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Initiated chemical vapor deposition of thermoresponsive poly (*N*-vinylcaprolactam) thin films for cell sheet engineering

Bora Lee^a, Alex Jiao^b, Seungjung Yu^a, Jae Bem You^a, Deok-Ho Kim^{b,c,d,*}, and Sung Gap Im^{a,*}

^aDepartment of Chemical and Biomolecular Engineering and KI for NanoCentury, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea

^bDepartment of Bioengineering, University of Washington, Seattle, WA 98195, USA

^cCenter for Cardiovascular Biology, University of Washington, Seattle, WA 98109, USA

^dInstitute for Stem Cell and Regenerative Medicine, Department of Bioengineering, University of Washington, Seattle, WA 98109, USA

Abstract

Poly(*N*-vinylcaprolactam) (PNVCL) is a thermoresponsive polymer known to be nontoxic, water soluble and biocompatible. Here, PNVCL homopolymer was successfully synthesized for the first time by use of a one-step vapor-phase process, termed initiated chemical vapor deposition (iCVD). Fourier transform infrared spectroscopy results showed that radical polymerization took place from *N*-vinylcaprolactam monomers without damaging the functional caprolactam ring. A sharp lower critical solution temperature transition was observed at 31 °C from the iCVD poly(*N*-vinylcaprolactam) (PNVCL) film. The thermoresponsive PNVCL surface exhibited a hydrophilic/hydrophobic alteration with external temperature change, which enabled the thermally modulated attachment and detachment of cells. The conformal coverage of PNVCL film on various substrates with complex topography, including fabrics and nano-patterns, was successfully demonstrated, which can further be utilized to fabricate cell sheets with aligned cell morphology. The advantage of this system is that cells cultured on such thermoresponsive surfaces could be recovered as an intact cell sheet by simply lowering the temperature, eliminating the need for conventional enzymatic treatments.

Keywords

Polymer; Cell sheet engineering; Thermoresponsive; iCVD

1. Introduction

A thermoresponsive material is one that undergoes a change of physical property when exposed to external thermal stimuli [1]. Most thermoresponsive polymers are amphiphilic

*Corresponding authors at: Department of Bioengineering, University of Washington, Seattle, WA 98195, USA. Tel.: +1 206 616 3113 (D.-H.Kim), Department of Chemical and Biomolecular Engineering and KI for NanoCentury, Daejeon 305-701, Republic of Korea Tel.: +82 42 3503936; fax: +82 423503910 (S. G. Im). deokho@uw.edu (D.-H. Kim), sgim@kaist.ac.kr (S.G. Im).

and exhibit a reversible phase transition from hydrophilic to hydrophobic state with increasing temperature, especially in an aqueous medium. The phase transition of thermoresponsive polymers is reported to originate from the intra- and intermolecular hydrophobic attractions of the monomer units [2]. The temperature at which this transition occurs is termed the lower critical solution temperature (LCST).

The use of thermoresponsive polymers has allowed novel methods in tissue engineering. Historically, cells are removed from culture systems using an enzymatic process which degrades the extracellular matrix of the cells in addition to dissociating cell–cell contacts. Detached cells thus become individualized and are typically seeded into supporting structures or scaffolds in order to fabricate three-dimensional (3-D) cellularized tissues. Although such a method is suitable for cell-sparse tissues, scaffolds reduce cell–cell contacts and do not properly recapitulate tissue structure of cell-dense tissues such as the heart and liver. Further, the use of scaffolds may restrict overall tissue dimensions due to the diffusion limits of oxygen and the transport issues of nutrients for cells seeded in the interior scaffold. Scaffolds also often elicit an inflammatory response, both with permanent implants and in response to non-toxic biodegradable scaffolds [3–5]. Scaffold-free approaches to 3-D tissue engineering, such as cell sheet engineering, may avoid these problems.

The term “cell sheet engineering” was coined by Teruo Okano for scaffold-free 3-D tissue engineering via the use of amphiphilic thermoresponsive polymers [6]. The principle behind cell sheet engineering is that the amphiphilic nature of the thermoresponsive polymer can dislodge intact cell sheets after the change in the conformation of polymer chains. For instance, a thermoresponsive polymer is hydrophobic above the LCST, allowing protein adsorption and cell attachment, whereas below the LCST medium infiltrates the hydrophilized polymer. The swollen surface causes the cells to actively detach from the surface while maintaining cell–cell contacts. Thus, confluent cultured cells will detach in a sheet, as the cell–cell connections are not disrupted or cleaved by an enzymatic process. The resulting cell sheets can be stacked to form 3-D tissues without the use of a scaffold [7,8]. Cell sheet engineering is a versatile technology and has been utilized with epithelial cells, ligament cells, hepatocytes, myocardial cells and other cell types [9–15].

Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of the best-known thermoresponsive polymers [16], as it has a LCST at 32 °C which falls between room temperature and body temperature. Due to its physiologically relevant LCST, PNIPAAm has triggered huge research interest, especially for various biomaterial applications, such as controlled drug delivery [17–21], cell sheet engineering [9,22–24] and bioseparation [25,26]. However, its acrylamide functionality is prone to hydrolysis in acidic conditions to release potentially toxic small amide molecules from the main polymer chain and cause unwanted side effects during long-term biomedical applications [27]. As an alternative, poly(*N*-vinylcaprolactam) (PNVCL) is an amphiphilic, water-soluble polymer with an LCST of approximately 31–34 °C in aqueous medium. Above the LCST, PNVCL is insoluble in water, whereas lowering the temperature below the LCST makes it water soluble. This transition is reversible and extremely fast. The *N*-vinylcaprolactam (NVCL) functionality of PNVCL consists of a hydrophilic amide group end-capped with a hydrophobic aliphatic hydrocarbon chain. The cyclic structure of caprolactam ensures the amphiphilic nature of PNVCL, and

the hydrolysis of PNVCL only results in the breakage of the caprolactam ring with no toxic volatile by-product [28]. In spite of its many advantages of PNVCL, the use of PNVCL for biomedical applications has not been as widely explored, and few reports exist on its application as a thin film [29]. Most of the current biomedical applications of PNVCL utilize micro-/nanoparticles [27,30]; the application of PNVCL polymer film is very rare. Lim et al. [31] have applied a copolymer of PNVCL and PNIPAAm for cell sheet engineering, but it is not clear what the role of PNVCL in the PNVCLco-PNIPAAm polymer was in their work. Most of the PNVCL materials have been synthesized using a liquid phase process comprising a complicated multi-step synthesis procedure [32].

In this research, we utilized a vapor-phase deposition process called initiated chemical vapor deposition (iCVD) to synthesize PNVCL homopolymer film onto various kinds of substrate. The one-step iCVD process could fabricate highly pure PNVCL homopolymer film without a complex purification step. The iCVD process utilizes a radical polymerization reaction in the vapor-phase by flowing vaporized initiator and monomer into the vacuum reactor. The scheme for the iCVD process is shown in Fig. 1 along with the polymerization reaction of N-vinylcaprolactam to PNVCL. Since the temperature of the substrate is in the range of 15–40 °C, various vulnerable substrates, such as paper, fabric and membranes, can be coated with iCVD polymer films without any damage [33,34]. Moreover, the vapor-phase process enables conformal coating of the polymer film onto various complex surface morphologies, including textured surface and nanopatterns [34–37], which is extremely desirable for the application of generating 3-D structured cell sheets.

The synthesis of PNVCL homopolymer films was successfully achieved for the first time via the iCVD process. Fourier-transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) analyses indicated that the PNVCL polymer was successfully formed from the vaporized NVCL monomer with the retention of the cyclic amide ring in PNVCL. The molecular weight of the deposited polymer was determined by gel-permeation chromatography (GPC) and the conformal coating of PNVCL on a nanopatterned substrate was confirmed by scanning electron microscopy (SEM). The phase transition of PNVCL across the LCST was monitored by both ultraviolet–visible spectroscopy (UV–Vis) and water contact angle (WCA) measurement. The LCST of thermoresponsive polymer was determined by the experimental value of the cloud point temperature (T_{CP}) through UV–Vis analysis. The cell behavior on the PNVCL substrate was observed by bright-field optical microscopy and the biocompatibility of the iCVD PNVCL polymer was determined by live/dead assay. Finally, the applicability of PNVCL to cell sheet engineering was also explored for the first time by checking the detachment of the cell sheet cultured on a PNVCL-coated substrate.

2. Experimental

2.1. iCVD polymerization

NVCL (98%, Aldrich) and *tert*-butyl peroxide (TBPO; 98%, Aldrich) were purchased and used without further purification as monomer and initiator, respectively. The iCVD polymerization took place in a custom-built iCVD reactor (Daeki Hi-Tech Co. Ltd) described previously [38]. Briefly, the NVCL monomer was heated to 80 °C and the formed

NVCL vapor was introduced to the reactor at a flow rate of 1.0 sccm. TBPO is volatile enough at room temperature to form a vapor, so it was fed into the reactor via metering valves at a flow rate of 0.8 sccm without any additional heating. To obtain a PNVCL film with high purity, NVCL and TBPO were fed into the reactor concurrently with a reactor pressure of 150 mtorr. The substrate was kept at a temperature of 35 °C to promote monomer adsorption, whereas the filament temperature was maintained at 200 °C. The thickness of the PNVCL film was monitored in situ using an He–Ne laser (JDS Uniphase). The substrates were cleaned with isopropanol and deionized (DI) water before undergoing 5 min of sonication each and drying with N₂ gas. The substrates were then treated with oxygen plasma. Depositing the polymer immediately after the plasma treatment allowed the grafting of the polymer to the substrate.

2.2. Characterization of iCVD PNVCL film

FTIR (Alpha FT-IR Spectrometer, Bruker Optics) of PNVCL film was measured in normal absorbance mode averaged over 64 scans. All spectra were baseline corrected.

The surface chemical compositions of the PNVCL films deposited by iCVD (iCVD PNVCL) were analyzed by XPS (MultiLab 2000, Thermo VG Scientific). The spectra were recorded using an Al K α X-ray radiation source (150 W, 14 kV, KE = 1486.6 eV) under a vacuum base pressure (5×10^{-10} torr). The XPS spectra were collected in the range of 0–1100 eV with a resolution of 1.0 eV and with a pass energy of 100 eV. The binding energy scale was calibrated with reference to the C1s line at 285.0 eV.

The molecular weight and the polydispersity index (PDI) of the deposited films were determined by GPC on a system equipped with a Waters 1515 isocratic high-performance liquid chromatography pump with a refractive index detector (Waters 2414). Tetrahydrofuran (JT Baker) was used as an eluent, with a flow rate of 1 ml min⁻¹. The temperature of the column oven was set to 30 °C. Monodisperse polystyrene standards (Polyscience) were used to generate the calibration curve. A 30 μ l volume of each sample was injected into the column after passing through a 0.2 μ m filtration membrane.

The morphology of iCVD PNVCL was assessed by SEM using a XL30SFEG (Philips) scanning electron microscope operating at 3.00 kV. All the samples were attached to a conducting sample holder with copper tape. The prepared samples were coated with gold under a vacuum using the sputtering system.

The LCST behavior of the iCVD PNVCL was investigated using UV–Vis spectroscopy (UV-3600, Shimadzu). The transmittance of 0.25 wt.% aqueous polymer solutions was measured at 500 nm at intervals of 2 °C in the range of 25–55 °C. The solution was allowed to be equilibrated at each temperature for 20 min before the transmittance was measured.

WCA measurements were performed on a contact angle meter (Phoenix 150, SEO) equipped with an environment chamber (temperature range 25–250 °C) using a 2.5 μ l DI water droplet. Measurements were taken both at room temperature (25 °C) and at 55 °C. An Si wafer coated with PNVCL was allowed to equilibrate at each temperature for 20 min before the contact angle was measured.

2.3. Cell culture

NIH 3T3 mouse fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) on tissue culture polystyrene (TCPS) dishes at 37 °C in 5% CO₂. PNVCL-grafted glass substrates were cut and placed in 35 mm diameter TCPS dishes and irradiated with UV at 264 nm for 30 min inside a biosafety cabinet to sterilize the substrates. PNVCL substrates were then soaked overnight in distilled H₂O. Prior to cell seeding, the H₂O was aspirated and the dry PNVCL substrates were placed in the incubator used for cell culture. Cultured cells (between passages 10–20) were trypsinized and seeded onto PNVCL-deposited glass substrates at a high density of 1.5×10^5 cells cm⁻² to achieve confluency within 24 h of culture. After being incubated at 37 °C in 5% CO₂, adherent cells were observed at 3, 6 and 24 h post-seeding with a phase contrast microscope (Eclipse TS100, Nikon).

2.4. Live/dead assay

A live/dead assay (Invitrogen) utilizing Calcein AM as a live cell marking dye and ethidium homodimer-1 (EthD-1) as a dead cell marking dye was used according to the manufacturer's instructions. Briefly, the kit reagents were added to sterile 1 × Dulbecco's Phosphate-Buffered Saline (DPBS) to create a 1 mM Calcein AM and 4 mM EthD-1 solution. The cells on PNVCL were washed gently with warm 1 × DPBS, then 200 µl of the Calcein AM/EthD-1 solution was added to the coverslips and incubated in the dark for 30 min at 37 °C and 5% CO₂. After incubation, cells were gently rinsed with 1 × DPBS and then imaged with a high-resolution wide-field fluorescent microscope (TiE, Nikon).

2.5. Detachment of cell sheets

For cell sheet transfer experiments, a plunger method previously published by the Okano group [39] was utilized to transfer partial cell sheets onto glass coverslips during the 30 min incubation at 25 °C. Briefly, cells were cultured to confluency on PNVCL surfaces (within 24 h of seeding). A 7.5% w/v gelatin in sterile 1 × DPBS was melted at 60 °C, filter sterilized and poured into a PDMS mold. A Teflon plunger was placed upon the melted gelatin and the ensemble was cooled at 4 °C for 10 min. The PDMS mold was then gently removed, leaving a solidified gelatin hydrogel attached to the Teflon plunger. Substrate medium was aspirated away and 200 µl of fresh, serum-free DMEM was added to the substrate samples to prevent the cells from dehydrating. The plunger was then placed on top of the substrate and cells, and the scaffold + plunger was incubated at 25 °C inside a biosafety cabinet for 30 min. After incubation, the gelatin plunger was gently removed from the substrate surface, capturing partially detached cell sheets. A scalpel was used to remove the gelatin from the Teflon plunger and the gelatin was placed cell sheet first onto a clean glass coverslip and allowed to attach for 30 min at 25 °C. After attachment, the gelatin plus coverslip was incubated at 37 °C and 5% CO₂, and the gelatin was allowed to melt. Coverslips plus cell sheets were rinsed gently twice with PBS, cultured with DMEM at 37 °C and 5% CO₂ for 24 h and then imaged.

3. Results and discussion

3.1. FTIR analysis

Fig. 2 shows the FTIR spectra of NVCL monomer and the PNVCL film deposited via iCVD. In the FTIR spectrum of the NVCL monomer, a characteristic carbonyl peak ($\text{C}=\text{O}$) in the amide group was detected at 1620 cm^{-1} . The peak for the unsaturated carbon $\text{C}=\text{C}$ was observed at 1651 cm^{-1} , and the stretching and bending vibrational peaks of $\text{C}-\text{H}$ bonds in the vinyl group ($=\text{CH}$ and $=\text{CH}_2$) were also detected at 3106 and 993 cm^{-1} , respectively. The peak corresponding to $\text{C}-\text{N}$ bond in the amide group was also found at 1478 cm^{-1} . The peak at 1441 cm^{-1} corresponds to the aliphatic $\text{C}-\text{C}$ bond in the caprolactam ring. In the spectrum of iCVD PNVCL, the peaks corresponding to the $\text{C}=\text{C}$ double bond of the monomer disappeared, as marked by red arrows in the FTIR spectrum (Fig. 2b), indicating successful polymerization of the monomer by the iCVD process. The retention of the caprolactam functionality in the PNVCL polymer was also confirmed from the FTIR spectrum of iCVD polymer. The characteristic carbonyl peak ($\text{C}=\text{O}$ stretching) in the amide bond was found at 1620 cm^{-1} . The aliphatic $\text{C}-\text{H}$ stretching peaks in the caprolactam ring were detected at 2931 cm^{-1} . The peak of tertiary amine ($\text{C}-\text{N}$) in the amide functionality was observed at 2850 cm^{-1} , which is close to that of NVCL monomer. The retention of caprolactam functionality in the iCVD PNVCL distinctly shows that the iCVD process did not damage the NVCL monomer during the polymerization process. A broad peak was observed in the spectrum of the iCVD polymer at 3431 cm^{-1} , which corresponds to the $\text{O}-\text{H}$ stretching vibration. Due to its extremely hygroscopic nature, PNVCL is known to absorb moisture readily. The peak corresponding to the absorbed moisture is located at 3431 cm^{-1} , which has been observed commonly in previous reports on PNVCL polymerized by various liquid-phase methods [28,40].

3.2. XPS survey scan and high resolution analysis

XPS analysis was also performed to determine the chemical compositions of the iCVD PNVCL film. As shown in Fig. 3, a repeating unit of PNVCL consists of eight carbons (C), one oxygen (O) and one nitrogen (N) atoms. The characteristic peaks of carbon ($\text{C}1s$ at 285.0 eV), oxygen ($\text{O}1s$ at 531.0 eV) and nitrogen ($\text{N}1s$ at 399.4 eV) in PNVCL were all detected in the survey scan spectrum shown in Fig. 3a. The atomic ratio of pNVCL film was calculated from the areas of $\text{C}1s$, $\text{O}1s$ and $\text{N}1s$ peaks in the XPS survey scan, multiplied by the appropriate sensitivity factors. The measured atomic ratio of $\text{C}:\text{O}:\text{N}$ was $75.8:13.5:10.7$. Compared to the composition predicted from the stoichiometry of the NVCL monomer, which is 80.0% of C, 10.0% of O and 10.0% of N, an excessive amount of oxygen was detected, which most likely originated from water absorption due to the highly hygroscopic nature of PNVCL. This observation is consistent with the FTIR results. XPS high-resolution scans of O, N, and C were also performed, and are shown in Fig. 3b–d. In case of the $\text{O}1s$ core-level XPS spectrum (Fig. 3b), one dominant peak at 531.0 eV corresponds to the sole oxygen bonding environment in PNVCL, while a small broad shoulder at 533.6 eV was also detected, most likely originating from the oxygen of the absorbed water to the PNVCL, which also supports the findings in the XPS survey scan and the FTIR measurement of the PNVCL film. In the $\text{N}1s$ core-level XPS spectrum (Fig. 3c), only one N bonding environment in PNVCL was observed, at 399.4 eV , representing the N in the amide group of

the caprolactam ring. The C1s core-level XPS spectrum (Fig. 3d) was deconvoluted to three major peaks of C: (1) a peak at 287.6 eV, representing C in a carbonyl bond (C=O) (1 in the inset of Fig. 3d); (2) a peak at 285.4 eV, corresponding to C in a C–N bond (2 in the inset of Fig. 3d); and (3) an aliphatic C1s peak at 284.6 eV (3 in the inset of Fig. 3d). The measured atomic ratio from the C1s core-level XPS spectrum was 0.19:0.39:1.00, which is consistent with the theoretical calculation from the chemical formula of PNVCL, which is 0.20:0.40:1.00 (Table 1b). Both the FTIR and XPS results clearly demonstrate that the PNVCL polymer was successfully synthesized by the iCVD polymerization process, maintaining the caprolactam ring in the PNVCL.

3.3. GPC analysis of PNVCL

The thermoresponsive behavior of PNVCL is reported to be highly dependent on the molecular weight of PNVCL. Since the molecular weight and its distribution depend on the synthesis methods, the phase transition curve of PNVCL varies significantly according to the polymerization scheme. In the case of the iCVD process, the used polymerization reaction is a radical polymerization reaction and the theoretical value of the PDI is 2.32. The molecular weight of iCVD polymer was reported to be in the range of 5000–100,000, depending on the monomers used in the iCVD process. In this work, the molecular weight and PDI of iCVD PNVCL were measured by GPC, with polystyrene as the standard. The number-averaged and weight-averaged molecular weight (M_n and M_w , respectively) of PNVCL were calculated to be about 14,000 and 29,000, respectively. The PDI was 2.04, calculated from the ratio of M_n to M_w . The PDI value was very close to the theoretical value of radical polymerization, which is one of the characteristics of iCVD polymers. The molecular weight was not extremely high, but in the range of typical molecular weight of iCVD polymers.

3.4. SEM analysis of iCVD PNVCL film

To confirm the formation of a conformal polymer coating, untreated bare nylon and nylon coated with PNVCL via iCVD were analyzed by SEM. Fig. 4 shows the SEM images of the untreated and coated fabrics. The initial surface morphologies of the bare fabric (Fig. 4a) are completely maintained even after the deposition of the PNVCL film (Fig. 4b). In the SEM images, no morphological damage was observed after the polymer deposition due to the gentleness of the processing conditions of iCVD [41]. A cross-sectional SEM image of nanometer-sized line pattern coated with 50 nm of iCVD PNVCL shows a well-defined coating (Fig. 4c). The top and side walls of the patterned substrates were coated to a constant thickness using the iCVD process. The conformal nature and gentle deposition condition of the polymer film onto the various complex surface morphologies using this method is extremely desirable for 3-D tissue scaffold applications.

3.5. UV–Vis analysis

PNVCL underwent phase transition by a temperature change across the LCST. Above the LCST, PNVCL became hydrophobic and precipitates of PNVCL were formed in aqueous solution, hence the PNVCL solution became turbid (bottom inset images of Fig. 5). Therefore, the LCST of PNVCL was determined by measuring the turbidity of the PNVCL in aqueous solution with an increase in the temperature of the PNVCL solution. The change in the turbidity was monitored by measuring the transmittance of the PNVCL solution via

UV–Vis spectroscopy. Fig. 5 displays the change in the transmittance of the PNVCL solution by increasing the temperature of the solution from 25 to 55 °C. Starting from 27 °C, an abrupt decrease in the transmittance was observed, and the turbidity was saturated at 43 °C. The inflection point in the S-shaped curve obtained experimentally is termed as the cloud point temperature, T_{CP} . From the curve in Fig. 5, the T_{CP} of the iCVD PNVCL polymer was determined to be 31 °C. The obtained T_{CP} matched well with the previous observations [30,42].

3.6. Static water contact angle measurement

The hydrophilic-to-hydrophobic phase transition of iCVD PNVCL was also monitored by measuring the change in static WCA. At 25 °C, which is below the LCST, PNVCL is hydrophilic and the static WCA was found to be around 25°. Analogously, at 55 °C, which is above the LCST, PNVCL is hydrophobic and the static WCA was found to be around 70° (top inset images of Fig. 5).

With the pre-treatment of the glass substrate with oxygen plasma, the iCVD PNVCL was grafted onto the surface of the glass substrate and the PNVCL film maintained its morphology below the LCST (25 °C). The iCVD PNVCL film was also intact in water above the LCST (55 °C). Fig. 6a and b shows still camera images of iCVD PNVCL films after incubation in DI water for 12 h below and above LCST, respectively. As shown in the figure, no apparent degradation or delamination was observed from the incubated samples. The physiologically relevant LCST of PNVCL enables its potential application to cell biology. It is important to ensure the thermoresponsiveness of PNVCL films in aqueous medium at a cell-culture temperature of 37 °C for biomedical and histological applications of PNVCL. For this purpose, PNVCL films deposited via iCVD were also incubated in DI water at 37 and 38 °C for 24 h, after which the WCA was measured. Unlike the WCA of bare Si wafer, which was about 47° regardless of the stage temperature (Fig. 6c), the Si wafers coated with PNVCL maintained their thermoresponsiveness even after the 24 h incubation at 37 and 38 °C. The WCA values of the PNVCL-coated wafers were demonstrated to be around 29° at 25 °C and higher than 60° at 55 °C. Although a slight change in the thermoresponsiveness was observed after the incubation in water, the major thermoresponsive property of PNVCL was retained. This stability test clearly indicates that the PNVCL film deposited via iCVD is capable of cell culture at 37 °C with minimal damage of the polymer surface.

3.7. Detachment of cell sheets using thermoresponsive PNVCL film

The initial attachment of cells on PNVCL was no different than on glass controls. Morphologically, 3T3 mouse fibroblasts demonstrated similar shapes and sizes when compared to the glass control, as shown in Fig. 7a. However, although seeded at the same density, the fibroblasts on the PNVCL retained more of a spindle shape than on the glass, where the cells appeared cobblestone-like. This indicates that the cells reached a higher confluence on glass than on PNVCL when seeded at the same density. Subsequently, to test whether the cell viability was impacted on PNVCL, a live/dead assay was performed. The live/dead assay provides two different fluorescent colors according to cell viability. Calcein AM and EthD-1 were used as the optimal dyes for mammalian cells in this research. The

Calcein AM is retained in live cells, representing green fluorescence; the EthD-1 produces red fluorescence in dead cells. Compared to glass controls, cells on PNVCL demonstrated equivalent viability. Qualitatively, few cells (<10) were dead on either PNVCL or glass substrates, indicating near 100% viability on both substrates. A few more cells are stained green on PNVCL than on glass. Therefore, the iCVD PNVCL film is non-cytotoxic towards NIH 3T3 mouse fibroblasts. Cells were also fixed on PNVCL substrates and stained to ensure sensitivity of the dead cell marker (Fig. 7b). In the cell sheet detachment experiments, partial detachment from the PNVCL substrates was observed. The detachment occurred within 30 min after the temperature was lowered to 25 °C, indicating a comparable rate to that of PNIPAAm. Cells were able to be detached from the substrate in an intact sheet and recovered with the gelatin plunger. Since the iCVD process is a surface-growing polymerization process, it is possible to tune the thickness of the polymer film exactly, and the thickness of the polymer film was also monitored in situ during the iCVD process by the interferometry. The dependency of attachment/detachment on the film thickness was assessed by changing the thickness of the polymer film from 30 nm to 100 nm, but no appreciable difference in the performance of cell detachment was observed. The recovered sheets were transferred to a new surface and replated. After replating, cells continued to proliferate outwards from the attached sheet (Fig. 7c). The partial detachment of the cell sheet on the PNVCL surface can be ascribed to a broad hydrophilic–hydrophobic transition temperature and the grafting density of PNVCL on the glass substrate. The LCST of PNVCL is known to be dependent upon the molecular weight of PNVCL, with the lower molecular weight PNVCL showing the higher LCST [43]. Since the iCVD process involves the free radical polymerization reaction, the synthesized polymer showed a range of molecular weights, as displayed in Table 2. The broad transition curve in Fig. 5 is due to the molecular weight distribution. The lower molecular weight part of the PNVCL did not show any hydrophilic transition and maintained the cell adhesion at lower temperature. This could be responsible for the partial detachment of the cell sheet. In addition, for the cell detachment experiment, the PNVCL polymer film was grafted onto the glass substrate by oxygen plasma pre-treatment. However, if the grafting was not perfect, the PNVCL film might be partially damaged during the cell culture, which could be another reason for the partial detachment. To overcome this problem, it is necessary to minimize the damage of PNVCL during the cell sheet experiment, which can be achieved by optimizing the pretreatment condition of the substrate for a better grafting density of PNVCL. Adding a small degree of cross-linker will also be helpful to improve the stability of PNVCL film. Increasing the molecular weight of the iCVD PNVCL film can also be achieved by controlling the iCVD process parameters, especially the flow rate ratio of the initiator to the monomer [36]. A sharper LCST transition can be obtained from this iCVD process optimization.

4. Conclusions

In this study, we presented an intelligent thermoresponsive surface by coating cell culture substrates with a PNVCL polymer film. The fabrication of the thermoresponsive PNVCL surfaces was achieved via the iCVD process for the first time. Characterization methods, such as FTIR and XPS, revealed that the polymer was successfully synthesized via iCVD

without causing any damage to the caprolactam ring. The thermoresponsive property of PNVCL was additionally confirmed by transmittance changes measured using UV–Vis analysis, with an LCST of 31 °C. Owing to these characteristics, the PNVCL films were demonstrated to be applicable to the field of cell sheet engineering. NIH 3T3 mouse fibroblasts attached well to the PNVCL and demonstrated their typical morphology at 37 °C. PNVCL films were also found to be biocompatible, as tested via a live/dead assay. Finally, partial cell sheet detachment, recovery and transfer using non-enzymatic processes were demonstrated by incubating the PNVCL scaffold at room temperature. This demonstrated the control of cell attachment and detachment on thermoresponsive PNVCL scaffolds in response to a temperature change.

The recovery of a cell sheet solely by lowering the temperature without the use of any enzymatic treatment is highly desired for the fabrication of three-dimensional tissue-like structures. Moreover, the recovered confluent cell sheets have the potential to be applied to novel therapies in clinical areas such as regenerative medicine. Therefore, the development of these novel smart surfaces and their use should aid the progress in a number of fields, including medicine and biology.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 1–7 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at <http://dx.doi.org/10.1016/j.actbio.2013.04.049>.

Fig. 1.

(a) Schematic diagram of iCVD polymerization of the PNVCL polymer from NVCL monomer. (b) Illustrations of the formation process of PNVCL in the iCVD chamber.

Fig. 2. FTIR spectra of (a) NVCL monomer and (b) iCVD PNVCL polymer. The solid line corresponds to the C=O bond and the dashed line represents the tertiary amine moiety. Important functional groups were retained in both spectra. Red arrows mark the C=C double bond and vinyl group in NVCL monomer. These peaks are absent in the iCVD PNVCL polymer.

Fig. 3.

The XPS spectra of PNVL: (a) XPS survey scan of the iCVD PNVL film. Oxygen, nitrogen and carbon 1s peaks are recorded in the spectra. XPS high-resolution scans of (b) O1s, (c) N1s and (d) C1s in PNVL, respectively.

Fig. 4. SEM images of (a) the surface of untreated nylon fabric, (b) the surface of iCVD PNVCL coated nylon mesh (scale bar = 500 μm) and (c) the cross-section of iCVD PNVCL coated on nanoscale line pattern (scale bar = 500 nm).

Fig. 5.

UV transmittance (at the wavelength of 500 nm) of aqueous solution containing 0.25 wt.% PNVCL, measured from 25 to 55 °C. The top inset images show the change in contact angle (CA) of iCVD PNVCL coated on Si wafer. The CA was about 25° at 25 °C (left) and 70° at 55 °C (right). The bottom inset images show the change in optical transparency of PNVCL aqueous solution at temperatures of 25 °C (left) and 55 °C (right). As shown in the images, the PNVCL solution is transparent below the LCST, while above it the solution is opaque.

Fig. 6.

Digital camera images of PNVCL-grafted Si wafer exposed to water at (a) 25 °C and (b) 55 °C show that the film remains intact both below and above the LCST. Water contact angle images of the three samples were also taken at 25 and 55 °C: (c) bare Si wafer after 24 h of exposure to water; (d) PNVCL film coated via iCVD on Si wafer after 24 h of exposure to water at 37 °C; and (e) PNVCL film coated via iCVD on Si wafer after 24 h of exposure to water at 38 °C. Unlike the bare sample, the wafers coated with PNVCL still display sharp transition even after 24 h of exposure to water.

Fig. 7. Cell study images. (a) The morphology of the cells on the glass and PNVCCL substrates is similar under normal culture conditions. (b) Cell viability staining via a live/dead assay. Green = live cells; red = dead cells. The viability on PNVCCL is similar to that on glass. (c) Partial detachment (left) and transfer (right) of the 3T3 fibroblast monolayer. Scale bar = 100 μ m.

Table 1

Elemental compositions determined by XPS: (a) a survey scan of the iCVD PNVCL film, (b) high-resolution data of the C1s peak.

(a)	Element content (At.%)		
	C1s	O1s	N1s
Theoretical value	80.0	10.0	10.0
Experimental value	75.8	13.5	10.7
(b)	Composition of the C1s peak (area%)		
	C=O	C–N	C–C
Theoretical value	0.20	0.40	1.00
Experimental value	0.19	0.39	1.00

Table 2

GPC results of the iCVD PNVCL polymer.

M_n (g mol ⁻¹)	14,347
M_w (g mol ⁻¹)	29,226
PDI	2.037