caprolactone) diols (PCL diols) [α, ω-dihydroxy poly (ε-caprolactone)] with average molecular weight of 3,000 g/mol and 2,000 g/mol were purchased from Scientific Polymer Products, Inc. Acrylate PEG-N-hydroxy-succinimide (acrylate-PEG-NHS, Mw 2000 Da) was obtained from JenKem Technology USA Inc, H-Gly-Arg-Gly-Asp-Ser-OH (GRGDS) peptide was obtained from Bachem California, and MPEG-acrylate (Mw 2000 Da) was obtained from from Creative PEGWorks. All were used without further purification. The synthesis of PCL2k and PCL3k macromers was carried out by the reaction of acryloyl chloride with the terminal hydroxyl groups of each oligomeric diol in the presence of triethylamine in anhydrous toluene according to the method as described in the literature.[18,19] GRGDS was conjugated to PEG monoacrylate by reacting the peptide with acrylate-PEG2k-NHS at a 1:1 molar ratio in 50 mM sodium bicarbonate (pH 8.5).[10–12,16] The product is denoted as acrylate-PEG2k-GRGDS. Synthesis of the PCL macromers and acrylate-PEG2k-GRGDS monomer are schematically illustrated in Scheme 1. The chemically crosslinked PCL3k/PEG2k-GRGDS and PCL2k/PEG2k-GRGDS networks with both hydrophilic and hydrophobic components were prepared use a free radical photocrosslinking reaction of the PCL macromers and acrylate-PEG2k-GRGDS monomer, combined with different weight ratios, and stoichiometric amount of tetrathiol and with 2,2-dimethoxy-2-phenylacetophenone (DMPA) photoinitiator. The reaction mixture was diluted in dichoromethane (DCM) and the clear solutions were irradiated with UV light (1200 $\mu W/cm^2$) at a wavelength of 365 nm, with a lamp-sample distance of 15 cm, and at room temeprature for 1 h. The procedure of the network synthesis is illustrated in Scheme 2. Other compositions were prepared for comparison using the same methodology. As an example, the PCL3k/MPEG2k (85/15) network, was prepared by photocrosslinking PCL3k diacrylate/DCM (170 mg in 100 μl) and MPEG2k monoacrylate/DMSO, (20 mg in 1 ml) as a homogeneous solution in a glass mold and in the presence of tetrathiol and 2 wt-% DMPA. A PCL3k homo-network was also prepared using the PCL3k diacrylate macromer and tetrathiol, following the same procedure.

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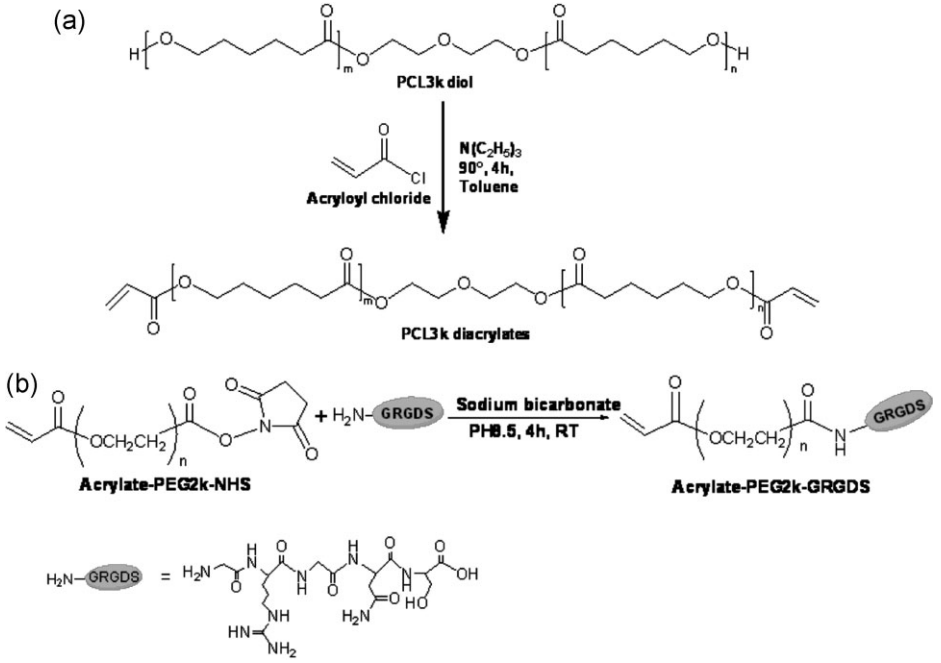
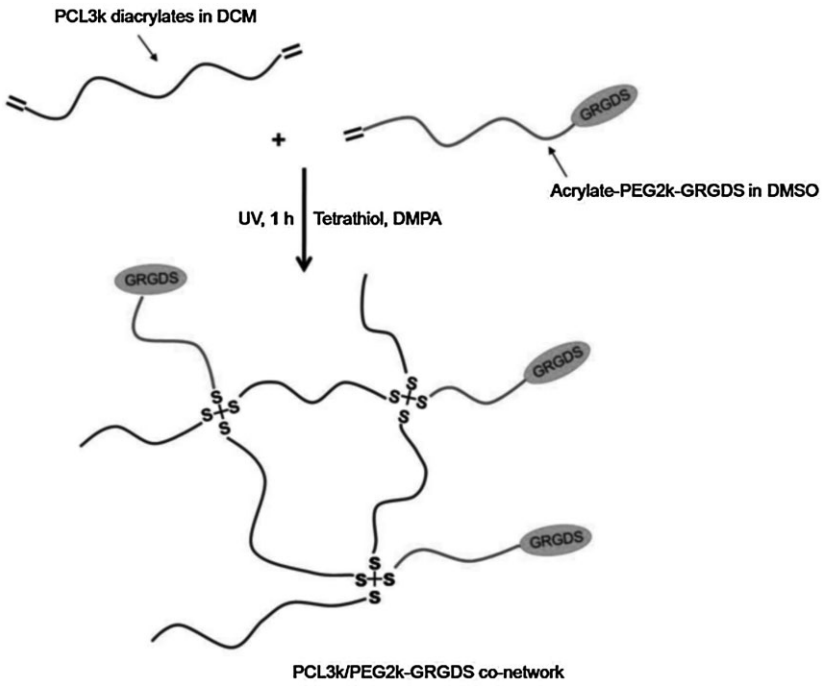


Scheme 1.
Synthesis of (a) PCL3k diacrylates,(b) acrylate-PEG2k-GRGDS.



Scheme 2.
Preparation of PCL3k/PEG2k-GRGDS networks with PEG2k-GRGDS pendant groups.

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Characterization

Thermal properties of dry and water-swollen (''wet'') PCL3k/PEG2k-GRGDS and PCL3k/MPEG2k samples, along with the PCL3k network, were characterized using differential scanning calorimetry (DSC, TA Instruments, Inc., Model Q100). Both the heating and cooling rate were 10 °C/min from −80 °C to70 °C. Melting temperatures (T_m) and latent heats of fusion (ΔH_m) of the samples were taken from the second heating traces. Linear viscoelastic thermo-mechanical properties were determined using dynamic mechanical analysis (DMA). Second heating traces were recorded and adapted to determine elastic modulus values. One-way shape memory (1W-SM) behavior of PCL3k/PEG2k-GRGDS networks was characterized by using the DMA apparatus operated in static mode, with shape fixing (R_f, a measure of the fixation of the temporary shape) and the strain recovery (R_r, a measure of recovery of the permanent shape of the polymer networks) determined as previously described.[20]

Gel Fraction and Degree of Water-Swelling

To determine the quality of crosslinking, gel fraction values of PCL3k/PEG2k-GRGDS and PCL3k/MPEG2k networks were measured using extraction and gravimetry, respectively. Specifically, a PCL3k/PEG2k-GRGDS or PCL3k/MPEG2k film was weighed (W_1), extracted repeatedly in DCM at 37 °C for 24 h, and dried under vacuum at 70 °C for 48 h. The dry film was weighed again (W_2) and the gel fraction value (G %) was calculated according to the following equation:

$$G\,(\%) = W_2/W_1 \times 100\%.$$

Subsequent to extraction, the film was allowed to equilibrate in deionized distilled water for 24 h and weighed (W_3) after excess water had been carefully swabbed away. The total water uptake (W_c %) of PCL3k/PEG2k-GRGDS and PCL3k/MPEG2k hydrogel was calculated by the following formula: $W_c\ (\%) = (W_3 - W_2)/W_2 \times 100\%$.

All measurements were performed in triplicate and averaged.

Cytocompatibility Studies

Hydrogel films were sterilized by 24 h ethanol soak followed by UV irradiation on the cell seeding side for 30 min before being seeded with cells. For all cell experiments, the mouse fibroblast C3H/10T1/2 cell line (ATCC) was employed under standard culture condition (37 °C, 95% relative humidity and 5% CO_2) following supplier guidelines. To assay cell attachment, the hydrogel films with and without peptide modification, as well as pure PCL3k network films, were placed in 96-well plates. The wells in each plate were divided into groups for corresponding samples, with each group run in triplicate: (1) positive control group: only test cells were added; (2) GRGDS-containing hydrogel: PCL3k/PEG2k-GRGDS (85/15); and (3) negative control group: PCL3k/MPEG2k (85/15) hydrogel. Cells were seeded on the top of each sample at a density of 1.2×10^4 cells/cm^2, cultured under standard culture condition for 24, 48 and 72 h, and then assayed by LIVE/DEAD staining.

Results and Discussion

Preparation of PCL3k/PEG2k-GRGDS Networks

Recently, the combination of shape memory capability of PCL with its biodegradable property has brought up great potential that is capable of opening up important applications for shape memory polymers in implantable biomedical areas. In particular, PCL has been extensively researched as a shape memory polymer for a (meth)acrylate-based thermoset or a polyurethane segment-based thermoplastic.[19,21] Herein, a PCL3k telechelic macromer was synthesized in order to develop chemically crosslinked PCL3k networks with structural and mechanical homogeneity. The reaction of PCL3k diol with acryloyl chloride led to the formation of PCL3k macromer (PCL3k

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diacrylates) that are vinyl group-end capped, which was verified by the presence of the vinyl groups in the δ 5.81–6.44 ppm range through ^{1}H-NMR spectrum (data not shown). The functionalization of acrylated PEG2k monomer with peptides was also confirmed using ^{1}H-NMR spectrum by the disappearance of proton peak of N-hydroxy succinimidyl ester at 2.83 ppm observed after the coupling reaction (data not shown).

The acrylic end-groups of both PCL3k macromer and acrylated PEG2k-GRGDS monomer were introduced to ultimately allow for a straightforward addition reaction with thiol groups through thiol-ene photochemistry in the presence of DMPA, [22,23] as shown in Scheme 2. Since PCL3k macromer has two terminal double bonds per molecule, the thiol-acrylate photopolymerization between PCL3k macromer and tetrathiol leads to the formation of a three-dimensional PCL3k network with tetrathiol as the netpoints. PEG2k-GRGDS, as a pendant group, is also linked by one chain end to an arm of a tretrathiol netpoint. Consequently, the PCL3k/PEG2k-GRGDS network has an architecture featuring PEG2k-GRGDS chains dangling on the 3D PCL3k network. In addition, we anticipated that the homogeneous network formation afforded by thiol-ene addition reactions would engender good strain/stress and shape memory properties for the network materials.[24,25]

Gel Fraction and Degree of Water-Swelling

The crosslinked networks proved to have good gel fraction values and moderate water-swelling capability for samples containing MPEG2k or PEG2k-GRGDS dangling chains. In particular, the gel fraction of PCL3k/PEG2k-GRGDS (85/15) was found to be $96.1 \pm 1.1\%$, PCL2k/MPEG2k was $95.1 \pm 1.46\%$, and PCL3k/MPEG2k had a gel fraction of $94.5 \pm 1.85\%$. By comparison, the PCL3k network had a very high gel fraction of $99.2 \pm 0.2\%$. Concerning water uptake, PCL3k/PEG2k-GRGDS (85/15) was found to swell $10.1 \pm 1.0\%$, very similar to PCL3k/MPEG2k at $10.17 \pm 1.25\%$. The PCL2k/PEG2k-GRGDS (85/15) composition swelled slightly more at $12.5 \pm 1.61\%$. In contrast, the PCL3k network was virtually impervious to water with $W_c = 0.2 \pm 0.4\%$. By thus, incorporating the GRGDS peptide in PCL/PEG2k-GRGDS hydrogels did not significantly affect the gel fraction and the water uptake of the prepared hydrogels. While the degree of water-swelling was small, we will show that this level of swelling lowered the PCL3k network melting point desirably for shape memory considerations.

Phase Behavior (DSC) and Microstructure (WAXD)

Thermal analysis was conducted using DSC to determine the phase behavior – principally melting and crystallization – of the materials as this depended on composition and water-swelling. Figure 1a displays the DSC curves of each of the PCL3k network, PCL3k/PEG2k-GRGDS and PCL2k/PEG2k-GRGDS networks, and as-synthesized PEG2k-GRGDS powder. The second heating trace for each network exhibited only one melting transition (T_m). For the PCL3k/PEG2k-GRGDS (85/15) network, the T_m, approximately 44.0 °C, is quite similar to that of the PCL3k homo-network. WAXD observations (data not shown) indicated that the T_m should be assigned to the melting of PCL crystallites by only showing the PCL characteristic peaks at $2\theta = 15.6°$, 22.7°, 23.4°, and 25.6°. Decreasing the PCL network chain molecular weight in the PCL2k/PEG2k-GRGDS case had the desirable effect of decreasing the melting point by about 10 °C to 31.8 °C. The heat of fusion (ΔH_m) was only slightly reduced suggesting good shape fixing as good as the sample with higher molecular weight PCL network chains, tested below. The T_m and ΔH_m values are summarized in Table 1. By comparison, PCL3k/MPEG2k network (no-GRGDS) showed two distinct melting endotherms (Figure 1b), indicating that the PCL3k/MPEG2k networks possessed segregated crystalline PEG and PCL phases. We postulate that the lower,

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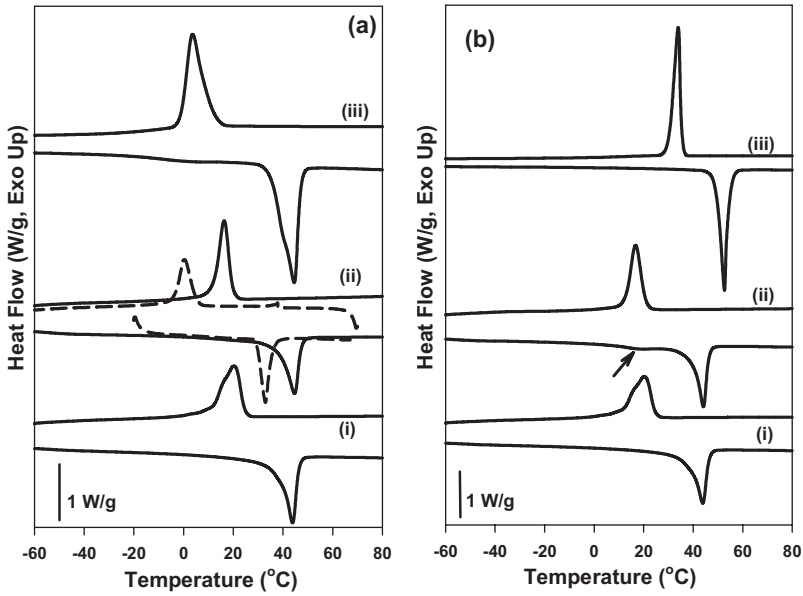

Figure 1.
DSC thermograms of (a) PCL3k/PEG2k-GRGDS networks and (b) PCL3k/MPEG2k networks. For comparison, PCL3k network, as-synthesized PEG2k-GRGDS powder and commercial MPEG2k powder are shown as well. (a) (i) PCL3k network, (ii) PCL3k/PEG2k-GRGDS (85/15) network (black) and PCL2k/PEG2k-GRGDS (85/15) network (dashed), (iii) PEG2k-GRGDS macromer; (b) (i) PCL3k network, (ii) PCL3k/MPEG2k (85/15) network, (iii) MPEG2k macromer, respectively.

Table 1.
DSC data of dry networks.

Sample Name	T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)
PCL3k network (100/0)	43.9	55.5	20.3	53.4
PCL3k/MPEG2k (85/15)	44.2	42.0	16.7	50.1
PCL3k/PEG2k-GRGDS (85/15)	44.6	52.4	16.4	48.7
PCL2k/PEG2k-GRGDS (85/15)	32.9	27.6	0.3	30.7

minor T_m transition (arrow in Figure 1b) is related to the melting of PEG crystallites, whereas the higher T_m was assigned to crystalline PCL phase. This postulation is supported by the observation that the lower Tm peak disappears upon swelling with water, while the higher temperature transition is unchanged. The DSC data for these samples are summarized in Table 2.

Intended use of these new shape memory networks in cell culture requires us to understand their properties in a water-swollen state. Thus, DSC analysis of water-saturated samples was accomplished and

Table 2.
DSC data of the swollen hydrogels.

Sample Name	T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)
PCL3k network (100/0)	43.3	51.3	12.3	50.5
PCL3k/MPEG2k (85/15)	42.8	41.9	10.0	43.4
PCL3k/PEG2k-GRGDS (85/15)	43.2	42.6	13.8	42.1
PCL2k/PEG2k-GRGDS (85/15)	31.8	20.8	−0.7	31.5

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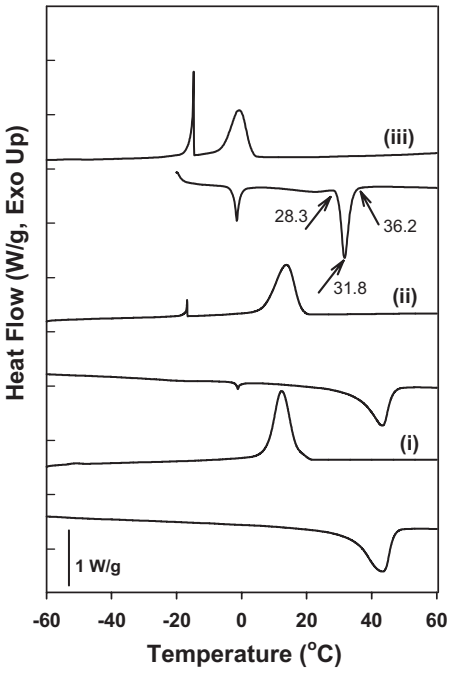

Figure 2.
First cooling (exotherms, upper) and second heating (endotherms, lower) DSC traces of PCL3k and PCL3k/PEG2k-GRGDS hydrogels contacted with water to equilibrium. (i) PCL3k network, (ii) PCL3k/PEG2k-GRGDS (85/15) hydrogel, (iii) PCL2k/PEG2k-GRGDS (85/15) hydrogel. For (a), trace (iii) during the second heating, the sample was first heated to 25 °C, held isothermal for 120 min, then cooled down to −20 °C, held isothermal for 3 min, and finally heated back to 70 °C.

traces for PCL3k/PEG2k-GRGDS, PCL2k/PEG2k-GRGDS, and PCL3k/MPEG2k hydrogels are shown in Figure 2. Each material exhibited only one, T_m, in the range of 36.0 °C to 43.3 °C. An obvious and sharp endothermic peak for the melting of ice crystals appeared at about 0 °C (Figure 2a and b). Compared with the DSC data of the corresponding dry samples, both T_m and ΔH_m decreased for PCL3k/PEG2k-GRGDS, PCL3k/PEG2k-GRGDS, and PCL3k/MPEG2k hydrogels due to the plasticizing effect of the water molecules within the hydrogels (Table 2). We conclude that PCL phase crystallized in the PCL3k/PEG2k-GRGDS and PCL2k/PEG2k-GRGDS networks both in dry and wet (swollen by water) state, indicating that PCL fraction in the network could provide desired mechanical and shape-memory properties.

As a potential bioactive implanted material for tissue engineering, the transition temperature of a candidate shape-memory polymer should be between room and body temperature for automatically inducing the shape change upon implantation without additional heating. In addition, such an activated (deployed) SMP should maintain its temporary shape completely, without unwanted shape recovery, at room temperature. In our study, the transition temperature, T_m, of each of PCL3k/PEG2k-GRGDS hydrogel, as shown in Figure 2a and Table 2, was around 40 °C, which is slightly above body temperature and enables on-demand control of the shape change by short time application of directly or indirectly supplemental heating.[26]

One of the near-term goals of this study is to investigate the cellular behavior in response to the mechanical cues resulting from the recovery of the substrates during cell culture. Therefore, our ongoing effort has been to adjust T_m of PCL3k/PEG2k-GRGDS hydrogels to a value slightly below body temperature, which would be both crucial for achieving this goal and useful for the design of clinical devices. As revealed by the DSC study (above) decreasing T_m of the PCL3k/PEG2k-GRGDS hydrogels could be achieved by decreasing the average molecular weight of PCL3k diacrylates used as precursors in the polymer network synthesis to PCL2k diacrylates. For example, PCL2k/PEG2k-GRGDS (85/15) network was prepared by photocuring the mixture of PCL2k diacrylates and acrylate-PEG2k-GRGDS in the presence of DMPA (2 wt. %, with respect to the total weight of the two precursors) and tetrathiol (1:1 molar ratio of thiol to double bonds), in which the weight ratio of PCL2k diacrylates to acrylate-PEG2k-GRGDS was 85 to 15. It was encouraging that PCL2k/PEG2k-GRGDS (85/15) network exhibited a very sharp thermal transition, T_m, at 32.9 °C

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(Figure 1(a), red line), which was significantly lower than that of PCL3k/PEG2k-GRGDS (85/15) (44.6 °C, shown in Figure 1a and Table 1). Meanhwhile, the T_m of PCL2k/PEG2k-GRGDS (85/15) swelled in water to a hydrogel state (water swelling ratio of 12.5%) was 31.8 °C (Figure 2(a), trace iii), with the beginning and ending temperature at about 28.3 °C and 36.2 °C, respectively, indicating the automatic shape recovery from its deformed state should occur when heated to body temperature, while the temporary shape can be maintained at room temperature.

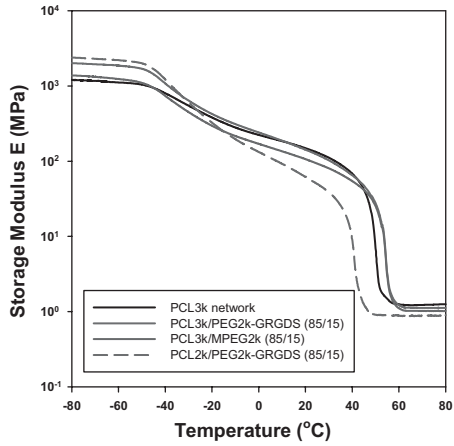

Figure 3.
Storage modulus (É) vs. temperature for various network samples indicated in the legend. Samples were first heated to 80 °C, cooled to −100 °C, and then heated to 80 °C. Data shown is for each second heating.

Mechanical Properties of the PCL3k/PEG2k-GRGDS Networks

All of the network samples exhibited similar tensile storage modulus (E) versus temperature traces, as shown in Figure 3. The storage modulus of each sample gradually reduced upon traversing a glass transition (T_g) starting around −50 °C and then sharply dropped around the melting temperature between 43 °C and 57 °C. Such behavior is typical for semicrystalline networks.[22] The T_g observed by DMA is attributed to the amorphous PCL polyester chains, even though it was not clearly revealed in the DSC curves of both PCL3k/PEG2k-GRGDS networks and PCL3k network (Figure 2a). Below T_g, the samples are glassy with the storage modulus well above 1 GPa. When reaching T_g, the storage modulus drops gradually with increasing temperature to a value below 200 MPa. Upon further heating, the storage moduli continue to decay, dropping dramatically at about 43 °C at the onset of T_m of PCL component, finally yielding a rubbery plateau at ∼ 1 MPa for temperatures above 60 °C. It could be seen that the E values in the semicrystalline state near room temperature were approximately two orders of magnitude larger than that those within the rubbery plateau, just above the melting temperature. This indicates that the stiffness can be dramatically changed in a narrow range of temperatures. For the PCL3k/MPEG2k network (Figure 3), the initial drop in modulus around −50 °C was quite similar that of the corresponding PCL3k/PEG2k-GRGDS network.; It is interesting to observe in Figure 3 that the PCL3k/PEG2k-GRGDS and PCL3k/mPEG2k samples had very similar melting behavior as determined by DMA – a finding consistent with the DSC results (Table 1). Meanwhile, the PCL2k/PEG2k-GRGDS sample behaved as predicted by DSC and showed melting transition approximately 10 °C lower than the samples incorporating the PCL3k macromer.

Shape Memory Behavior

All of the network samples exhibited excellent one-way shape memory (1W-SM) behavior with shape recovery being driven by covalent crosslinking and shape-fixing being enabled by crystallization of constituent PCL network chains. Figure 4 shows the repeated 1W-SM cycles of PCL3k/PEG2k-GRGDS (85/15) network along with that of the PCL3k network. The remarkably reproducible shape memory behavior of each sample was observed from the repeated three cycles obtained under the same condition. All samples also

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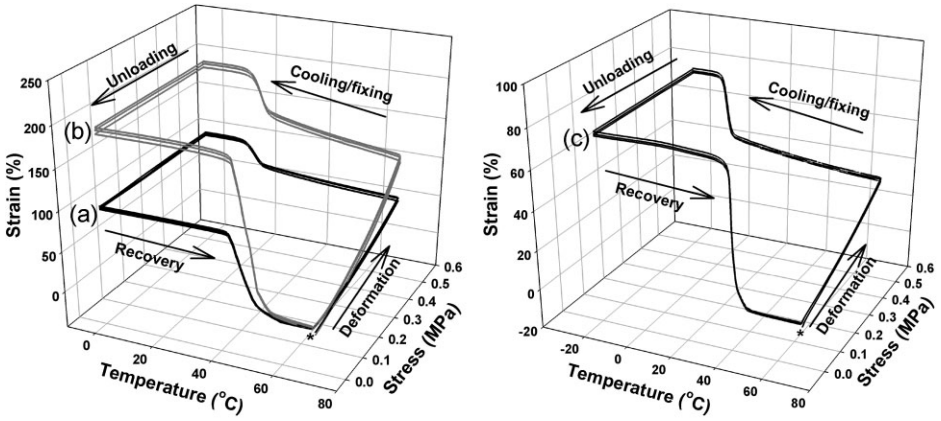

Figure 4.

One-way shape memory behavior for (a) PCL3k network, (b) PCL3k/PEG2k-GRGDS (85/15) network, and (c) PCL2k/PEG2k-GRGDS (85/15) network under a constant stress (500 kPa). The asterisk indicates the beginning of the 1W-SM experiment. For curves (a) and (b), the 1W-SM cycles were repeated three times from 70 °C to 0 °C at a constant ramping rate of temperature (2 °C/min), while for curve (c), the temperature was ramped from 70 °C to −20 °C at 2 °C/min during both heating and cooling steps.

showed similar 1W-SM behaviors with an increase of strain while cooling/fixing, a result of the crystallization of PCL network chains under stress.[22,27,28] Both shape fixing and recovery were close to 99% for the GRGDS-containing networks. We observed a significantly lower fixing (crystallization) temperature for the PCL2k/PEG2k-GRGDS (85/15) sample and this is attributed to the lower crystallization temperature.

Cell Attachment

The morphologies of fibroblasts seeded on the hydrogels with and without GRGDS or on TCPS (positive control) at 24, 48, and 72 h are shown in Figure 5. The cells seeded on TCPS qualitatively showed the highest cell attachment and most extensive spreading at each time point. Compared to the hydrogels without GRGDS, GRGDS-functionalized hydrogels showed qualitatively higher cell attachment after 24 h. Cells seeded on the hydrogels without GRGDS had an apparent decrease in density with culture time from 24 h to 72 h. Furthermore, the cells on these materials became qualitatively smaller and less spread after 48 and 72 h. These results suggest that the fibroblasts have a weak interaction with these materials, and further exemplified the importance of the GRGDS peptide for cells adhesion and spreading on the material.

Conclusion

In summary, shape memory hydrogels bearing dangling GRGDS units were successfully developed. The resulting hydrogels had a structure with covalent immobilization of GRGDS peptide sequences to the PCL3k network via flexible PEG2k spacer chains. DSC results showed that the transition temperature, T_m, of the PCL3k/PEG2k-GRGDS hydrogel was in the range of 39.2 °C to 43.2 °C, which is above body temperature and enables on demand control of the shape change by supplemental heating. Analysis of shape memory properties with DMA revealed that the PCL3k/PEG2k-GRGDS hydrogel had excellent, reproducible 1W-SM effect. Both shape fixing and recovery of the hydrogels were about 99%. In addition, the cell attachment and proliferation studies revealed that the presentation of GRGDS molecules in the hydrogels via flexible PEG spacers facilitated fibroblasts adhesion,

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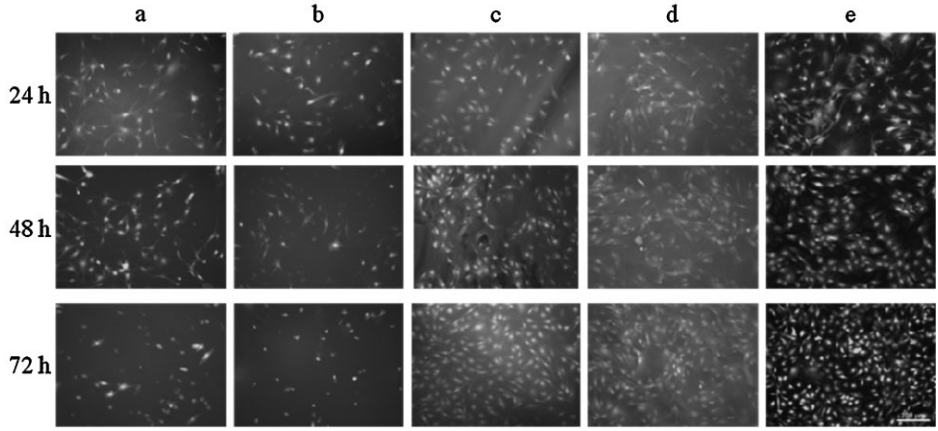

Figure 5.
Cell morphology of fibroblasts cultured for 24, 48 and 72 h on the surface of (a) PCL3k network, (b) PCL3k/MPEG2k (85/15) hydrogel, (c) PCL3k/PEG2k-GRGDS (85/15) hydrogel, (d) PCL2k/PEG2k-GRGDS (85/15) hydrogel, and (e) TCPS. The cell seeding density was 1.2×10^4 cells/cm^2. All the figures are of the same magnification. The scale bar represents 200 μm for all the images.

spreading and growth on the hydrogel surface.

Cells adhered and spread on hydrogels with the GRGDS peptide and tuning the transition temperature of PCL2k/PEG2k-GRGDS (85/15) hydrogel between room and body temperature led to significant recovery at body temperature, suggesting a possible system for applying dynamic mechanical stimuli to adherent cells during cell culture. The multifunctional hydrogel presented here combined good bioactivity and potential for biodegradability with the excellent shape-memory property that hold promise to be applied in tissue engineering, especially as biomedical implants or for guiding cells by the incorporation of both bioactive signal domains and shape-memory effects to develop artificial tissues and organs with appropriate functionality.

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