Light-induced dynamically tunable micropatterned surface for the regulation of the endothelial cell alignment

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Abstract: The surface topography has a great effect on the behaviour of adherent cells. We report an efficient method to prepare shape memory surfaces with dynamically changed micropatterns, which can be induced by near-infrared (NIR) light by varying the power density. After polydopamine (PDA) was coated on the cross-linked polyethylene glycol-poly-(ε-caprolactone), the surface temperature increases by 40°C at room temperature when 808 nm light with 1.0 W/cm² is used because of the photothermal properties of PDA. This temperature increase is enough for the shape recovery of the pressed micropatterns. The depth of the recovered micropatterns is controllable by adjusting the power density of the 808 nm light. The NIR-induced micropatterns efficiently regulate the morphology and alignment of endothelial cells.

Therefore, NIR-induced shape memory surfaces have the potential to be used in remote-controlled devices.

1 Introduction

Bioinspired smart surfaces have become a research hotspot because of their wide applications including anti-bioadhesion [1–3], cell capture/release [4], self-cleaning [5], and controllable oil/water separation [6]. Surface microstructures directly endow the surfaces with many properties including directional adhesion [7], water collection [8, 9], and water-directional spreading [10, 11]. In the past few years, shape memory polymers (SMPs) such as poly-(ε-caprolactone) (PCL) [12–14], poly(ethylene-vinyl acetate) [15], and polyacrylate [16, 17] have been used to fabricate dynamically changed micropatterned surfaces. Micropatterns including microgrooves, arrays of micropillars, microwells, and microwrinkles [12, 13, 17–25] have been fabricated by using SMPs. Since the properties of the micropatterns and dynamically changed surfaces, SMP-micropatterned surfaces have been used to adjust the cell behaviour including cell alignment, cell proliferation, and cell differentiation [12–14, 21, 23, 24, 26].

Thermally induced SMP-micropatterned surfaces are generally deformed by compressing or stretching above the transformation temperature (Ttrans) and temporary surfaces are obtained by cooling down while maintaining the stress. The surface micropatterns then recover to the original patterns by heating above the Ttrans. However, traditional heating methods using hot water or an oven are limited, especially practical working circumstances. Thus, indirect heating methods are established to extend the application of SMPs by adopting electricity, alternating magnetic fields, and light. Near-infrared (NIR) light is suitable for in vivo biomedical applications because of the excellent tissue penetration [27]. Therefore, many efforts have been made to develop NIR-induced SMP composites based on the addition of photothermal fillers such as carbon nanotubes, graphene, and gold nanorods [28–31]. These SMP composites absorb NIR light and convert the optical energy into heat to realise the shape recovery of the SMP composites. However, these photothermal fillers are always blended with SMP matrices and suffer from poor biocompatibility.

As an alternative method to fabricate light-responsive SMPs, coating is a more direct and simpler method. Perovskite and polydopamine (PDA) have been coated on the surface of SMPs to endow them with light responsibility [32, 33]. The PDA coating, first reported by Messersmith et al. in 2007, exhibits a firm adhesion on almost all materials, which is based on the catechol groups. Moreover, PDA has a strong photothermal effect and generates heat under NIR stimulation. Thus, NIR light triggers the shape recovery of deformed SMPs that were modified with a PDA coating [33–35]. Wei et al. [33] coated PDA on cross-linked poly-(ε-caprolactone) films and reported that the thickness of the PDA coating and light intensity affect the photothermal effect.

In this study, we aim to fabricate NIR-induced shape memory micropatterns with dynamical changed properties, as shown in Fig. 1. Vinyl group-functionalised 6-arm polyethylene glycol-poly-(ε-caprolactone) acryloyl chloride (6-arm PEG-PCL-AC) was synthesised by ring-opening polymerisation and esterification. Subsequently, 6-arm PEG-PCL-AC and poly(ethylene glycol) diacyrate (PEGDA) were cross-linked on a micropatterned mould to form chemically cross-linked polyethylene glycol-poly-(ε-caprolactone) (cPEGPCL) with changeable hydrophilicity. The PDA was coated on the micropatterns to endow them with photothermal properties. The crystallinity, hydrophilicity, and shape memory of PEGDA were analysed. The photothermal transition properties and surface shape memory recovery induced by NIR light were tested. The cytotoxicity and cell alignment on micropatterns with controlled depths regulated by NIR light were studied.

2 Materials and methods

2.1 Materials

The 6-arm PEG (M₆ ≈ 6000) was procured from the Liming Research Institute of Chemical Industry. The ε-caprolactone (99.9%, Aldrich) was purified by distillation over freshly powdered CaH₂ under reduced pressure. Acryloyl chloride (AC, 98%, Adamas), stannous octoate [Sn(OC₈)₂, 95%, Adamas], 2,4,6-trimethylbenzylidiphosphine oxide (TPO, 97%, Aldrich), PEGDA (M₆ ≈ 200, Aladdin), triethylamine (99%, Adamas), and dopamine hydrochloride (DOPA.HCl, 98%, Aladdin) were used as received.

2.2 Preparation of cPEGPCL/PDA

The 6-arm PEG-PCL-AC was synthesised as described in our previous report [36]. The mixture of 6-arm PEG-PCL-AC, poly-
PEGDA, and TPO with a mass ratio of 1.0:2.0:0.01 was heated in an oven at 60°C. The molten mixture was placed on a micropatterned silicon template and reacted under ultraviolet (UV) light (λ=365 nm) for 60 min after being pressed with flat silicon. Subsequently, cPEGPCL films with square micropatterns (15 × 15 × 10 μm²; spacing: 5 μm) were obtained. The PDA coating was prepared by simply dipping cPEGPCL into 2 mg/ml DOPA buffer solution (0.02 mM, pH = 8.5) at room temperature. After a reaction time of 12 h, the cPEGPCL/PDA films were washed with deionised water and dried in a vacuum oven before use.

### 2.3 Characterisation of cPEGPCL/PDA

1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM-300 spectrometer (Bruker Co., Germany). Fourier transform infrared spectra (FTIR) were obtained using a Bruker AM-300 spectrometer (Bruker Co., Germany). Fourier-transform infrared spectra (FTIR) were obtained using a Bruker AM-300 spectrometer (Bruker Co., Germany). The morphology of the micropatterned surfaces was characterised with scanning electron microscopy (SEM). The shape recovery ratio (Rr) and shape recovery ratio (Rf) of the thermally induced shape memory effect were calculated with the following equations:

\[
R_r(\%) = \frac{H_r - H_f}{H_o} \times 100\% . \tag{4}
\]

\[
R_f(\%) = \frac{H_o - H_f}{H_i - H_o} \times 100\% . \tag{5}
\]

where \(H_o\) and \(H_r\) are the original and pressed heights of the ridge, respectively, and \(H_f\) is the recovered height under 808 nm irradiation.

### 2.4 Photothermal properties of cPEGPCL/PDA

The cPEGPCL/PDA films with a size of 20 × 5 × 0.35 mm² were irradiated by using an 808 nm NIR excitation device (Hi-Tech Optoelectronics, China) for 10 min to investigate the photothermal properties. The temperature changes were recorded with an infrared thermal imager (Fluke Ti480, USA). The power density of the 808 nm light was adjusted by changing the current of the excitation device. To investigate the photostability, cPEGPCL/PDA was irradiated with 808 nm light with a power density of 1.0 W/cm². After the surface temperature of the film reached the maximum value and stabilised, the excitation device was turned off and the film naturally cooled down to room temperature. During this process, the surface temperature was recorded with an infrared thermal imager. The results were obtained by repeating this process five times.

### 2.5 Shape memory property tests

The thermally induced shape memory properties of cPEGPCL were firstly measured. The film was heated at 60°C for 1 min, deformed in half (180°), and fixed at 0°C for 1 min to obtain the temporary angle (θt). The film was then recovered at different temperatures. The residual angle is defined as θr. The shape fixity ratio (Rf) and shape recovery ratio (Rr) of the thermally induced shape memory effect were calculated with the following equations:

\[
R_f(\%) = \frac{θ_f}{θ_t} \times 100\% , \tag{2}
\]

\[
R_r(\%) = \frac{θ_t - θ_r}{θ_t} \times 100\% . \tag{3}
\]

The NIR-induced shape memory tests of the surface micropattern were performed with micropatterned cPEGPCL/PDA films with dimensions of ∼3 × 3 × 0.35 mm². For the deformation, the film was first treated in a thermo-compressor at 60°C for 1 h, followed by compression with pre-cleaned flat silicon at 1.0 MPa for 600 s. The temperature was then turned off to cool the film down to room temperature under pressure. Therefore, a temporary surface shape was obtained. For the restoration, the temporary surface was irradiated for 600 s with an 808 nm laser with different power densities to obtain the surface shapes with different shape recovery ratios. The morphologies of the different surfaces were characterised with SEM. The shape fixity ratio (Rf) and shape recovery ratio (Rr) of the tunable surface shape were calculated with the following equation; at least three different locations on the images (3000×) for each sample were measured.

### 2.6 Cell culture on the micropatterned surface

The cytotoxicity of cPEGPCL was evaluated using Alamar Blue (AB) assay [37]. The films (3 × 3 × 0.35 mm²) were firstly soaked in phosphate-buffered saline (PBS) with 2% antibiotic/antimycotic solution for 1 day and then sterilised overnight using an UV lamp (365 nm wavelength, 12 W power) for 12 h, followed by at least three times washing with PBS before use. For the cell culture, 200 μl of foetal bovine serum (FBS) was dropwise added onto each film and incubated for 1 h at 37°C to enhance cell adhesion to the films. Subsequently, the harvested endothelial cells (ECs) were seeded on the surfaces of the films with a density of 1 × 10⁴ cells/well in a 48-well plate (Costar) and cultured in F12 solution for 1 day and then sterilised overnight using an UV lamp. Moreover, the medium was replaced at a certain time to provide adequate nutrition for the cells. For the cell viability study, the culture medium was replaced with AB solution (10% AB, 80% media 199, Gibco, and 10% FBS; v/v) after 1, 4, and 7 days. Subsequently, 200 μl samples of the supernatant were collected from each well to quantitatively determine the cell viability. Furthermore, the ECs were stained with calcein (Sigma, USA) and propidium iodide (Sigma, USA), and then observed with a
fluorescence microscope (LSM 800, Zeiss, Germany) to visualise the cell morphology and proliferation.

To regulate the alignment of the ECs, the flat cPEGPCL/PDA films, temporary pressed micropatterned films, and recovered micropatterned films irradiated with an 808 nm laser with 0.4, 0.7, and 1.0 W/cm² for 600 s were selected. The films were sterilised as mentioned above. After co-culturing for 4 and 7 days, the ECs were firstly fixed with 2.5% glutaraldehyde for 12 h and then stained with rhodamine–phalloidin (Sigma, USA) and 4',6-diamidino-2-phenylindole (Sigma) to label F-actin and the nuclei, respectively. Each experiment was performed in duplicate. At least three fluorescence images (200×) were used to calculate the percentage of the cells captured by the tunable micropatterns of the film surfaces.

3 Results and discussion

3.1 Characterisation of the cPEGPCL films

The chemical structure of 6-arm PEG-PCL was confirmed by FTIR and ¹H NMR, as shown in Fig. 2–4. The FTIR spectra show a C–O–C characteristic peak at 1106 cm⁻¹ for PEG chains. The peaks at 1106 and 1631 cm⁻¹ can be ascribed to the O=C=O and C=C stretching vibrations of the PCL chains, respectively, demonstrating that 6-arm PEG-PCL-AC was successfully synthesised by ring-opening polymerisation and vinyl groups formed on the end of the PCL chains. The chemical structure was further confirmed by ¹H NMR spectroscopy (Fig. S2). The grating ratio of the vinyl groups was calculated to be 95% by using the ratios of the peak areas of –CH=CH₂ (5.80–6.40 ppm)–CH₂– (4.04 ppm). The vinyl groups of 6-arm PEG-PCL-AC make it possible to prepare cross-linked films by free radical polymerisation.

To prepare chemical cross-linked cPEGPCL films, vinyl group-terminated 6-arm PEG-PCL, PEGDA, and TPO were mixed and dissolved in dichloromethane. After removing the solvent by evaporation, the mixtures were reacted under UV light with a wavelength of 365 nm. As shown in Fig. 5a, the characteristic peak at 1631 cm⁻¹ is absent in the spectra, suggesting the successful polymerisation of 6-arm PEG-PCL-AC and PEGDA. The phenomenon that the obtained films swell but dissolve in dichloromethane also demonstrates the chemical cross-linking. We further studied the effect of PEGDA on the melting point and crystallinity (Figs. 5b–d). The characteristic diffraction peaks at 21.5° and 23.8° are both ascribed to the PCL chains. The PEGDA barely affects the melting peak of cPEGPCL. However, the crystallinity of cPEGPCL decreases from 44.17±3.47% to

Fig. 2 FTIR spectra of 6-arm PEG, 6-arm PEG-PCL, and 6-arm PEG-PCL-AC

Fig. 3 ¹H NMR spectrum of 6-arm PEG-PCL-AC

Fig. 4 DSC curves of cPEGPCL films with 0%, 10 wt.%, and 20 wt.% PEGDA in the wet state

Fig. 5 Characterisations of cPEGPCL films with varied amounts of PEGDA

a Attenuated total reflection–FTIR spectra
b DSC curves
c XRD curves of the cPEGPCL films with 0%, 10 wt.%, and 20 wt.% PEGDA
d Crystallinity calculated from the XRD results and gel content of cPEGPCL
36.95 ± 4.00% with an increasing amount of PEGDA from 0 to 20%, whereas the gel content increases from 75.92% ± 2.62% to 87.14% ± 2.02%.

After the addition of PEGDA to cPEGPCL, the hydrophilicity was characterised by measuring the water contact angle (Fig. 6a). The water contact angle decreases from 97.10° ± 7.69° to 78.76° ± 5.77° after 20% PEGDA was added to cPEGPCL. The increase in the hydrophilicity may influence the shape recovery in the water environment due to the destruction of crystalline result from the penetrated water molecules. Figs. 6b–c show the shape recovery of deformed cPEGPCL with PEGDA at different temperatures. The shape of deformed cPEGPCL with 10 and 20% PEGDA starts to recover at 41 and 44°C, respectively, while cPEGPCL without PEGDA starts to recover at 47°C. In other words, by increasing the PEGDA content, the shape recovery occurs at a lower temperature. Based on Fig. 6c, cPEGPCL with 20% PEGDA exhibits the largest shape recovery ratio at the same temperature.

The above-mentioned results indicate that the addition of the hydrophilic polymer of PEGDA to cPEGPCL has little effect on the melting point. However, PEGDA has a notable effect on the crystallinity and hydrophilicity. Therefore, the improved hydrophilicity based on the addition of PEGDA influences the shape memory properties, especially in a water environment. Furthermore, the broader responsive range makes it possible to adjust the recovery ratio by controlling the environment temperature. So the cPEGPCL film with 20% PEGDA is selected to prepare the cPEGPCL/PDA film, which is characterised by a weak broad absorption peak at 3200–3700 cm⁻¹, which is ascribed to N–H stretching vibration of the PDA coating as shown in Fig. 5a.

A schematic illustration of the shape memory effect of cPEGPCL based on the above-mentioned results is shown in Fig. 7. The cPEGPCL chains and irreversible covalent bonds play the roles of ‘temporary phase’ and ‘fixed phase’, respectively. When elevating the temperature, the crystalline domains of cPEGPCL chains melt, which provides segment mobility. After being deformed, the temporary shape is fixed by cooling to low temperature (e.g. 0°C). When elevating the temperature again, the shape of cPEGPCL recovers to the original shape because the irreversible covalent bonds reach thermodynamic stability.

3.2 Photothermal conversion of the cPEGPCL/PDA films

The photothermal conversion of cPEGPCL/PDA was investigated by irradiation with an 808 nm laser with varied power densities for 600 s. The temperature of cPEGPCL/PDA increases by ~40°C in ~100 s when irradiated at 1.0 W/cm². In contrast, the temperature of cPEGPCL increases by only 1.1°C. The DSC curves in Fig. 5 show that the shape of the deformed cPEGPCL can be almost recovered when the temperature increases by ~40°C. Thus, temperature elevation by irradiation with 808 nm NIR light for 600 s is enough to realise shape recovery. When NIR light with 0.2, 0.4, 0.6, 0.8, and 1.0 W/cm² was used, the ΔT versus time curves show similar tendencies. Under 1.0 W/cm² irradiation, ΔT could reach 38.2°C in 80 s and there was still a relatively stable ΔT of 39.8°C at 600 s (Fig. 8b). We also observed that ΔT under different power densities exhibits a linear relationship, with a correlation coefficient of 0.98 (Fig. 8c), which makes it possible to control the surface temperature by varying the power density to further adjust the surface structure. To verify the light stability of cPEGPCL/PDA for long-term application, five cycles of heating and cooling at a power density of 1.0 W/cm² were performed. The temperature increases by ~40°C during each cycle (Fig. 8d),
indicating that cPEGPCL/PDA exhibits a good photothermal conversion.

### 3.3 NIR-induced surface shape memory effect

As mentioned above, the cPEGPCL has good shape memory properties on the microscale due to heating and the PDA coating serves as photothermal transition substance. To assure the shape memory effect on the surface micropatterns, PDA was coated on a micropatterned surface and NIR light was used to induce the shape recovery of the micropatterns. The film was firstly pressed at 60°C and 1.0 MPa in a thermocompressor for 1 h to deform and then cooled to room temperature under pressure to fix the temporary patterns. Figs. 9a and b show the topographic and cross-sectional images of cPEGPCL/PDA after irradiation with 808 nm light at varying power densities. After irradiation with 808 nm light for 600 s, the micropatterns recover from the flat shape to the patterned shape based on the elevation of the power density. When light with a 1.0 W/cm² was used, the micropatterns fully recovered to the original shape. The micropatterns were not destroyed during the shape recovery. The cross-sectional SEM images of the surface also reflect the shape change. By measuring the height of the ridge (labelled ‘H’ in the images), the shape recovery ratio was calculated, as shown in Fig. 9c. The shape recovery ratio is ~100% when light with 0.8 W/cm² was used. Moreover, the pressed film that was soaked in PBS solution at 37°C has a shape recovery ratio of only 10.68% ± 2.77%, indicating that the micropattern depth would not change during cell culturing.

### 3.4 Effect of tunable micropatterned surfaces on the cell viability and memory effect of ECs induced by NIR

The ECs play an important role in the endothelialisation of blood vessels; therefore, the regulation of ECs is important. Flat and micropatterned cPEGPCL films and flat and micropatterned cPEGPCL/PDA films were selected to investigate the proliferation and viability of ECs. The fluorescence images in Fig. 10 show cell proliferation and viability. The number of cells increases with the co-culturing days. In addition, the introduction of the PDA coating leads to an insignificant difference in the cell viability compared with the PDA-free coated group, indicating the low toxicity of the PDA coating. Furthermore, the introduction of micropatterns has a slight positive effect on the proliferation of the cells. These results demonstrate that these films have good cytocompatibilities. The cell viability of these four types of films is above 90% after 7 days, further indicating excellent cell compatibility.

The morphology of the cells could affect protein expression and cell functions. The pattern depths of the flat cPEGPCL/PDA films, temporary pressed films, and NIR-induced shape memory micropatterned films at a power density of 0.4, 0.7, and 1.0 W/cm² are 0, 2.48 ± 0.24, 3.87 ± 0.43, 5.01 ± 0.55, and 9.95 ± 0.09 μm, respectively. A cellular cytoskeleton was stained to investigate the effect of the micropattern depth on the cell alignment. Fig. 11 shows that the ECs cultured on the flat and pressed films are random and their spreading area is larger, whereas the spreading area of cells cultured on the recovered micropatterned films with larger micropattern depths is smaller and the cells tend to be captured by the micropattern. In other words, when the depth of the micropattern is small, the morphology of the cells is not affected by the micropattern and the cells can migrate freely. In contrast, when the depth of the micropattern increases, the cells are tightly bound by the micropattern and the migration of the cells is limited, causing the square-like morphology of the cells and a more ordered arrangement. In addition, for the same group, the number of captured cells increases from 4 to 7 days, suggesting that the cells gradually migrate to the micropattern.

The ratio of the cells captured by micropatterns (N₁) to total cells (N₀) was determined to quantitatively describe the cell capture ability of micropatterns. After 7 days, the ratio of N₁ to N₀ of the four types of films is 0%, 35.33% ± 5.55%, 40.80% ± 1.03%, 49.08% ± 15.76%, and 95.92% ± 3.28%, respectively, indicating that the ability of capturing cells of the micropatterns notably enhances along with the increasing micropattern depth, which is consistent with the results obtained above. Therefore, all results demonstrate that the morphology and arrangement of cells can be regulated by tunable micropatterns, thereby affecting the protein expression and cell functions.

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Fig. 9 SEM images of cPEGPCL films with varied amounts of PEGDA and the resulted recovery ratio

- a) Topographic images
- b) Cross-sectional SEM images of cPEGPCL/PDA after irradiation with 808 nm light for 600 s at different power densities of 0.2, 0.4, 0.6, 0.7, 0.8, and 1.0 W/cm², respectively
- c) Recovery ratio of the micropatterns calculated from the SEM images using (5)

Fig. 10 Live-dead fluorescence images of the ECs showing the cell viability of different surface shapes after 1, 4, and 7 days. The live and dead cells were stained green and red, respectively
Fig. 11  Cell viability and the ratio of the cells captured by micropatterns 

- Fluorescence images of the F-actin distribution of ECs on different surface shapes after 4 and 7 days. The F-actin and nuclei were marked red and blue, respectively.
- Cell viability on different surface shapes after 1, 4, and 7 days based on AB assay
- Ratio of the cells captured by micropatterns (Nc) to total cells (N0) was determined by using fluorescence images (magnification: 200×) after 4 and 7 days of cultivation (at least three pictures were averaged)

4 Conclusion

NIR-induced SMPs with micropatterns with dynamically changed shapes were prepared by coating PDA on micropatterned cPEGPCl. Based on the addition of PEGDA to 6-arm PEG-PCL-AC, the cPEGPCl exhibits an improved gel content and hydrophilicity, which facilitates the shape recovery process at a lower temperature. After the PDA was coated on the micropatterned surfaces, the surface temperature increases by 40°C when 808 nm light with 1.0 W/cm² is used, which is due to the photothermal properties of PDA. Based on the photothermal effect, the surface micropatterns with temporary shapes recover to the original shapes. The recovery ratio is controlled by adjusting the power densities. The surface micropatterns with different depths have notable effects on the regulation of the alignment of ECs.

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6 References

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