

ADVANCES IN THE SYNTHESIS AND APPLICATION OF POLYCAPROLACTONE
SHAPE MEMORY POLYMER BIOMATERIALS

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in
partial fulfillment of the requirements for the degree of Doctor of Philosophy in the
department of Chemistry.

Chapel Hill
2012

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ABSTRACT

DUY M. LE: ADVANCES IN THE SYNTHESIS AND APPLICATION OF
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(Under the direction of Dr. Valerie Sheares Ashby)

Shape memory polymers (SMPs) are a promising class of responsive materials that have recently gained widespread interest for their unique applications in biomaterials science. The present work describes the synthesis and characterization of novel poly(ϵ -caprolactone) (PCL) SMPs. A series of PCL thermosets were synthesized from linear and branched PCL prepolymers of different molecular weights. The PCL networks showed excellent control over the transition temperature and outstanding shape memory properties. A double replica soft lithographic technique was then used to fabricate dynamic PCL surfaces capable of transitioning between microarrays of different sizes and shapes. An equally important objective of this research was to demonstrate the viability of these SMP surfaces as a highly versatile and controlled means of dynamic cell culture, specifically for the purpose of investigating cell-topography interactions. The morphology of human mesenchymal stem cells was topographically directed through the application of the surface shape memory effect at physiological temperature. Lastly, gold nanoshell/PCL nanocomposites were synthesized for remote activation of the shape memory effect via near-infrared irradiation. The nanocomposites demonstrated excellent shape fixation and recovery in response to low laser power intensities at nanoshell weight fractions of 0.5 and 1.0 wt%.

*This dissertation is dedicated to my parents,
for their love, sacrifice, and inspiration.*

ACKNOWLEDGMENTS

I would like to express sincere and earnest gratitude to all of the individuals who have helped make this dissertation possible. First, I would like to thank my research advisor, Prof. Valerie Sheares Ashby, for her support over the past five years. I am also greatly indebted to Prof. Kam Leong for his encouragement, mentorship, and guidance. A special thank you is also owed to Prof. Joeseeph DeSimone for his collaboration and continued support.

It is also a great pleasure to thank the numerous contributors herein; Dr. Karina Kulangara for all of her contributions to the dynamic cell culture project; Andrew Adler for widefield image analysis; and Prof. Christopher Fecko and Michael Tycon for their collaboration on the NIR SMP project. Amongst the many kind members of the UNC and Duke community I would also like to thank Dr. Yapei Yang, Dr. Christopher Luft, Dr. Wonhee Jong, Dr. Timothy Merkel, Dr. Carrie Donely, Dr. Syandan Chakraborty, and Melathi Chellapan.

This acknowledgement would not be complete without recognizing my parents for all their unconditional love and for always believing in me. I would also like to express my deep appreciation to the Le, Cointot, and Bloome families for their generous support and encouragement. Above all, I would like to thank my wife, Kayla Sue, who is both my greatest supporter and my best friend. Your boundless care and optimism has been the source of great strength and inspiration, and I am incredibly blessed to have you in my life. Thank you for all that you do.

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List of Abbreviations

AFM	Atomic force microscopy
Arg	Arginine
Asp	Aspartic acid
BPA	Bisphenol A
BD	Butane diol
CA	Cinnamic acid
CAA	Cinnamylidene acetic acid
CHM	Cyclohexane methacrylate
CNT	Carbon nanotube
DEAP	Diethoxyacetophenone
DMA	Dynamic mechanical analysis
DSC	Differential scanning calorimetry
EL	Ethacridine lactate
EN	Enoxacin
Gly	Glycine
HD	Hexane diol
HDMI	Hexamethylene diisocyanate
hMSC	Human mesenchymal stem cell
MDI	Methylene diphenyl diisocyanate
NF	Nitrofurantoin
NGDE	Neopentyl glycol diglycidyl ether
NHC	N-heterocyclic carbene

NIR	Near infrared radiation
NMR	Nuclear magnetic resonance
PBS	Phosphate buffer solution
PCHM	Poly(cyclohexane methacrylate)
PCL	Poly(ϵ -caprolactone)
PCLDM	Poly(ϵ -caprolactone) dimethacrylate
PDI	Polydispersity index
PDMS	Poly(dimethylsiloxane)
PEGMA	Poly(ethylene glycol) methyl ether methacrylate
PFSA	Perfluorosulfonic acid
PGA	Poly(glycolide)
PLA	Poly(lactide)
PLLA	Poly(L-lactide)
POSS	Polyhedral oligomeric silsesquioxane
PTHF	Poly(tetrahydrofuran)
PTMG	Poly(tetramethylene glycol)
PU	Polyurethane
ROP	Ring opening polymerization
SEM	Scanning electron microscopy
Ser	Serine
SMP	Shape memory polymer
SPR	Surface plasmon resonance
TBD	Triazabicyclodecene

List of Symbols

χ_c	Degree of crystallinity
D_m	Degree of methacrylation
ϵ_R	Elongation at break
ΔH_m	Enthalpy of melting
ϵ_u	Fixed strain after unloading
T_g	Glass Transition Temperature
$\tan \delta$	Loss factor
f	Magnetic frequency
H	Magnetic flux density
T_m	Melting Temperature
$\langle M_n \rangle$	Number average molecular weight
ϵ_p	Permanent strain after the recovery step
I_r	Repeat ^1H NMR methylene intensities of a PCL chain
R_f	Shape Fixity Ratio
R_r	Shape Recovery Ratio
E'	Storage modulus
ϵ	Tensile strain
σ	Tensile stress
I_t	Terminal ^1H NMR methylene intensities of a PCL chain
ϵ_m	Total strain induced during mechanical deformation
T_{trans}	Transition Temperature
ϵ_s	Yield strain

σ_s	Yield stress
E	Young's modulus

Chapter 1

Introduction to Shape Memory Polymers

1.1 The Shape Memory Effect of Polymers

Shape memory polymers (SMPs) are an emerging class of smart materials that can change shape in a predetermined way when exposed to the appropriate external stimulus (**Fig. 1-1**).^[1] Since their introduction in 1984 by the CDF Chimie Company (France), SMPs have become an active field of materials research with applications spanning many areas of science and engineering. More recently, SMPs have gained widespread interest for their broad implications in biomaterials science.^[2,3] This has largely resulted from coupling the shape memory effect with additional functionalities such as biocompatibility, tunable degradation, and controlled drug release.^[4,5]

While, the shape memory effect has been activated electronically, magnetically, and electromagnetically, the most common shape memory trigger is thermal activation.^[6,7,8,9,10] There are two features that are ubiquitous to all thermally activated SMPs.^[11] First, the polymer must possess chemical or physical crosslinks which act as net points and prevent chain slipping during mechanical deformation and recovery. The second essential feature is a thermally-reversible phase transition. This transition temperature (T_{trans}) is typically associated with the polymer glass transition temperature (T_g) or melting temperature (T_m). The molecular mechanism for the shape memory effect is depicted in **Fig. 1-2**.^[2]

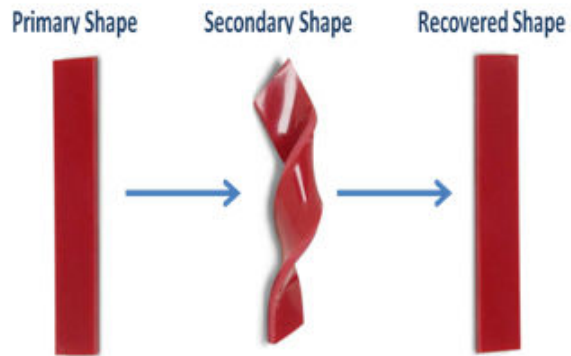


Fig. 1-1. Images showing a primary rectangular film, a temporary spiral shape, and the recovered primary rectangular shape.

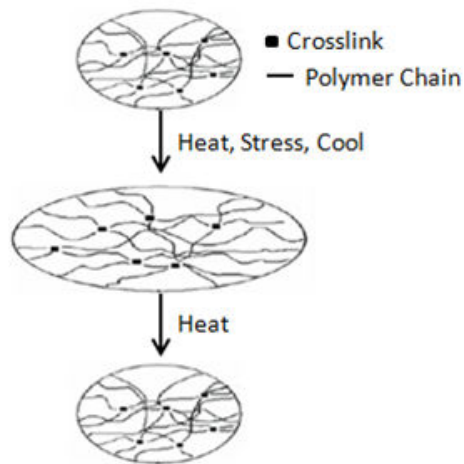


Fig. 1-2. Molecular mechanism of the shape memory effect of polymers.

A shape memory cycle begins by crosslinking liquid prepolymer into the desired primary (permanent) shape. The primary shape is then mechanically deformed into a secondary (temporary) shape at temperatures which exceed T_{trans} . Subsequently, the sample is cooled below T_{trans} while still under mechanical load. Retention of the secondary shape is achieved through a sharp reduction in molecular mobility via crystallization or vitrification of the polymer. Conversely, primary shape recovery is attained by heating the unconstrained network above T_{trans} . The resulting increase in chain mobility allows the entropic energy lost during deformation to be converted into a mechanical restorative force that re-establishes the original shape of the network.^[12] Recovery stresses for SMPs are typically between 4 and 10 MPa.^[13] The shape memory stress-strain curve for poly(cyclooctene) is shown in **Fig. 1-3**.^[14]

The most common figures of merit for shape memory performance are the shape fixity (R_f) and shape recovery (R_r) ratios.^[14] R_f is a measure of secondary shape retention, while R_r is an evaluation of primary shape recovery. The equations for these metrics of shape memory are as follows:

$$R_f = \frac{\epsilon_u}{\epsilon_m} \cdot 100 \quad (1)$$

$$R_r = \frac{\epsilon_u - \epsilon_p}{\epsilon_m - \epsilon_p} \cdot 100 \quad (2)$$

R_f is defined as the ratio of the fixed strain after unloading (ϵ_u) to the total strain induced during mechanical deformation (ϵ_m).

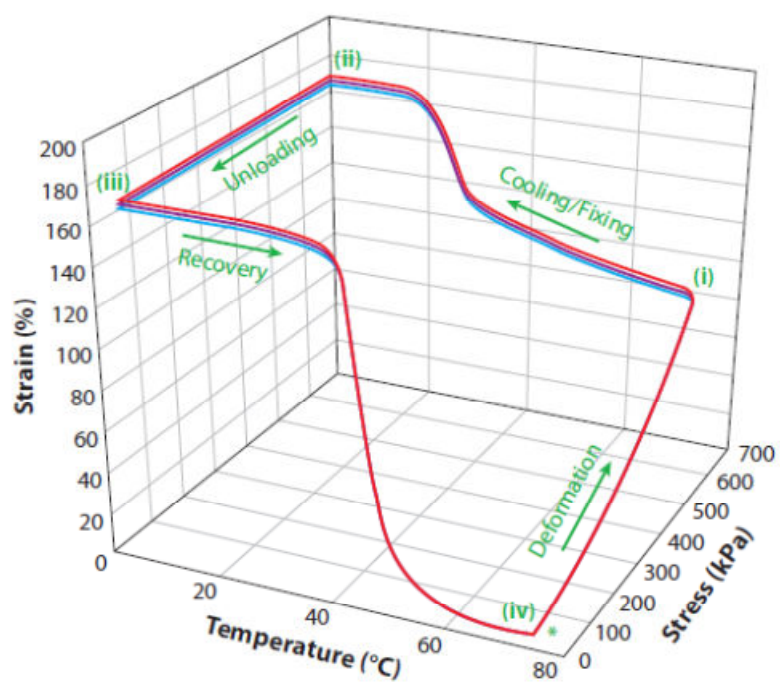


Fig. 1-3. Thermomechanical cycle for poly(cyclooctene).

R_r is defined as the ratio of the difference between the fixed strain after unloading (ϵ_u) and the permanent strain after the recovery step (ϵ_p) to the difference between the total strain induced during mechanical deformation (ϵ_m) and the permanent strain after the recovery step (ϵ_p).

In addition to specific thermomechanical processing, the shape memory effect is also highly dependent upon many confluent factors, including polymer composition, morphology, and architecture.

1.2 Shape Memory Polymer Design

1.2.1 Basic Synthesis and Classification

There are four general categories of SMPs which are classified by the nature of the crosslinks and the origin of T_{trans} .^[13] The categories of SMPs are as follows:

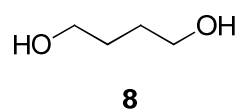
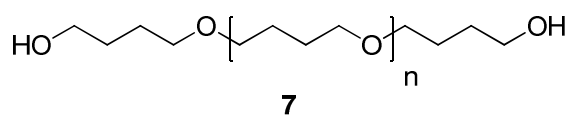
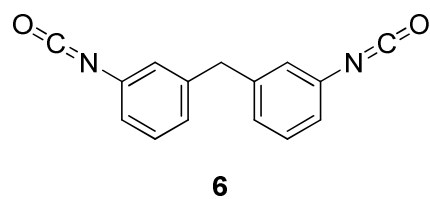
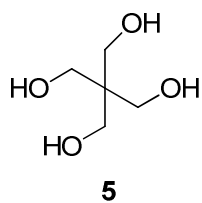
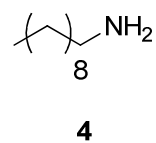
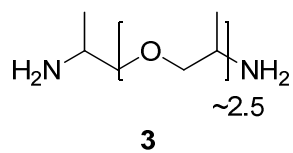
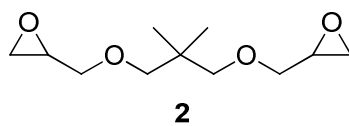
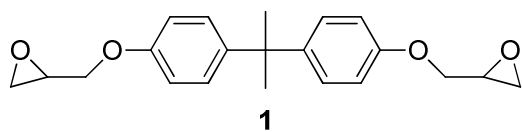
1. Amorphous thermosets
2. Semi-crystalline thermosets
3. Amorphous thermoplastic elastomers
4. Semi-crystalline thermoplastic elastomers

The first class of SMPs, are amorphous chemically crosslinked networks that employ the T_g as the shape memory switch. This group of SMPs are exemplified by epoxy-based systems and have demonstrated excellent shape memory properties and. Recently, Rousseau and co-workers synthesized an epoxy system from Bisphenol A (BPA)-diepoxide (**1**), neopentyl glycol diglycidyl ether (NGDE) (**2**), Jeffamine (**3**), and decylamine (**4**) with excellent shape memory properties.^[15] Polyurethanes (PU) are also extensively used as SMPs due to the wide range of properties that can be accommodated by selecting the

appropriate hard and soft segments. Buckley and co-workers reported a PU system with exceptional shape memory properties using the trifunctional crosslinker 1,1,1-trimethylol propane (**5**), methylene diphenyl diisocyanate (MDI) (**6**), and a poly(tetrahydrofuran) (PTHF) macrodiol (**7**).^[16]

Class two SMPs are chemically crosslinked semi-crystalline polymers with a T_m dependent transition temperature. As a result of the semi-crystalline microstructure, these materials inherently have a fixed modulus which is an order of magnitude lower than thermoplastic elastomers.^[13] These polymer networks can be synthesized by using a multivalent crosslinker or by crosslinking linear or branched prepolymers. Common examples of class 2 SMPs are crosslinked poly(ethylene), poly(ethylene-vinyl acetate), and poly(cyclooctene).

In the third class of SMPs, the net points are physical and originate from reversible intermolecular interactions such as van der Waals, dipole-dipole interactions, and hydrogen bonding.^[14] Here, T_{trans} is associated with the T_g of a soft segment, which is typically situated well below the highest thermally-reversible phase transition of the hard segment to avoid chain slipping at the working temperature. A recent example is the synthesis of a PU network containing MDI (**6**) as the hard segment, poly(tetramethylene glycol) (PTMG) (**7**) as the soft segment, and 1,4-butanediol (BD) (**8**) as the chain extender.^[17] It was shown that the ratio of hard to soft segment and the molecular weight were the most important determinants of shape memory performance. Class three of SMPs also inherently possesses lower mechanical properties when compared to classes 1 and 2 due to hard segment disruption during mechanical deformation.



The last class of SMPs are physically crosslinked semi-crystalline copolymers. The physical netpoints are maintained by intermolecular interactions while the phase change behavior is dictated by the T_m of a semi-crystalline soft segment. Due to the highly versatile nature of PU chemistries, these systems dominate this class of SMPs. Analogous to the example provided for class three SMPs, a semi-crystalline thermoplastic elastomer can be synthesized by substituting a semi-crystalline diol, such as poly(ϵ -caprolactone) (PCL), for the soft segment and coupling with MDI (6) and BD (8).

1.2.2 Practical Considerations

Shape recovery stress is an important design criterion because it dictates the capacity for shape recovery and the recovery speed. This has been achieved most simply by increasing the crosslink density. However, doping a polymer system with nanoparticulate fillers such as carbon fibers, nanoclays, and carbon nanotubes (CNTs) has also been investigated.^[12,18,19] The addition of reinforcing materials increases the modulus of the elastic state, but has a more subtle effect on the fixed modulus. Therefore, the use of SMP composites can enhance SMP performance without perturbing the modulus of the material above T_{trans} .

High performance properties such as extremely sharp and uniform phase transitions are sometimes desirable for specific applications. The width of the thermal transition is determined by the distribution of relaxation times associated with components of the network. Chain length and molecular interactions play a large role in designing appropriate relaxation times. Generally, semi-crystalline SMPs display much sharper and more uniform

shape recovery in comparison to glass transition mediated shape changes. However, the rate of crystallization must be longer than the time-scale of the shape transition.

Effective heat transfer is of paramount importance for thermally activated SMPs. The thermal conductivity of most polymers is considerably low at less than 0.3 W/K·m.^[14] Traditional shape memory alloys such as nickel-titanium (Nitinol[®]) are over 60 times more conductive than SMPs. Low thermal conductivities are a serious limitation of SMPs and have recently been addressed through the construction of composite materials. Most notable is the incorporation of CNTs, which are attractive for their high electrical and thermal conductivities, low loading prerequisite, and increased SMP recovery stresses.^[20]

1.3 Recent Advances in Shape Memory Polymers

1.3.1 Triple and Quadruple Shape Memory Polymers

In recent years, polymers with triple and quadruple shape memory have been realized and constitute a special new class of materials.^[21] The multi-shape memory effect is based on a polymer network with at least two phase segregated domains. These domains are also dictated by the T_g or T_m and each contributes an individual T_{trans} . Thermomechanical processing is essentially the same as the dual shape memory effect and leads to a transition from a shape A, to a shape B, to a shape C, and so forth. Langer and Lendlein reported a triple shape memory effect polymer synthesized from the radical polymerization of PCL dimethacrylate (PCLDM) (**9**) with cyclohexyl methacrylate (CHM) (**10**).^[22] The T_m of the PCLDM segments was found to be 50 °C and corresponds to the first shape transition, $T_{trans-A}$. The T_g of the PCHM segment was determined to be 140 °C and is responsible for the second shape transition, $T_{trans-B}$. The same authors also synthesized a triple shape memory

material by crosslinking PCLDM and poly(ethylene glycol) methyl ether methacrylate (PEGMA) (**11**). The resulting network had two melting temperatures and likewise two transition temperatures. The first T_{trans} was dictated by the T_m of PEGMA and the second was determined by the T_m of PCLDM which were found to be approximately 32-39 and 53-56 °C, respectively. Triple shape polymer materials have also recently been reported by Mather, Kolesov, and Kim.^[23,24,25,26]

In 2010, Xie demonstrated that a perfluorosulfonic acid ionomer (PFSA) (**12**), commercially known as Nafion, can undergo up to four shape transitions.^[27] Xie showed that PFSA, with only one thermally-reversible phase transition, can execute highly controlled dual, triple, and quadruple shape memory transitions without any further alteration to the polymer. PFSA is a thermoplastic material with a very broad T_g , ranging from approximately 55 to 130 °C, as represented by the $\text{Tan } \delta$ curve in **Fig. 1-4**. The $\text{Tan } \delta$ curve is representative of the wide distribution of thermally induced relaxation events, each of which can be thought of as a discrete memory element that can be used to program a temporary shape. Images of the quadruple shape memory effect and the corresponding stress-strain behavior as a function of temperature are shown in **Fig. 1-5**.^[27]

1.3.2 Functionally Graded Shape Memory Polymers

The shape memory effect typically corresponds to one discrete thermally-reversible phase transition; however, functionally graded SMPs possess a distribution of spatially dependent compositions and associated phase transitions. Mather and co-workers developed a graded shape memory device using NOA-63, a polyurethane-based thiol-ene crosslinked SMP.^[28]



Fig. 1-4. Storage modulus (E') and $\tan \delta$ of PFSA (Nafion) as a function of temperature.

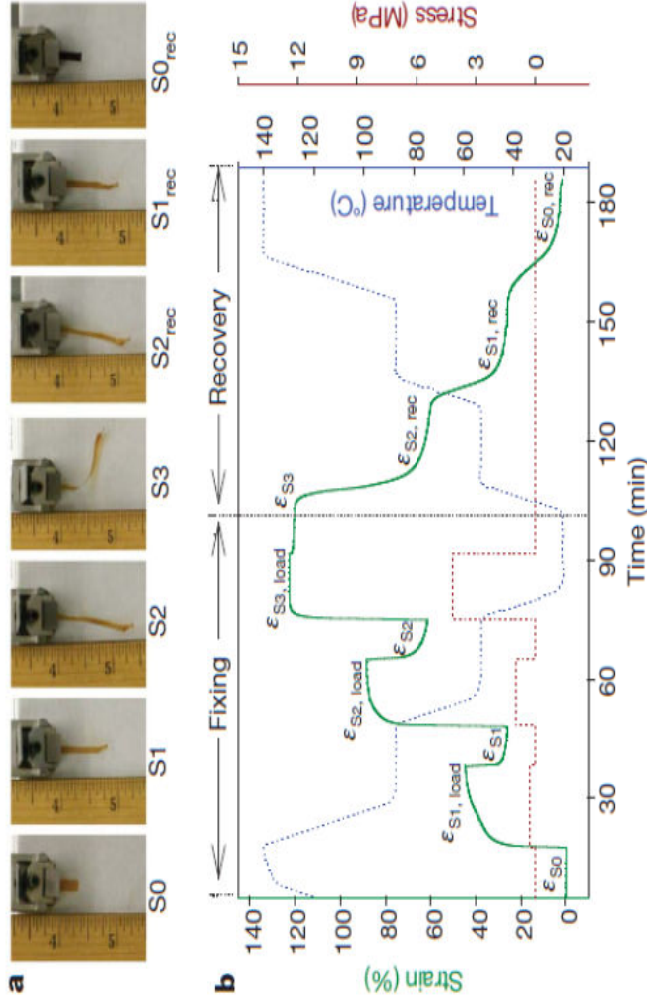


Fig. 1-5. Quadruple shape memory properties of PFSA. A) Visual demonstration. S0: permanent shape; S1: 1st temporary shape ($T_{f1}=140$ °C); S2: 2nd temporary shape ($T_{f2}=107$ °C); S3: 3rd temporary shape ($T_{f3}=68$ °C); S2rec: recovered 2nd temporary shape ($T_{r1}=68$ °C); S1rec: recovered 1st temporary shape ($T_{r2}=107$ °C); S0rec: recovered primary shape ($T_{r3}=140$ °C). B) Corresponding stress and strain behavior as a function of time and temperature.

Photochemical crosslinking of NOA-63 at room temperature resulted in a T_g of approximately 30 °C. It was found that the curing temperature significantly affected the extent of the reaction. Polymerizations carried out at room temperature were significantly slowed due to vitrification. At higher temperatures chain mobility and diffusion increased and allowed the reaction to proceed to a greater extent. This resulted in a higher crosslink density and consequently a higher T_g . Therefore, the T_{trans} of spatially discrete regions of a polymer device can be programmed separately by controlling the curing temperature.

A rectangular strip of NOA-63 was photochemically crosslinked and cut at regular intervals resulting in polymer fingers of equal length and width. The polymer strips were then post-cured at different temperatures which lead to a spatially resolved T_{trans} gradient ranging from approximately 30 to 55 °C. The polymer fingers were programmed to unfold sequentially and upon heating the strips consecutively unfolded as is shown in **Fig. 1-6**.^[28] Possible applications for these functionally graded materials are low cost temperature sensors that can be implemented in situations where conventional methods are not feasible or when continuous monitoring is not amenable such as sterilization or temperature dependent food packaging.

1.3.3 Shape Memory Polymer Surfaces

There are several examples in the literature of methods to program patterned SMP surfaces. One of the earliest is the process invented by 3M, where radically polymerized poly(siloxane) surfaces were embossed by imprint lithography.^[29] The resulting polymer surface was capable of transitioning between features on the order of several hundred microns in response to heating.

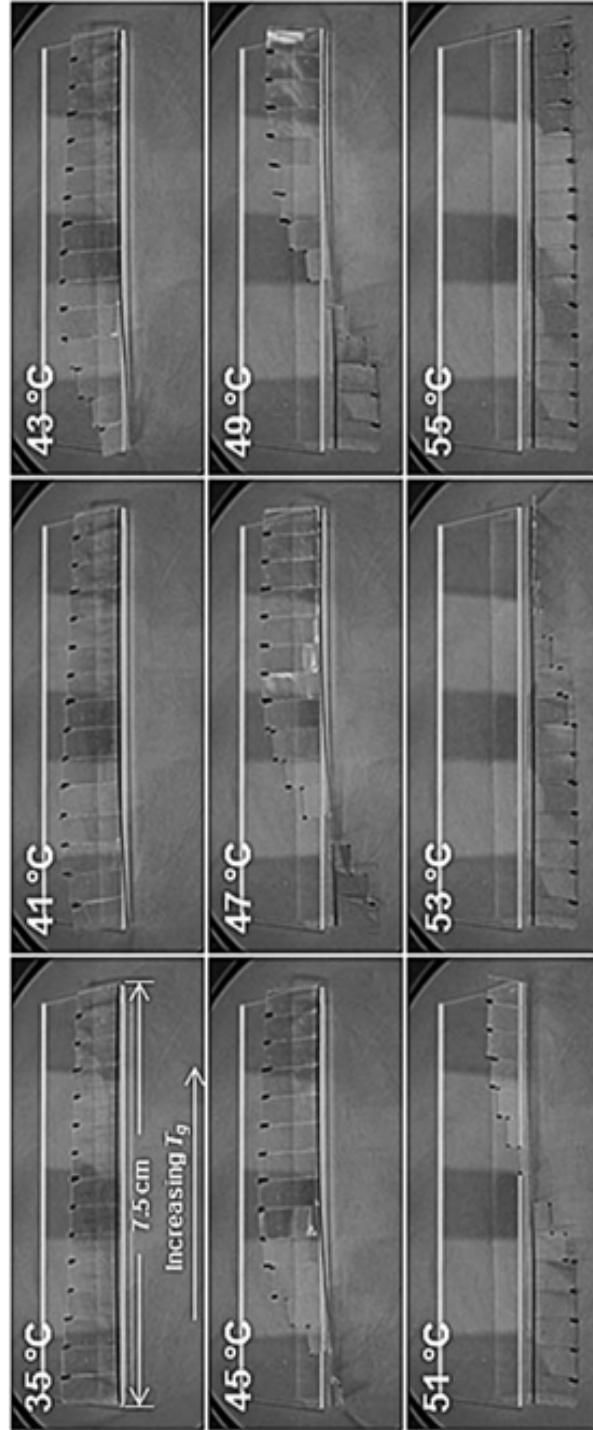


Fig. 1-6. Visual demonstration of the gradient recovery behavior of a functionally graded NOA-63 SMP. The arrow in the first image (35 °C) indicates the direction of the T_g gradient.

In 2005, Gall and co-workers reported a method to create reversible nanoindentations (~ 200 nm x 15 nm) in thermoplastic epoxy SMP surfaces using an atomic force microscopy (AFM) tip.^[30] As the temperature was increased gradually from 25 °C to the T_g (70 °C), nearly full recovery of the planar surface was observed (**Fig. 1-7**).^[30]

PU-Ni nanoparticle SMP composites were used to form microprotrusions from the surface (**Fig. 1-8**).^[31] The microprotrusions were magnetically deformed to produce leaning pillars that recovered to the original vertical orientation when heated above the T_g . Shape memory pillars were also fabricated using Tecoflex 72D, a cycloaliphatic PU block copolymer synthesized from two phase segregated glassy components.^[32] The hard segment contained methylene bis(*p*-cyclohexyl isocyanate) and 1,4 BD ($T_g = 120$ -140 °C) and the soft segment was comprised of PTMG ($T_g = 51$ °C). Pillar microarrays (10 x 100 μ m) were fabricated by a double-replica embossing method. Silicon wafers were etched with the pillar microarray pattern and fluorinated by vapor exposure to heptadecafluoro-1,1,2,2-tetrahydrooctyltrichlorosilane to minimize adhesion to the master mold. Poly(dimethylsiloxane) (PDMS) (Sylgard 184) was cast onto the etched silicon wafer and crosslinked to create a soft replica mold. Tecoflex was then cast into the PDMS replica mold and thermally-cured at 200 °C. The Tecoflex pillar microarrays were deformed by applying mechanical stress using a glass plate at 70 °C above T_{trans} and subsequently cooled below T_{trans} to retain the temporary leaning orientation through vitrification. The original vertical orientation was recovered by heating the film 70 °C above T_{trans} . SEM images of the shape memory effect are shown in **Fig. 1-9**.^[32] Henderson and co-workers have also reported the fabrication of shape memory channel microarrays (~ 140 μ m wide) embossed into NOA-63 surfaces.^[33]

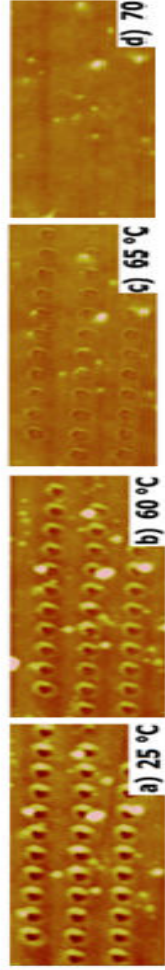


Figure 1-7. AFM images of a flat surface recovered from pits (200 x 15 nm) at a) 25, b) 60, c) 65, and d) 70 °C.



Figure 1-8. SEM images of PU-Ni microprotrusions A) Primary shape, B) temporary shape, C) recovered shape.

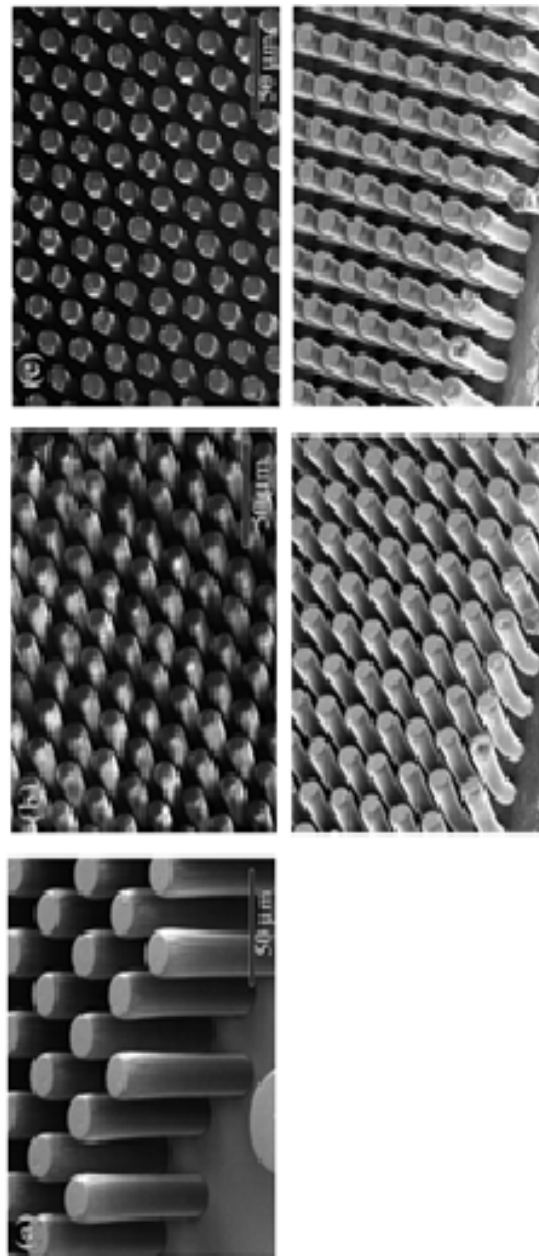


Figure 1-9. SEM images of Tecoflex shape memory micropillar microarrays (10 x 100 μm). A) Permanent shape, B) temporary shape, C) recovered shape. Recovered at 120 °C.

The channels reverted back to the permanent planar topography after being heated for 9.5 h at 37 °C.^[33]

1.3.4 Biodegradable Shape Memory Polymers

The synthesis of SMPs from biodegradable polymers has become an attractive means of precluding the need for surgical implant removal and have largely been developed around poly(glycolide) (PGA), poly(L-lactide) (PLLA), and poly(ϵ -caprolactone) (PCL).^[14]

The T_{trans} of SMPs containing PCL as the switching segment can readily be tuned by adjusting the molecular weight of the PCL segment. A common synthetic strategy for PCL-based SMPs is the ring opening polymerization (ROP) of ϵ -caprolactone to the corresponding diol and subsequent treatment with a diisocyanate or a functionalized acrylate to furnish a poly(ester urethane) or PCL diacrylate, respectively (**Fig. 1-10**).^[34] Photochemical crosslinking of the precursor is a common method to synthesize networks from diacrylate precursors.^[14] Lendlein and co-workers demonstrated that PCL dimethacrylates of different molecular weights have excellent mechanical properties with near quantitative R_f and R_r values.^[35] Jing and co-workers synthesized PCL-based PUs and showed that the thermal properties were dependent on molecular weight. Melting temperatures ranged from 43-48 °C with PCL segments of 2,000 to 10,000 g mol⁻¹, respectively.^[36]

Recently, PCL-based systems with star shaped architectures have gained considerable interest. Li and co-workers reported a novel biodegradable 3-arm poly(ester-urethane) SMP containing PCL soft segments and a T_{trans} of 36-38 °C.^[37]

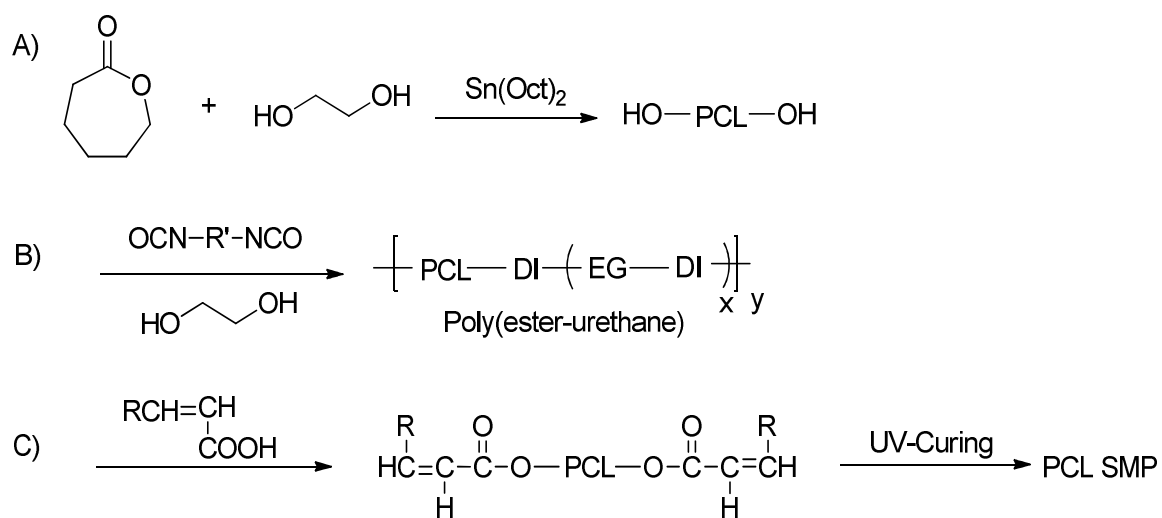


Fig. 1-10. Synthetic routes available to prepare poly(ester-urethanes) and PCL thermosets.

The poly(ester-urethane) was synthesized by glycerol initiated ROP of ϵ -caprolactone in the presence of enzymatic catalyst Novozyme 435. Subsequently, the PCL triol was treated with MDI and hexane diol (HD) to form the hard segment. The star shape design was shown to be critical in achieving a T_{trans} near biologically relevant temperatures. The authors suggested that the large molecular weight of the 3-arm PCL segments helped to preserve crystallinity at temperatures several degrees lower than that which is accessible by linear PCL networks. The synthetic route to a 3-arm poly(ester-urethane) SMP is shown in **Fig. 1-11**.^[37]

While PCL is well-suited for biomedical SMPs, it does possess certain limitations to its applicability. Namely, its long degradation rates are not appropriate for certain applications. Jing and co-workers prepared a PLA-based SMP through the diol route and found that it had superior shape memory properties when compared to PCL.^[38] However, the T_g of PLA is near 60 °C and is not easily altered without changing the architecture of the polymer. Jing also investigated PLA-co-PCL random copolymers as a means of tuning the transition temperature.^[39] By modifying the PCL and PLA chain lengths, the authors were able to gain better control over the T_m and degradation rate.

In 2010, Song and co-workers reported an 8-arm star PLA-SMP polymer containing a rigid polyhedral oligomeric silsesquioxane (POSS) core.^[40] The rigid POSS nanoparticle contained eight hydroxy groups and was used to initiate the ROP of the lactide monomer in the presence of tin octanoate. Crosslinking was achieved by reacting the POSS-PLA polyol with HDI to give the corresponding SMP network (**Fig. 1-12**). The nanoparticle core improved uniformity and spatial distribution of the PLA chains and helped to mitigate excessive chain entangling that might otherwise hinder shape recovery.

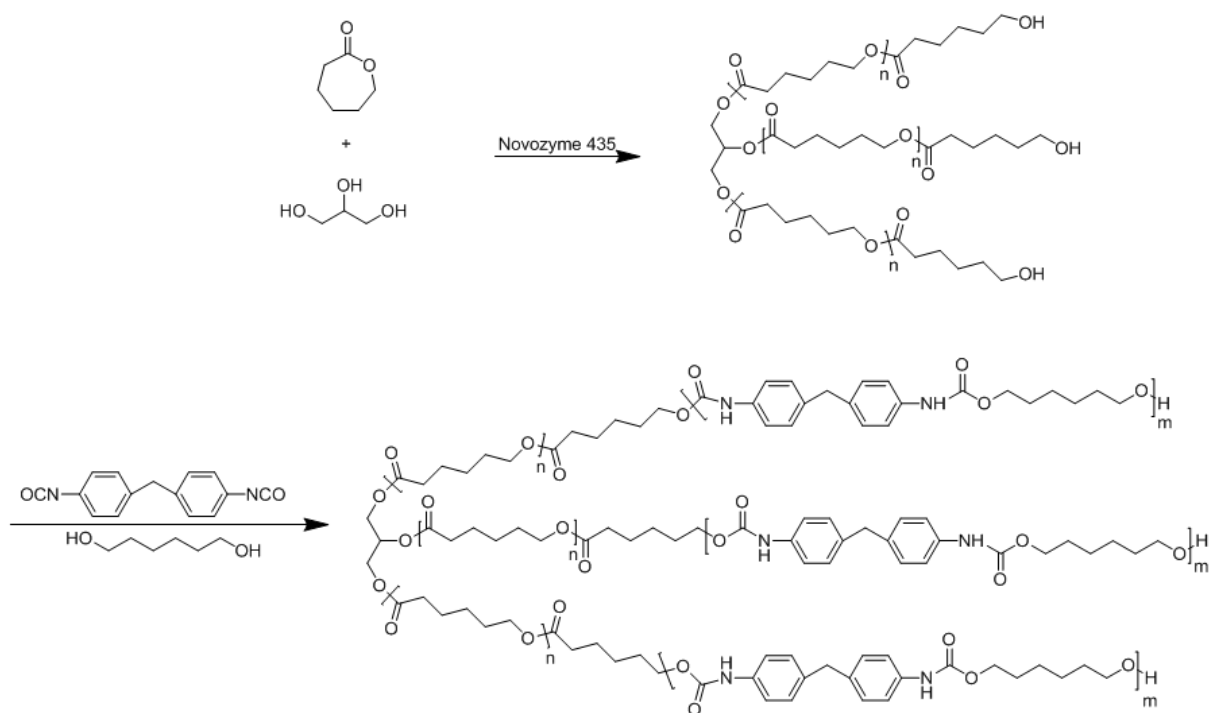


Fig. 1-11. Synthetic route to a 3-arm poly(ester-urethane).



Fig. 1-12. Preparation of a PLA-SMP with a rigid POSS nanoparticle core.

Initiation using the POSS nanoparticle core rendered the PLA segments completely amorphous and yielded T_g values as low as 42.8 °C as indicated by the maximum of the Tan δ curve (**Fig. 1-13**). Dynamic mechanical analysis (DMA) revealed that the POSS-PLA SMP exhibited a more pronounced drop in storage modulus (E'), a narrower T_g , and a greater than 10 °C drop in T_g when compared to a structurally similar PLA-SMP initiated with a flexible 8-arm organic core. These findings suggest that the size and rigidity of the initiator can dictate not only T_{trans} , but can also lead to improved shape memory properties by preventing excessive chain entanglement. The authors also reported the first example of a biofunctionalized SMP by co-polymerizing with 3-azido-1-propanol to give the azide functionalized PLA network. Subsequently, a fluorescently labeled alkyne functionalized peptide (Arg-Gly-Asp-Ser) was coupled to the surface via a 1,3 dipolar (Huisgen) cycloaddition.

1.4 Biomedical Applications of Shape Memory Polymers

In 2002, Langer and Lendlein first demonstrated the potential of SMPs as thermally responsive biodegradable medical devices.^[41] Novel synthetic self-tightening suture materials were constructed from multi-block poly(ester-urethane) copolymers containing oligo(dioxanone) hard segments and oligo(caprolactone) soft segments. The fibers showed near quantitative shape memory properties and a T_{trans} of 41 °C. The SMP suture can be applied in the temporary form, and upon heating, tighten to secure a knot by recovering the permanent shape (**Fig. 1-14**). Recovery stress was carefully controlled to ensure full wound closure while minimizing the risk of adjacent tissue necrosis or hernia from scar tissue formation.

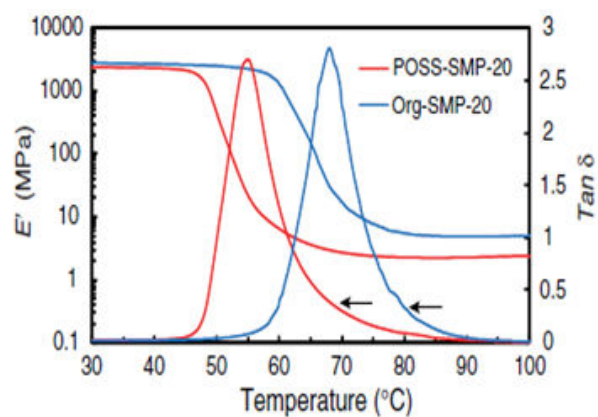


Fig. 1-13. E' and $\tan \delta$ as a function of temperature for PLA based SMPs containing 8 arm POSS-nanoparticle and organic cores.

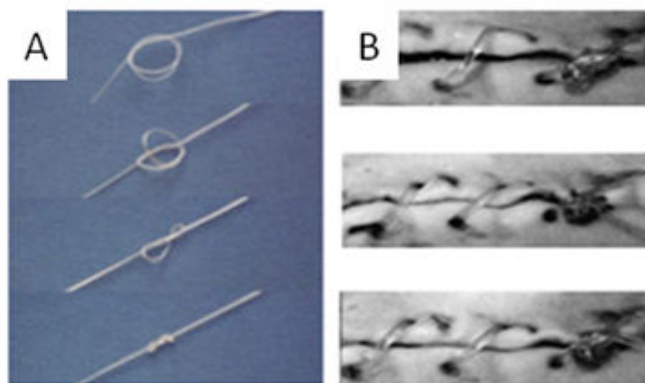


Fig. 1-14. Images of A) self-tying sutures and B) their application to a pig skin wound at body temperature.

SMPs have also been investigated as materials for self deploying vascular, cardiovascular, and neurovascular stents (**Fig. 1-15**).^[42,43,44,45] Large SMP stents can be implanted in a minimally invasive, compact form that self-deploys at physiological temperature without any need for auxiliary instrumentation. SMP stents can also more accurately match tissue dimensions and compliance in comparison to metallic stents that are deployed by balloon angioplasty.

Tissue scaffolds could also potentially be implanted in a minimally invasive fashion using SMPs. To date, only four studies have concentrated on the ability of cells to tolerate PU and PCL SMPs.^[46,47,48,49] Neuss and co-workers were the first to investigate the behavior of mouse fibroblasts, human mesenchymal stem cells (hMSC), and human/rat mesothelial cells on bulk PCL samples to assess its suitability as a minimally invasive implant.^[50] It was shown that the polymer was biocompatible with all cells types, and fully supported cell viability, proliferation, and differentiation. Furthermore, the recovery of stretched films did not show any deleterious effects on the differentiation capacity of the adherent hMSCs.

Ohya and co-workers investigated the shape memory properties and drug release profile of a multi-arm poly(ester-urethane) copolymer.^[51] The star polymer was synthesized using an oligoglycerin initiator to ROP ϵ -caprolactone in the presence of tin octanoate. The 8-arm star PCL polyol was then crosslinked through the addition of HMDI. In accord with the previous 3-arm PCL SMP example, the multi-arm PCL SMP demonstrated excellent shape memory properties and a T_{trans} of 37 °C (**Fig. 16a**). To examine the drug release kinetics, model drug theophylline was loaded at 10 and 20 % into prepolymer mixture, solution drop casted into a mold, and thermally cured.

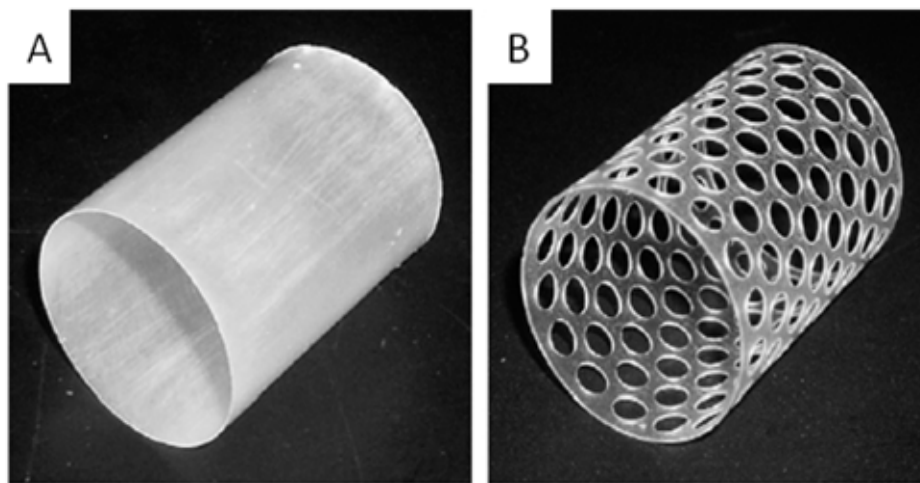


Fig. 1-15. Images of A) solid and B) perforated polymer vascular stents.

Slow and sustained drug release was observed in PBS at 37 °C over 35 days without any initial burst of drug (**Fig. 1-16b**). Lendlein and co-workers described the first amorphous biodegradable polymer with shape memory functionality under physiological conditions.^[52] Crosslinked star shaped oligo[(rac-lactide)-*co*-glycolide] copolymers were loaded with three drugs of different hydrophobicities: enoxacin (EN), nitrofurantoin (NF), and ethacridine lactate (EL). EN and EL showed a burst release of less than 20% and NF showed even lower initial release. EL and NF exhibited a slow near linear release profile over the course of 30 days. In contrast, the more hydrophobic EN partitioned to the aqueous medium sparingly for the first 25 days and then showed a large increase in release rate which coincides with increased water uptake due to degradation. The onset of mass loss due to degradation took place after 25 to 35 days and varied with the network payload. Despite excellent shape memory properties in air, in aqueous conditions water plasticization led to reduced fixation values. In 2010, Lendlein and co-workers also evaluated different methods of SMP network drug loading.^[53] It was generally concluded that drug loading by swelling leads to low payloads and high burst release, while drug loading before crosslinking allows for more control over payload and a low burst release. Accordingly, the former method would be best employed in applications which require a high initial burst of therapeutic and the latter approach is better suited for situations that require control over drug loading and low burst release.

The foregoing section is a very small sample of the growing body of literature featuring the development of biodegradable SMPs and their diverse applications. The far-reaching implications of SMPs in biomaterials and biomedical devices are only presently being realized.

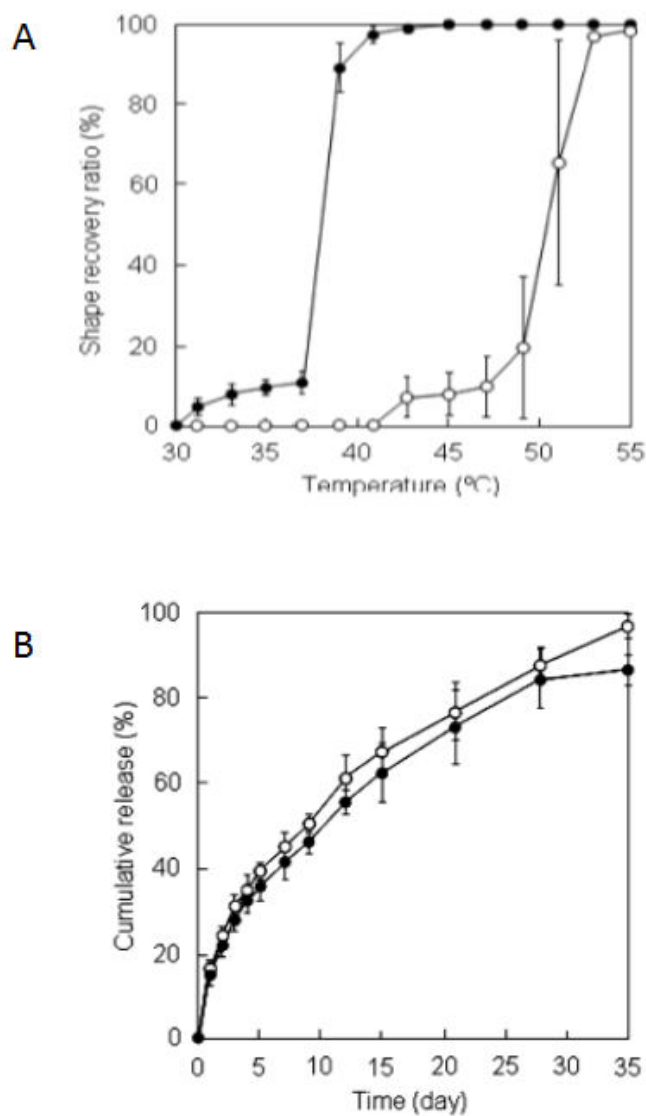


Fig. 1-16. A) R_r of 8-arm PCL based SMPs with $\langle M_n \rangle = 10,300 \text{ gmol}^{-1}$ ($\bullet$) and $18,700 \text{ gmol}^{-1}$ ($\circ$). B) In vitro drug release profile of theophylline from 8-arm PCL based SMPs with $\langle M_n \rangle = 10,300 \text{ gmol}^{-1}$ ($\circ$) and $18,700 \text{ gmol}^{-1}$ ($\bullet$).

Future advancements in the field may enable new and exciting approaches to treating illness, disease, and injury.

1.5 Dissertation Organization

This work is organized into five chapters:

1. The first chapter is a general discussion of shape memory polymers with special emphasis on biomedical applications. The molecular basis of the shape memory effect is discussed, in addition to basic classification, and general synthetic approaches for each sub-category. Practical considerations are also briefly discussed including: heat transfer, recovery stress, phase transition uniformity, and recovery speed. Recent innovations in the field are highlighted with examples from the literature, such as, multi-shape memory polymers, functionally graded shape memory polymers, shape memory surfaces, and biodegradable shape memory polymer materials. Biomedical applications of shape memory polymers are reviewed including shape memory polymer suture materials, stents, tissue scaffolds, and drug delivery platforms.
2. Chapter 2 describes the synthesis and characterization of novel photo-chemically crosslinked poly(ϵ -caprolactone) networks. The synthetic routes to linear and branched poly(ϵ -caprolactone) materials are presented. Thermal, mechanical, surface and shape memory properties are discussed.
3. The third chapter describes the application of the poly(ϵ -caprolactone) shape memory polymers described in chapter two in the fabrication of thermally responsive microarrays.

The application of these devices in studying the response of human mesenchymal stem cells to dynamic topography is also discussed. Surface fabrication and chemical modifications are described in addition to *in vitro* cytotoxicity studies. The impact of dynamic topography on cell morphology is also described herein.

4. Chapter four focuses on the development of near infrared activated shape memory polymers. The fabrication of gold nanoshell/poly(ϵ -caprolactone) nanocomposites is described. The mechanical, thermal, and shape memory properties of the films are outlined. Shape recovery and heat dissipation of the films in response to NIR irradiation are also discussed.

5. The last chapter discusses several possible directions for future research.

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Chapter 2

Synthesis and Characterization of Linear and Branched Polycaprolactone Shape Memory Polymers*

2.1 Introduction

PCL has been used extensively in biomedical applications for nearly four decades.^[1] Its popularity as a biomaterial, stems largely from its facile and relatively inexpensive synthetic routes, biocompatibility, controlled degradation kinetics, and tailorable physiochemical properties.^[2] PCL can be prepared by bulk or solution ROP using a variety of propagating species such as cationic, anionic, or free-radical polymerization.^[3] Organometallic catalysts such as tin octanoate, are routinely used to catalyze the polymerization of polylactones.^[4,5] Recently, *N*-heterocyclic carbene (NHC) organocatalysts, such as triazabicyclodecene (TBD), have also been successfully implemented in the living ROP of cyclic esters and have demonstrated highly controlled monomer activated catalysis.^[6,7] Waymouth, Hedrick, and co-workers reported the ROP of δ -valerolactone, ϵ -caprolactone, and lactide using TBD which resulted in polyesters of highly controlled $\langle M_n \rangle$ and narrow polydispersity indices (PDI).^[8] The authors also suggested a bifunctional nucleophilic mechanism where the imine nitrogen attack on the ester carbonyl generates an active intermediate. This intermediate then reacts with an alcohol molecule, liberating the polymerized ester (**Fig. 2-1**).^[9]

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D.M. Le, K. Kulangara, A.F. Adler, K.W. Leong, V. Sheares Ashby, *Adv. Mater.* **2011**, 23, 3278-3283.

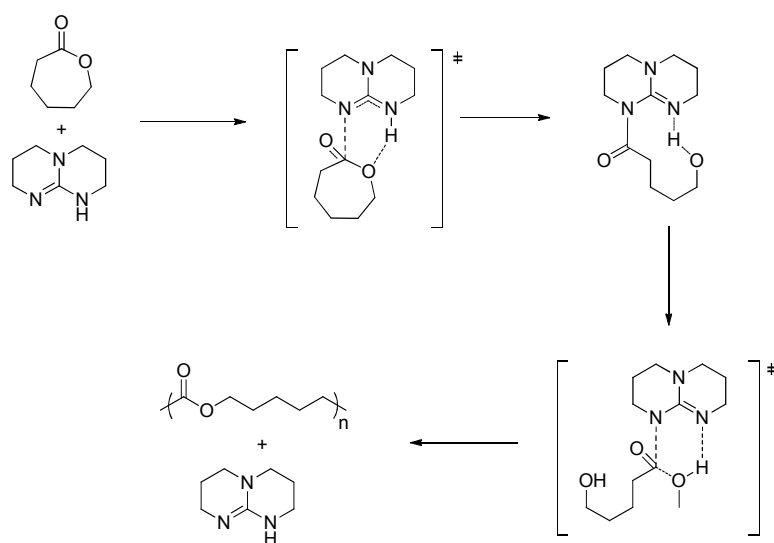


Fig.2-1. Proposed mechanism for TBD catalyzed ϵ -caprolactone polymerization.

Recently, nanoparticle initiated ROP of ϵ -caprolactone has also become a popular strategy to diversify the properties of PCL SMP networks. Mather and Wang, recently reported POSS and silica initiated ROP of ϵ -caprolactone to give PCL films with excellent mechanical strength and shape memory performance.^[10,11]

PCL is a hydrophobic semi-crystalline polymer with moderate tensile strength (~23 MPa), an equilibrium T_m between 59-64 °C, and a T_g of -60 °C.^[12] The low T_g and T_m of PCL makes it highly amenable to device fabrication at relatively low temperatures. PCL is also a biodegradable polymer due to the presence of hydrolytically labile aliphatic ester linkages and typically degrades completely within 2-4 years. The rate of hydrolysis can be altered by copolymerization with more hydrophilic lactones such as lactide or glycolide. The well-documented biocompatibility of PCL also makes it a highly desirable material for implantable devices. Hutmacher and co-workers investigated the short and long term biocompatibility of PCL tissue scaffolds using several different animal models.^[13-16] No deleterious histological effects were observed at fifteen weeks up to two years after implantation.

This unique combination of properties has made PCL a popular choice for shape memory polymer biomaterials and has been widely employed as a switching segment in thermoplastic polyurethanes, covalently crosslinked networks, and multi-block copolymers.^[17-22] Moreover, the low T_m of PCL can be easily tuned to physiologically relevant temperatures, making it an attractive materials for biomedical applications. Activation of the shape memory effect is often programmed at temperatures which are slightly above body temperature to circumvent early deployment and is done by intermittent localized heating. However, T_{trans} is more commonly positioned at body temperature for self-

deploying applications.^[23-25] The distinct advantage of this approach is in using the body's thermal energy to initiate the self-deployment of an implantable device.

Herein, we describe the synthesis and characterization of novel photo-polymerizable PCL-based SMP biomaterials. These materials can be divided into two categories: those based on linear PCL prepolymers for high temperature shape memory activation and those based upon star-shaped PCL prepolymers for body temperature shape memory activation. The linear PCL materials were prepared by solution ROP in the presence of a diol while the branched PCL materials were prepared by bulk ROP in the presence of a triol. In either case, telechelic PCL macropolyols were methacrylate end-capped and subsequently photochemically crosslinked to form PCL SMP thermosets. Both routes allowed for precisely tuned thermomechanical properties of the prepolymers and the resulting networks. The thermal, mechanical, and shape memory properties of the networks are described herein.

2.2 Experimental Section

2.2.1 Materials. ϵ -Caprolactone (99%), tin octanoate and anhydrous glycerol were purchased from Sigma-Aldrich. Acetic acid, methylene chloride, methanol, and acetone were purchased from Fisher Scientific. ϵ -Caprolactone was dried over CaH_2 for 24 h and distilled prior to use. Perfluoropolyether (PFPE) and PDMS molds were prepared in house.

2.2.2 *Preparation of linear poly(ϵ -caprolactone) diols.* A series of PCL diols of varying $\langle M_n \rangle$ (gmol^{-1}) were synthesized by ROP using TBD, as described in literature.^[61] All reaction preparation was performed under nitrogen and all materials were flamed dried prior to use. In a 250 mL round bottom flask, dry ϵ -caprolactone was diluted with anhydrous

toluene to make a 1M solution. In a 100 mL round bottom flask 0.5 mol % TBD and the appropriate quantity of anhydrous ethylene glycol were stirred in a small amount of anhydrous toluene until the solids were completely dissolved. Subsequently, the organocatalyst and ethylene glycol solution were added to the ϵ -caprolactone and allowed to stir at room temperature until the reaction reached approximately 90% conversion. The reaction was quenched with several milliliters of glacial acetic acid. The PCL diol was then precipitated in cold (-78 °C) methanol, isolated by vacuum filtration, and dried in a vacuum desiccator at 0.1 mmHg overnight.

2.2.3 Preparation of star-shaped poly(ϵ -caprolactone) triols. A series of star-shaped PCL prepolymers were synthesized by bulk ROP of ϵ -caprolactone using tin octanoate and the trifunctional initiator glycerol. Under nitrogen atmosphere, glycerol, tin octanoate, and ϵ -caprolactone were added to the reaction vessel and heated to 120 °C for 2.5 h, whereupon the reaction was quenched with acetic acid, and the product was precipitated in cold (-78 °C) methanol, isolated by vacuum filtration, and dried in a vacuum desiccator at 0.1 mmHg overnight.

2.2.4 Preparation of methacrylate end-functionalized polyesters. Polyol precursor was refluxed with 4.5 mol equivalents of 2-isocyanatoethyl methacrylate and 0.1 mol % tin octanoate in anhydrous methylene chloride for 2.5 h. The product was precipitated in cold (-78 °C) methanol, isolated by vacuum filtration, and dried in a vacuum desiccator at 0.1 mmHg overnight.

2.2.5 Preparation of chemically crosslinked polyester networks. PCL networks were prepared by casting molten methacrylate end-capped PCL precursor and photoinitiator diethoxyacetophenone (DEAP) (0.1 wt %) into a teflon mold. Subsequently, the prepolymer melt was irradiated with 30 mW/cm² UV light (365 nm) under N₂ atmosphere for 10 min. PFPE and PDMS replica molds were used as photo-curing templates or to emboss secondary surface patterns. Mechanical force was applied at 130 °C for 15 min followed by rapid cooling to -78 °C for an additional 60 min. The primary, secondary, and thermally recovered shapes (40 °C) were imaged using brightfield microscopy.

2.2.6 Characterization. ¹H NMR spectra were acquired in deuterated chloroform on a Bruker 400 AVANCE. A Waters Gel Permeation Chromatography (GPC) system was used to measure linear polymer molecular weights relative to polystyrene standards. The measurements were taken at 40 °C with THF as the mobile phase in four columns. End group analysis of tri-hydroxyl PCL materials was done using ¹H NMR (CDCl₃). <M_n> was determined using the following relationship:

$$< M_n > = \left[\frac{I_r}{I_t} + 1 \right] \times 114 \times 3 + 92 \quad (1)$$

where, I_r and I_t are the repeat and terminal ¹H NMR methylene intensities of the PCL chain, respectively.^[26] The values 114, 92, and 3 represent the molar mass of a monomer unit, the molar mass of the glycerol initiator, and the number of PCL arms, respectively. Water-in-air contact angles were measured at room temperature using a KSV instrument imaging system using the sessile drop method. The contact angle was monitored with from 0 to 5 min.

Mechanical stress/strain analysis was performed on an Instron 5566 at a crosshead speed of 10 mm/min at varying temperatures. The Young's modulus was calculated using the initial linear portion of the stress/strain curve (0-5% strain). Each measurement was performed on 2 dog bone molds and averaged. Degree of crystallinity (χ_c) was determined using the following relationship:

$$\chi_c = \frac{\Delta H_m}{135} \quad (2)$$

where, ΔH_m (Jg^{-1}) is the heat of fusion of the experimental PCL and 140 Jg^{-1} is the ΔH_m of a perfect PCL crystal.^[27] Thermal characterization was performed on a TA instrument Q200 differential scanning calorimeter (DSC), under nitrogen atmosphere from -20°C to 80°C with heating and cooling rates of 5°Cmin^{-1} and 10°Cmin^{-1} , respectively.

Shape memory performance was analyzed by thermomechanical tensile analysis using an Instron analyzer. The equations for R_f and R_r are as follows:

$$R_f = \frac{\epsilon_u}{\epsilon_m} \cdot 100 \quad (3)$$

$$R_r = \frac{\epsilon_u - \epsilon_p}{\epsilon_m - \epsilon_p} \cdot 100 \quad (4)$$

R_f is defined as the fixed strain after unloading (ϵ_u) to the total strain induced during deformation (ϵ_m). R_r is defined as the ratio of the difference between the strain after unloading (ϵ_u) and the permanent strain after recovery (ϵ_p) to the difference between the total strain induced during deformation (ϵ_m) and the permanent strain after recovery (ϵ_p).^[28] Dog

bone molds (1 x 37 x 3 mm) were heated to 60 °C and extended to a strain of 35%. The sample was then allowed to cool under load to room temperature. Subsequently, the load was removed and the fixed strain was recorded to determine the shape fixity (R_f). To measure shape recovery (R_r), linear samples were heated above T_{trans} while branched samples were immersed in water at 40 °C for 10 min.

2.3 Results and Discussion

2.3.1 Poly(ϵ -caprolactone) Synthesis and Thermal Characterization

PCL diols were prepared by ethylene glycol initiated solution ROP of ϵ -caprolactone in toluene (1M) using organocatalyst TBD at room temperature (**Fig. 2-2**). Three arm star-shaped PCL triols were synthesized by glycerol initiated bulk ROP of ϵ -caprolactone in the presence of tin octanoate at 120 °C for 2.5 h. (**Fig. 2-3**). Both methods resulted in well-behaved polymerizations and yielded PCL macropolyols with highly controlled physiochemical properties. The resulting polyol prepolymers were analyzed by ^1H NMR and showed spectrum which are characteristic for hydroxyl-terminated PCL materials (**Fig. 2-4, 2-5**).^[29-32] The presence of hydroxy-end groups were confirmed by ^1H NMR signal $\delta = 3.66$ ppm. Control over $\langle M_n \rangle$ could be achieved by manipulating the monomer to initiator stoichiometry. The resulting telechelic prepolymers showed excellent agreement with the target $\langle M_n \rangle$ values. Reactions were terminated with acetic acid and demonstrated low polydispersity indices ($\text{PDI} \leq 1.2$). After purification and drying, the hydroxy-terminated PCL materials were refluxed in anhydrous methylene chloride with 2-isocyanatoethyl methacrylate and tin octanoate for 2.5 h.

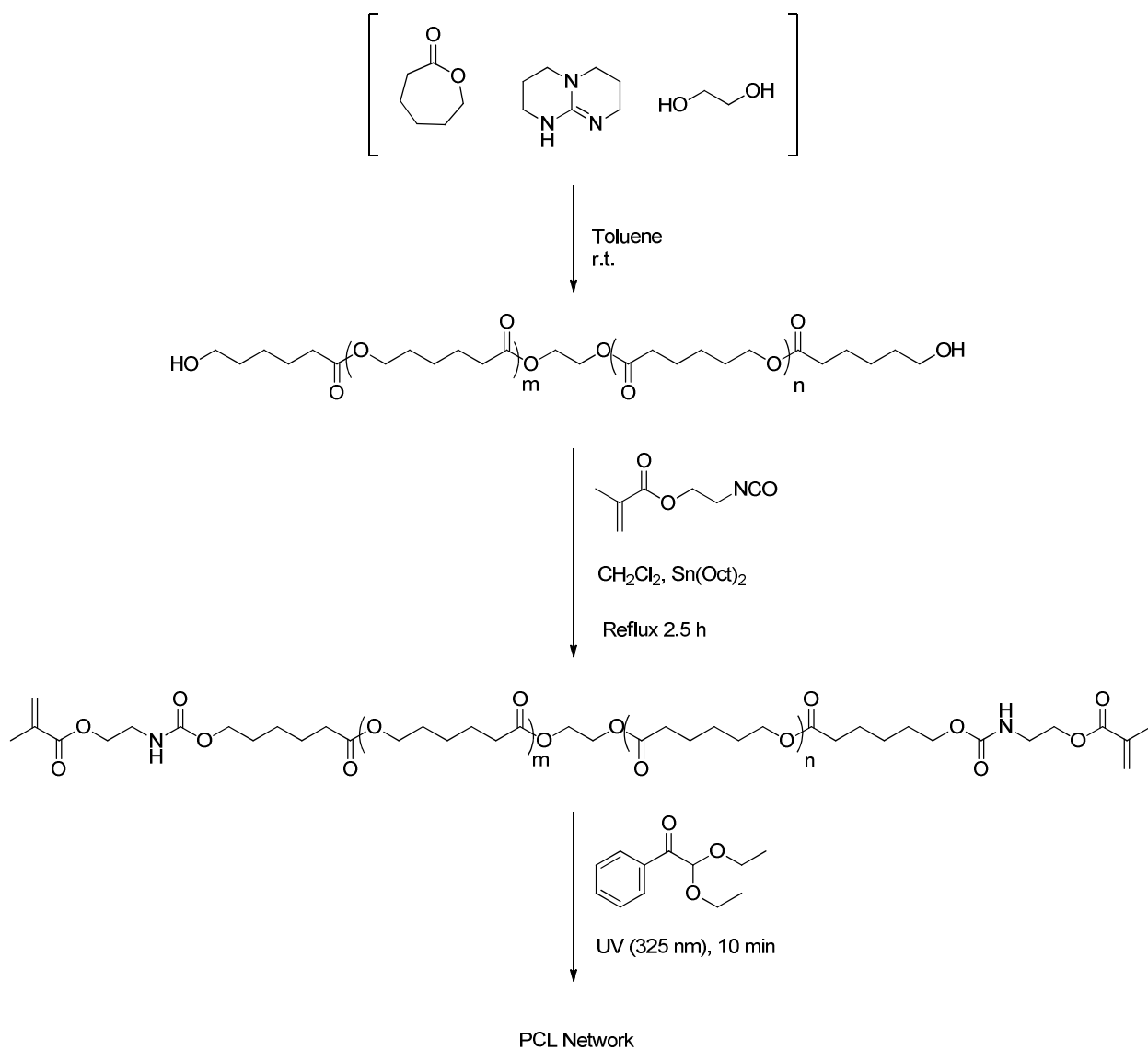


Fig. 2-2. Synthesis of PCL SMP networks from linear precursors.

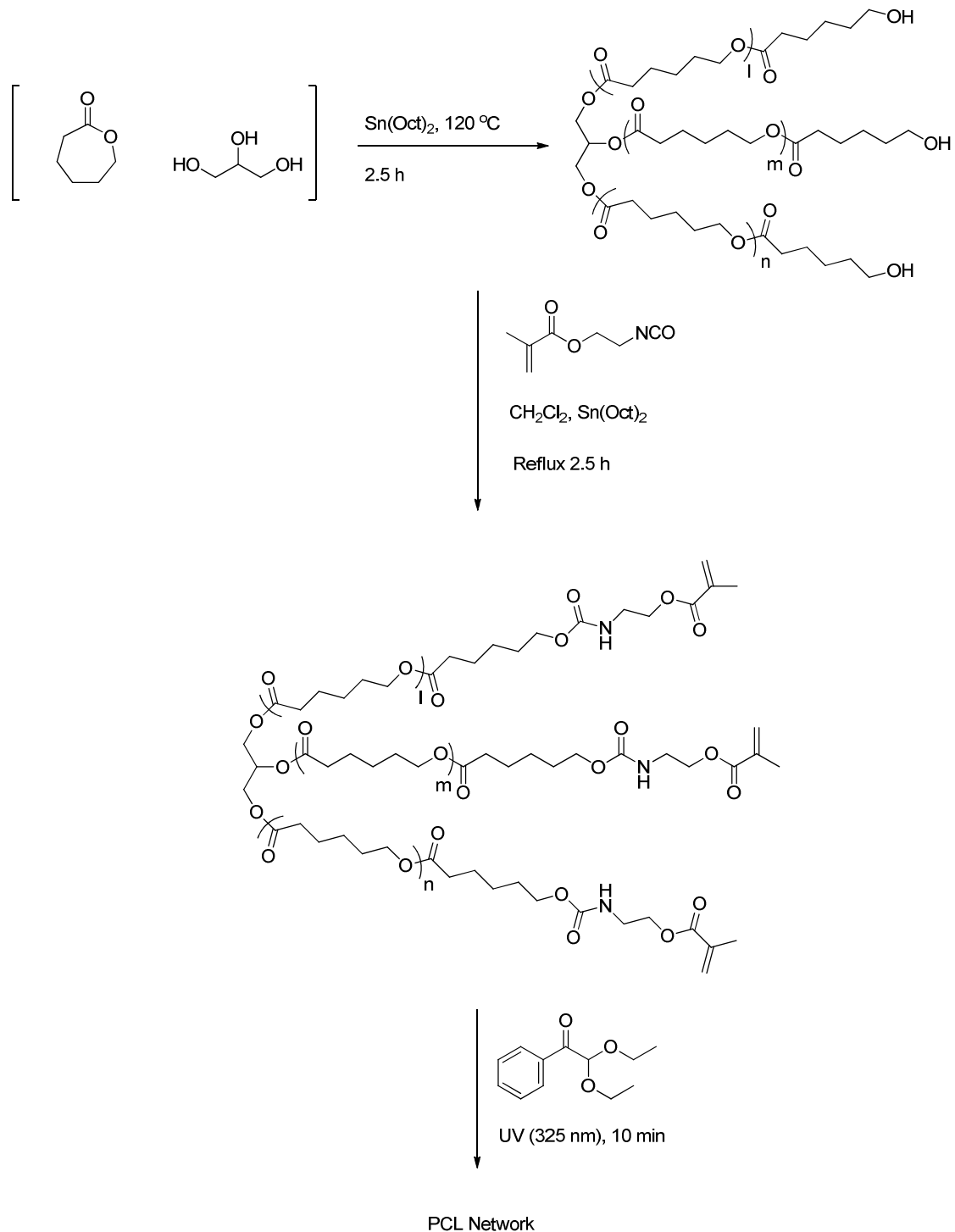


Fig. 2-3. Synthesis of PCL SMP networks from 3-arm star-shaped precursors.

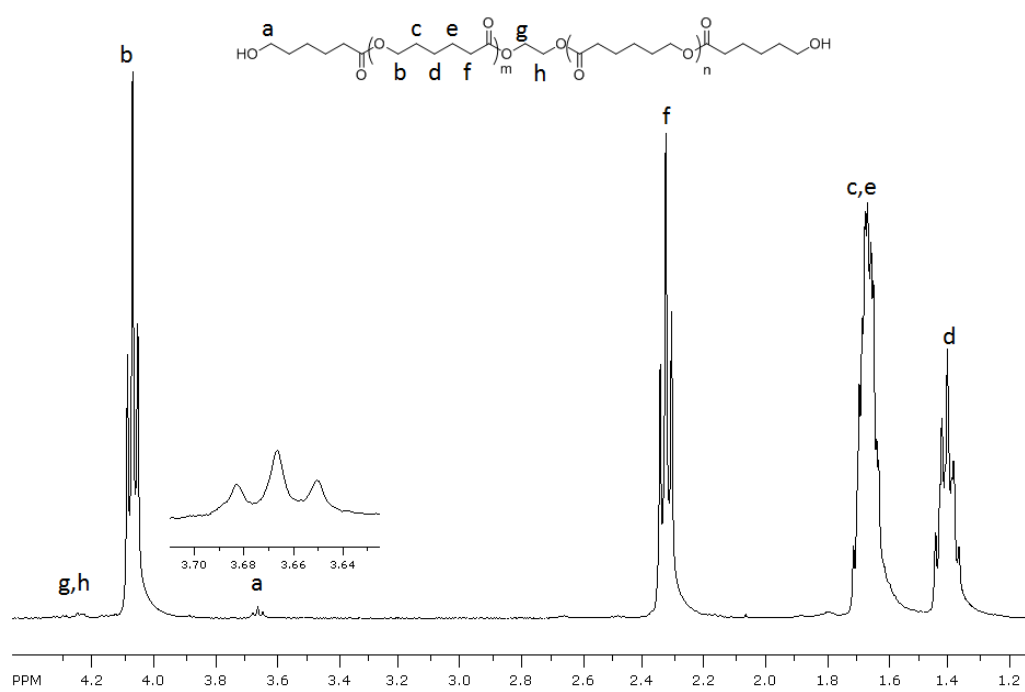


Fig. 2-4. ^1H NMR spectrum of linear PCL diol.

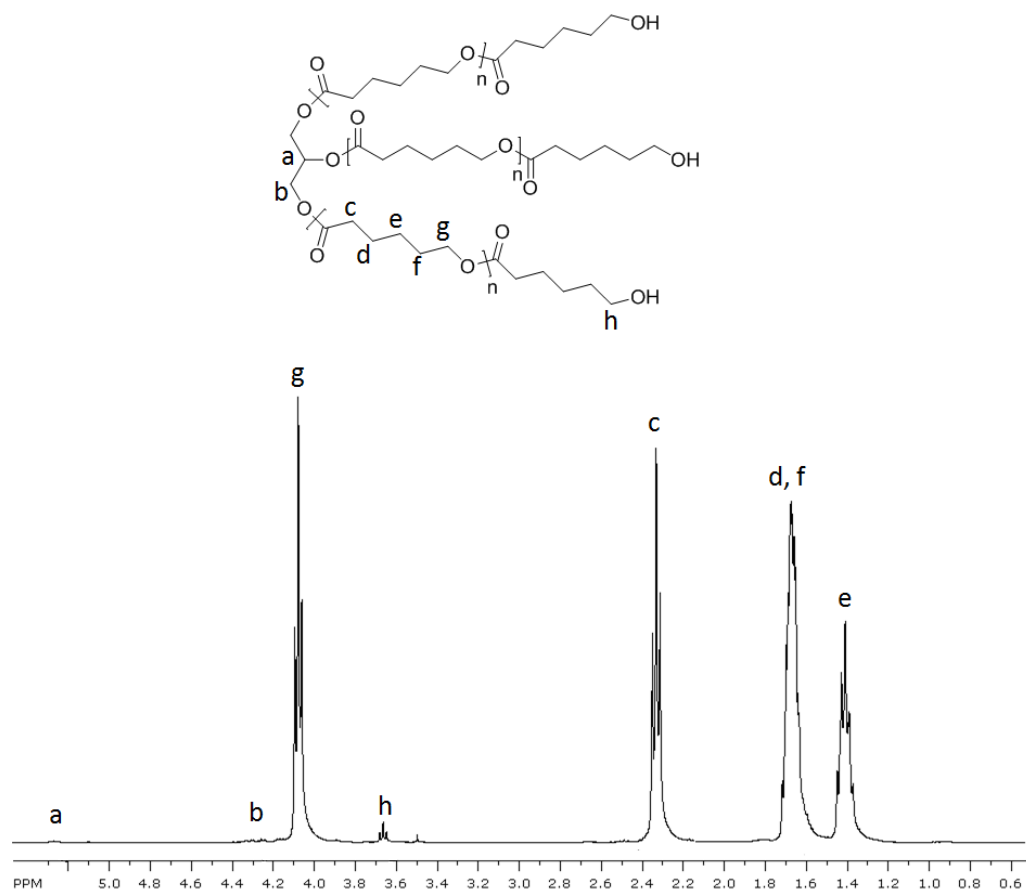


Fig. 2-5. ^1H NMR spectrum of branched PCL triol.

Methacrylation was indicated by the vinyl ^1H NMR peaks at $\delta = 5.62$ and 6.14 ppm, in addition to $\delta = 1.9$ and 3.51 ppm corresponding to the methyl protons of the methacrylate group (**Fig. 2-6, 2-7**). The degree of methacrylation was determined by comparing the ratio of the methyl peaks of the methacrylate group at $\delta = 3.51$ ppm and the residual hydroxyl peaks at $\delta = 3.66$ in the crude ^1H NMR. The degree of methacrylation was found to be between 68% and 82%. Methacrylation did not significantly alter the $\langle M_n \rangle$ or polydispersity of the prepolymers.

Differential scanning calorimetry (DSC) showed a systematic dependence of the network T_m on the $\langle M_n \rangle$ of the prepolymers. Increasing the chain length promoted the formation of larger and more stable PCL crystallites and consequently a higher T_m . This was reflected through the degree of crystallinity (χ_c) which also demonstrated a concomitant increase with increasing chain length. Networks synthesized from linear dimethacrylate prepolymers of $\langle M_n \rangle = 2,500$ (LPCL-3k) did not demonstrate any crystallinity. Smaller prepolymer chain lengths resulted in a higher network crosslink density which completely suppressed the formation of PCL crystallites. Networks with larger linear precursors of 6,600 (LPCL-7k) and 7,700 (LPCL-8k) g mol^{-1} exhibited a T_m of 59 and 57 $^{\circ}\text{C}$, respectively. Networks with star-shaped PCL prepolymers with $\langle M_n \rangle$ of approximately 7,000 (SPCL-7k) g mol^{-1} were rendered completely amorphous, while prepolymers of 12,500 (SPCL-13k) and 14,300 (SPCL-14k) g mol^{-1} resulted in T_m values of 36 and 47 $^{\circ}\text{C}$, respectively. SPCL-14k exhibited a shoulder at approximately 55 $^{\circ}\text{C}$, which represents a small population of unreacted prepolymer. It is likely that crosslinking was partially quenched due to the high viscosity of the melt.

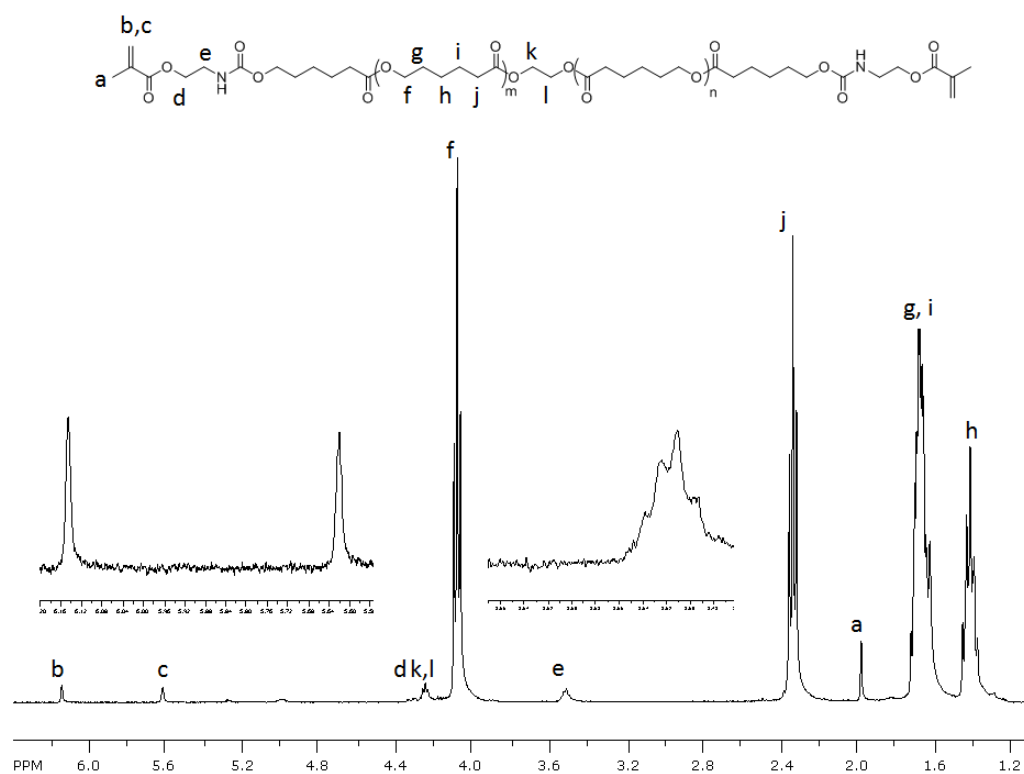


Fig. 2-6. ^1H NMR of linear PCL dimethacrylate.

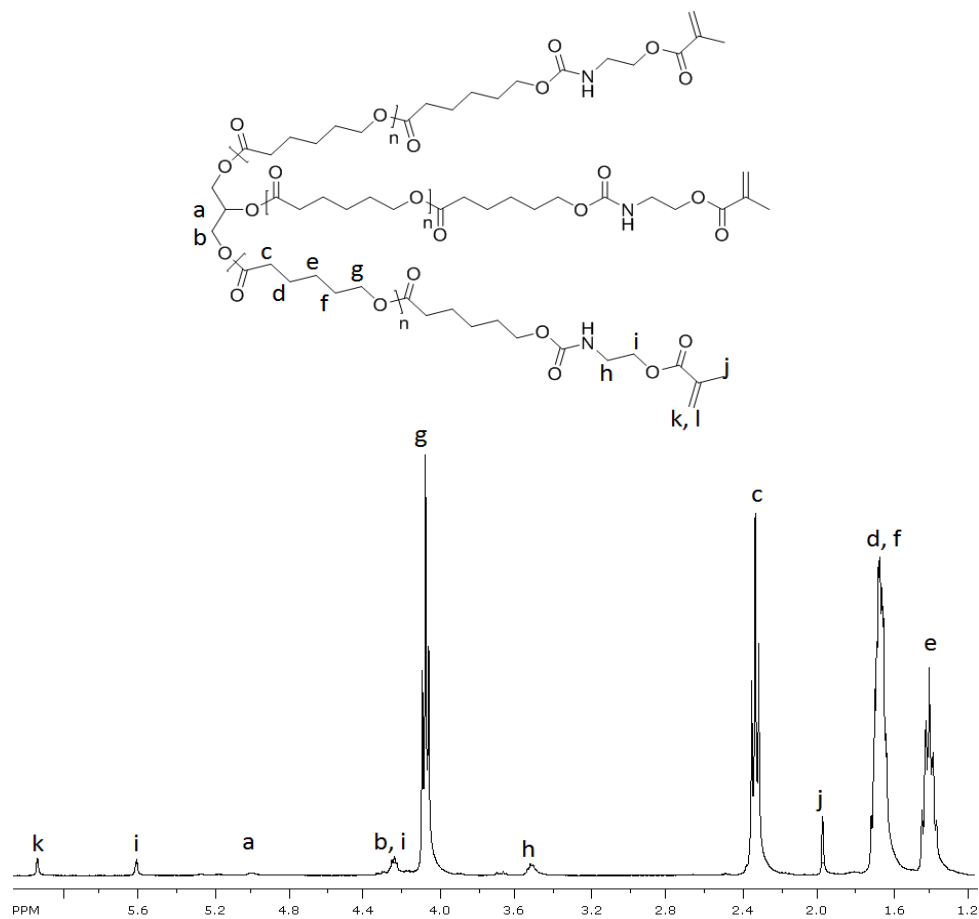


Fig. 2-7. ^1H NMR of linear PCL trimethacrylate.

Moreover, it was shown that the range of T_m values attainable by manipulating the $\langle M_n \rangle$ of linear prepolymers was limited to temperatures well above body temperature. This obstacle was overcome by employing a branched star-shaped prepolymer architecture, which yielded networks with substantially lower T_m and χ_c when compared to linear materials of similar $\langle M_n \rangle$. A T_m near body temperature was achieved in SPCL-13k through the combined effect of a branched prepolymer architecture and the precise manipulation of chain length (**Fig.2-8**). These results are in agreement with previous studies demonstrating that highly branched prepolymer architectures can yield T_m values much lower than their linear counterparts.^[24,26,32,33,34] Thermal characterization of the PCL networks is summarized in **Table 2-1**.

2.3.2 Thermomechanical and Shape Memory Characterization

Mechanical properties were characterized by tensile analysis at 25 °C and 60 °C with a constant strain rate of 10 mm/min. At 25 °C Young's modulus (E) generally increased with increasing chain length. Networks possessing longer PCL chains favored the formation of larger and more stable PCL crystallites which contribute to the mechanical strength of the film. For this reason, yield stress (σ_s), yield strain (ϵ_s), and elongation at break (ϵ_r) were also generally higher for networks possessing larger prepolymer chain lengths. Clearly, the crystalline domains of the film act as physical crosslinks and enhance the mechanical behavior of the films at temperatures below T_m . These conclusions are supported by ΔH_m values which show that networks with longer chain lengths also possess a larger χ_c . At 60 °C, all of the films were well above their respective T_m and completely amorphous.

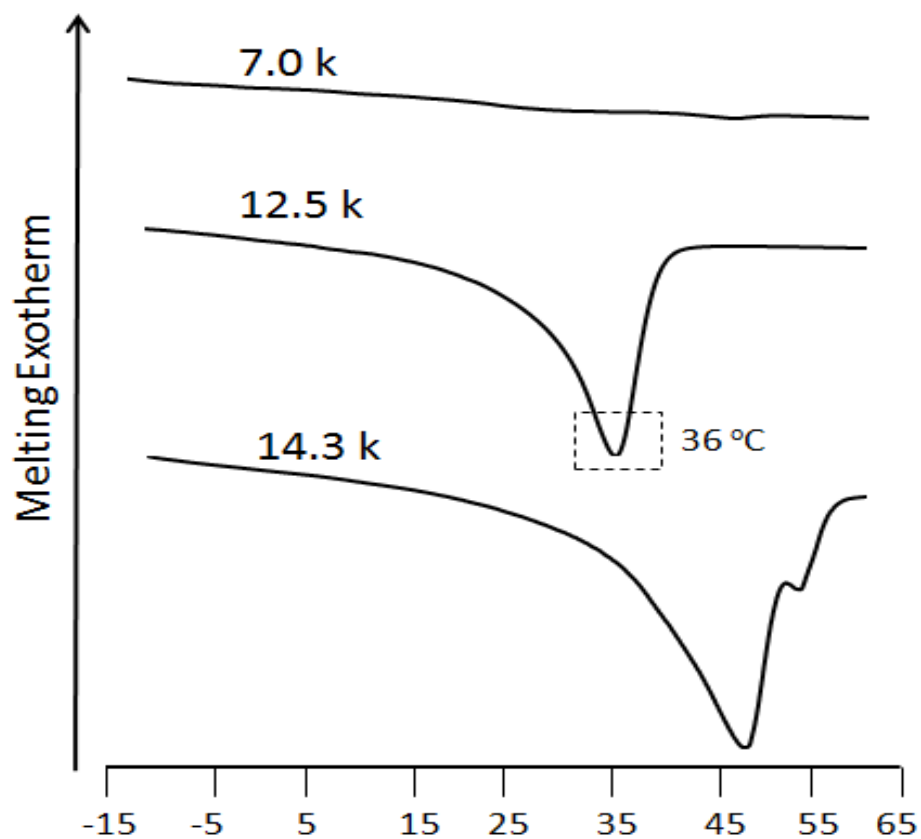


Fig. 2-8. Melting exotherm of SPCL-7k, SPCL-13k, and SPCL-14k.

Table 2-1. Prepolymer and Network Characterization

Polymer	$\langle M_n \rangle$ ($\times 10^{-3}$) (gmol^{-1}) ^{ab}	[M]/[I]	Time (h)	PDI ^a	D _M (%) ^b	T _m (°C) ^c	χ_c (%) ^{cd}
LPCL-3k	2.5 ^a	24.4	7.5	1.1	75	--	--
LPCL-7k	6.9 ^a	63.3	18	1.2	75	50	40
LPCL-8k	7.7 ^a	77.9	29	1.1	80	53	42
SPCL-7k	7.0 ^b	61	2.5	1.1	77	--	--
SPCL-13k	12.5 ^b	110	2.5	1.2	80	36	22
SPCL-14k	14.3 ^b	125	2.5	1.2	82	47	29

^a $\langle M_n \rangle$ is based on GPC calibrated with polystyrene with a correction factor of 0.5 ^b Measured by ¹H NMR (CDCl₃) ^c Measured by Differential Scanning Calorimetry (DSC) ^d Based on theoretical PCL (0.135kJg⁻¹)

Conversely, at high temperatures E significantly decreased with increasing $\langle M_n \rangle$. These results are due to the strong dependence of E at high temperatures on crosslink density. Networks prepared from shorter precursors have much higher crosslink densities and consequently a larger E when compared to films synthesized from larger chains. σ_s showed no dependency on $\langle M_n \rangle$ at high temperature due to the absence of crystalline domains. However, ϵ_s and ϵ_R increased with increasing chain length. Here, it was shown that while the mechanical properties at 25 °C are controlled by crystallinity, the mechanical properties at higher temperatures are dominated by the crosslink density.

Shape memory performance was quantified by thermomechanical tensile analysis. Dog bone molds were subject to 30% strain at 60 °C and then cooled to room temperature. The load was then removed and shape recovery was examined by heating the samples to 60 °C for networks having linear prepolymers and 40 °C for networks having branched prepolymers. Films LPCL-3k and SPCL-7k did not possess the crystallinity necessary to retain the elongated secondary shape and consequently did not demonstrate any shape memory properties. Moreover, due to the high crosslink density these materials were also prone to premature rupture during mechanical deformation. Films LPCL-7k and LPCL-8k were considerably more crystalline and demonstrated excellent R_f and R_r values when subjected to 60 °C for 10 min. Likewise, SPCL-13k ($T_m = 36$ °C) demonstrated near quantitative shape memory properties at 37 °C. SPCL-14k also possessed R_f values of > 98%; however, the high crystallinity ($T_m = 49$ °C) of these materials led to negligible R_r at 40 °C. When heated at 50 °C SPCL-14k, the films recovered the primary shape with $R_r > 98\%$.

The results of mechanical characterization at low and high temperatures and shape memory performance are summarized in **Table 2-2**.

Table 2-2. Mechanical and Shape Memory Characterization

Polymer	25 °C				60 °C				Shape Memory	
	E (MPa)	σ_s (MPa)	ϵ_s (%)	ϵ_R (%)	E (MPa)	σ_s (MPa)	ϵ_s (%)	ϵ_R (%)	R_f (%)	R_r (%)
LPCL-3k	56	4	14	10	18	11	3	9	--	--
LPCL-7k	653	--	--	2	16	1	4	10	99	98
LPCL-8k	441	10	18	46	3	1	17	19	99	98
SPCL-7k	16	2	16	16	18	1	8	15	--	--
SPCL-13k	134	7	17	37	7	2	40	40	99	98
SPCL-14k	209	7	17	58	4	1	32	43	99	< 1

2.4 Conclusions

SMPs based on chemically crosslinked polymeric networks of linear or branched PCL precursors were developed. The thermal properties demonstrated a strong dependence on $\langle M_n \rangle$ and chemical architecture. Generally, films having larger prepolymers showed a higher T_m that resulted from an increase in crystallinity. Moreover, films synthesized from branched precursors showed the ability to maintain crystallinity at temperatures much lower than films prepared from linear materials. By varying these network parameters it was possible to readily tune T_{trans} to values ranging between 36 and 53 °C.

These sets of materials are suitable for applications which require switching temperatures above physiological temperature (LPCL-7k, 8k, SPCL-14k) and at physiological temperature (SPCL-13k). The thermomechanical properties of the films were strongly dependent upon the relationship between crystallinity and crosslink density with crystallinity being the major determinant of mechanical properties at $T < T_{trans}$ and crosslink density at $T > T_{trans}$. Linear materials generally showed a much higher E than branched materials due to their higher crystallinity. Films possessing shorter chain lengths and high crosslink densities did not possess crystalline domains and therefore did not demonstrate any shape memory properties. Conversely, films which possess crystalline domains demonstrated near quantitative R_f and R_r at temperatures greater than T_{trans} .

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Chapter 3

Dynamic Topographical Control of Mesenchymal Stem Cells by Culture on Responsive Polycaprolactone Surfaces*

3.1 Introduction

There is clear, emerging evidence in the literature supporting the influence of surface topography on various cell phenotypes.^[1-5] Recent advancements in mechanobiology have relied heavily on synthetic extracellular matrix (ECM) mimics to investigate how cellular phenomena are dependent upon surface geometry.^[6] Concurrent developments in micro-and nanofabrication techniques have enabled the construction of well-defined surface arrays which aim to emulate the extracellular microenvironment. Numerous patterns of different sizes and shapes including grooves, posts, and pits have been used to study the *in vitro* response of various cell types such as: fibroblasts, osteoblasts, epithelial cells, neuronal cells, and more recently stem cells.^[7-14]

Cell-topography interactions have far-reaching implications in cell biology and biomedical engineering. Many biological processes such as embryogenesis and angiogenesis are strongly influenced by these interactions.^[4,15,16] Additionally, abnormalities in ECM sensing have been linked to many disease states such as cardiomyopathy, muscular dystrophy, and cancer.^[17-19] Topography is also currently being explored as a means to mechanically direct stem cell fate and will be important in the design of next generation

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D.M. Le, K. Kulangara, A.F. Adler, K.W. Leong, V. Sheares Ashby, *Adv. Mater.* **2011**, 23, 3278-3283.

tissue engineering scaffolds.^[13,20-23] However, there remain significant fundamental questions surrounding cell-topography interactions for which innovative, dynamic biomaterials may offer new insights not previously accessible by static substrates. Accordingly, there has been an increased effort to design dynamic substrates that can communicate active physical cues to cells in a more biomimetic context.^[24-28]

3.1.1 Micro-and Nanotopography in Dictating Cellular Response

Cell-topography interactions have commonly been studied on static ordered parallel channels of varying size, interfeature spacing, and chemical composition.^[2-4] Channels attempt to mimic the fibrillar structures found within the extracellular matrix (ECM).^[29] Numerous studies have reported marked cell alignment in response to culture on micro-and nano channel arrays (**Fig. 3-1**).^[30-32] The preferential alignment of cultured cells along the major axis of an anisotropic surface feature is referred to as contact guidance.^[33] This phenomena is believed to be caused by topography-induced modifications in cell adhesion and cytoskeletal arrangement.^[33,34] Recently, Ding and co-workers described contact guidance through thermodynamic considerations.^[35] The authors showed that the initial energetic barrier experienced during cell spreading on patterned surfaces was quantitatively similar to simple liquids. Furthermore, it was demonstrated that the free energy (ΔG) required to spread parallel to the channels was significantly lower than the energetic gradient required to cross perpendicularly. In addition to cell morphology and alignment, ordered channels have also been shown to affect proliferation, migration, polarization, and gene expression.^[36]

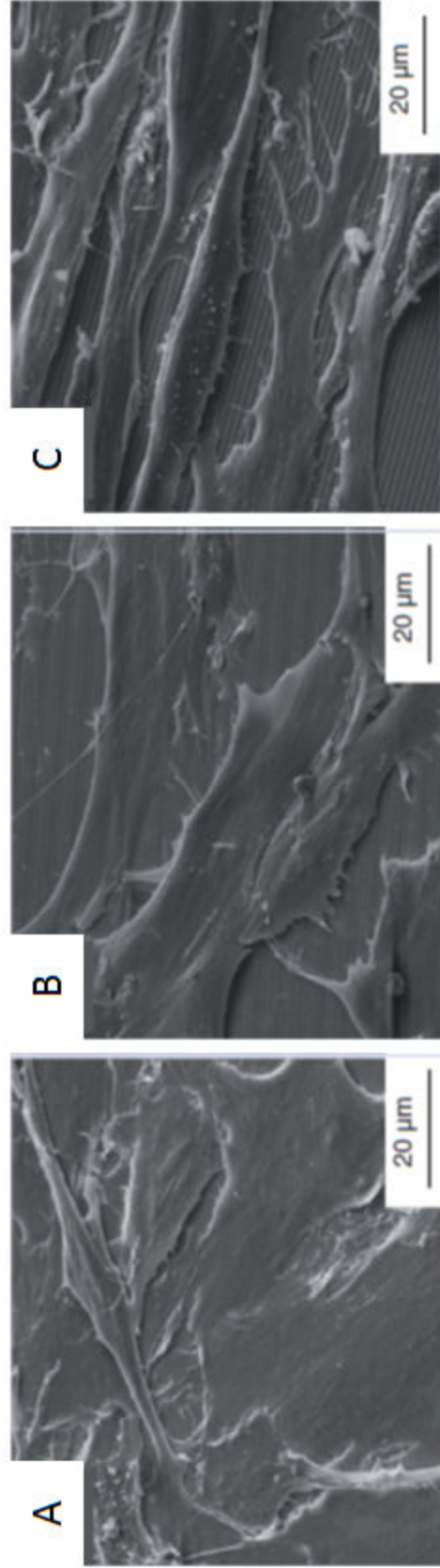


Fig. 3-1. Scanning electron micrographs of A) hMSC spreading on a flat PDMS surface, the B) moderate alignment and elongation of hMSC on 6 μm wide PDMS lines, and the C) marked alignment and elongation of hMSCs on 1 μm wide PDMS lines. Scale bar is 20 μm.

Recently, the topographical control of gene expression and differentiation in multi-potent stem cells has become the focus of intense research. The interest in stem cells relates to their unique potential as cell-based therapies and for their fundamental value in understanding basic cellular processes.^[37] Leong and coworkers investigated the effect of micro-and nanoscale topography on the transdifferentiation of human mesenchymal stem cells (hMSC) to non-default neuronal lineages.^[13] hMSCs cultured on 350 nm wide PDMS surfaces demonstrated a high propensity for alignment (86.5%) in the direction of the PDMS channel axis in comparison to flat surfaces which showed negligible alignment (**Fig. 3-2**). Aligned hMSC cells also showed pronounced elongation in comparison to unpatterned samples, with 18.7 and 3.3% elongation respectively. Likewise, cell nuclei exhibited elevated levels of elongation (1.43%) in comparison to control samples (0.23). The channels were able to drive hMSC phenotype to neuronal lineages as determined by the presence of mature neuronal markers β -Tubulin III (Tuj1), nestin, synaptophysin, glial fibrillary acidic protein (GFAP) and by the upregulation of microtubule-associated protein 2 (MAP2) as compared to unpatterned samples (**Fig. 3-3**). Interestingly, proliferation and differentiation also showed significant size dependence, where decreasing feature size led to lower proliferation rates and increased neuronal differentiation as shown by increased Brdu incorporation and MAP2 expression, respectively. Although the signaling pathway was not determined, the authors hypothesized that cytoskeletal tension and nuclei elongation were the key events in creating the appropriate signal transduction chain. Chen and coworkers demonstrated that the lineage commitment of hMSCs is tightly correlated with cell shape and cytoskeletal tension.^[38]

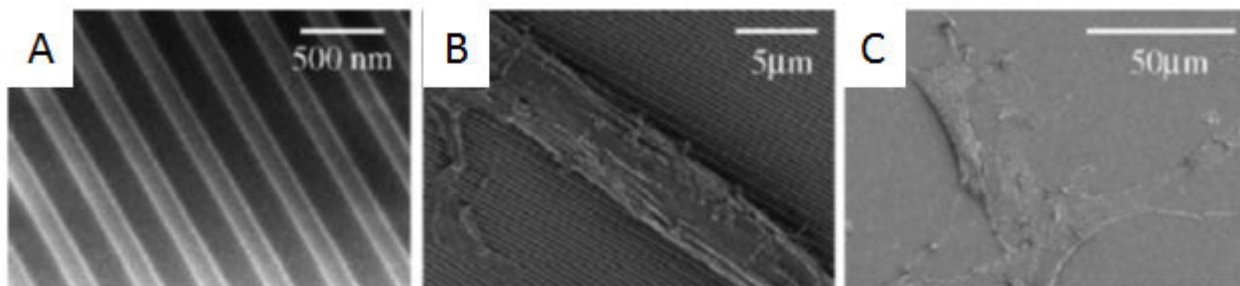


Fig. 3-2. Scanning electron micrographs of A) 350 nm PDMS nanochannel array; hMSCs cultured on B) nanopatterned PDMS, and C) unpatterned PDMS.

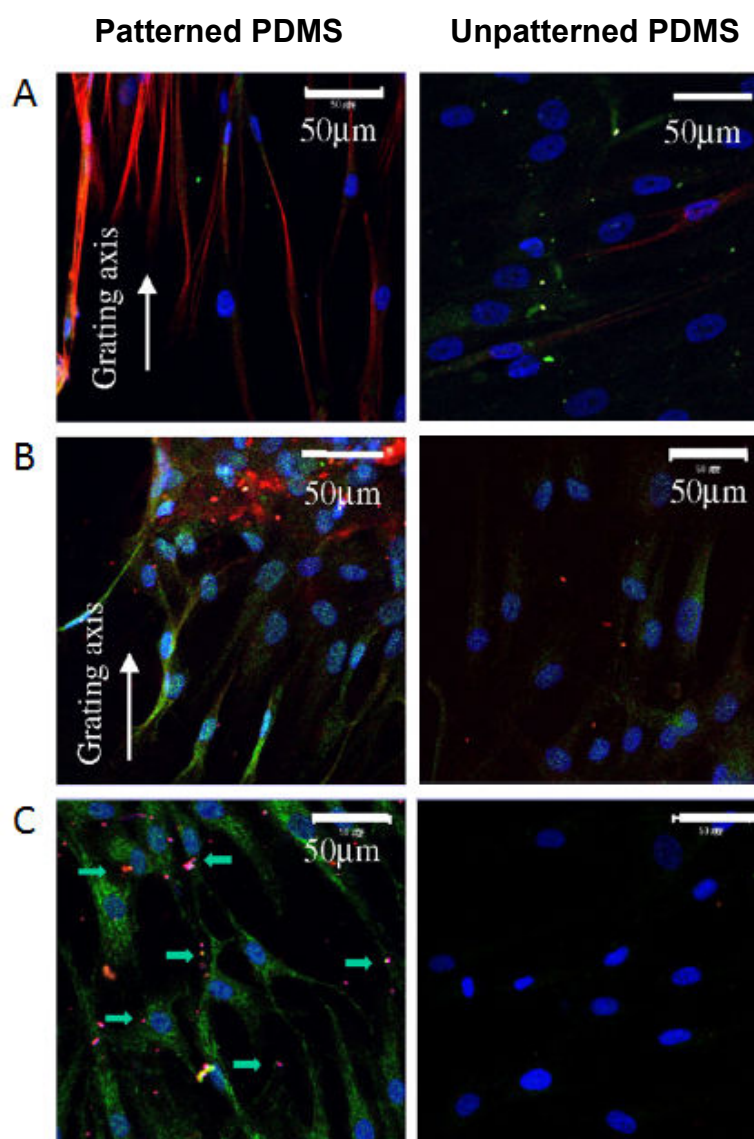


Fig. 3-3. Immunofluorescent staining of neural protein markers A) Tuj1 (red), GFAP (green), B) Nestin (red), MAP2 (green), and C) synaptophysin (red) on nanopatterned and unpatterned PDMS films. DAPI nuclei counterstaining is shown in blue.

Microcontact printed PDMS islands were used to control the degree of cell spreading against the substrate. hMSCs were plated as single cells per island and spread to varying degrees depending on the size of the island. It was shown that adipogenesis occurred only on small islands, osteogenesis occurred only on large islands, and a mixture of both lineages was found on intermediate sized islands (**Fig. 3-4**). Larger microislands increased cell-substrate interactions, cytoskeletal tension, and RhoA expression, while the reverse was found for smaller microislands. Moreover, RhoA and its downstream effector ROCK were shown to be the hMSC commitment switch between osteogenic or adipogenic fate (**Fig. 3-5**). Negative RhoA activity was consistent with low cytoskeletal tension and adipogenesis, while active RhoA activity was found in parallel with high cytoskeletal tension and osteogenesis.

This interpretation is consistent with reports by Mrksich and coworkers.^[39] hMSCs were cultured on microcontact printed PDMS rectangles with increasing aspect ratios and pentagonal shapes of different curvature. Cells cultured in square patterns displayed 46% osteogenesis while cells cultured in rectangles with aspect ratios of 3:2 and 4:1 demonstrated 56% and 61% osteogenesis, respectively. Likewise, hMSCs cultured on pentagonal structures with large convex sides, resembling a flower, demonstrated 62% adipogenesis, while structures with straight lines for edges and star shaped structures with concave sides and pointed vertices demonstrated 50 and 62% osteogenesis (**Fig. 3-6**). These findings suggest that hMSCs cultured on structures of high aspect ratio or curvature favored osteogenesis while structures of low aspect ratio and curvature favored adipogenesis.

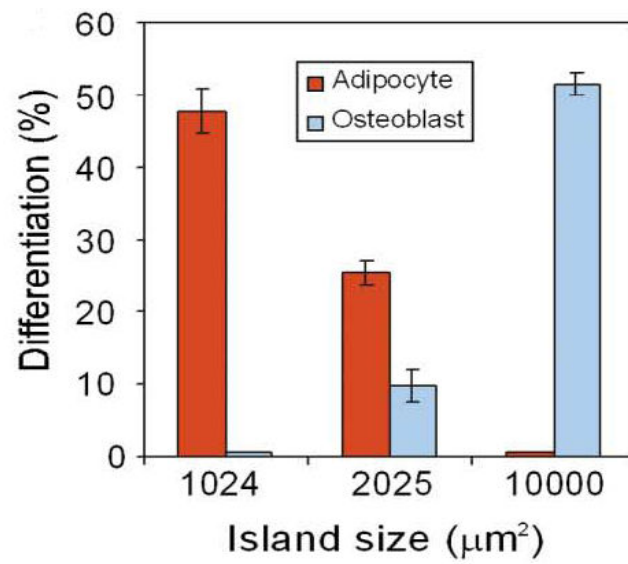


Figure 3-4. Effect of PDMS island size on the differentiation pathway of hMSCs.

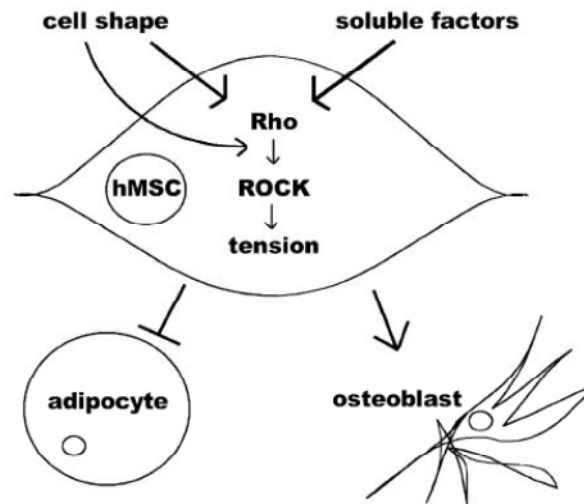


Fig. 3-5. Model of a topographically mediated switch to adipogenic or osteogenic fate.

Immunofluorescence of hMSCs cultured on flower and star shaped pentagonal structures were used to observe the effect of surface geometry on stress fiber and focal adhesion formation, as indicated by F-actin (green) and vinculin (red), respectively (**Fig. 3-6**). Cells cultured on star shaped structures showed larger stress fibers and focal adhesion plaques than cells cultured on flower like structures. Cellular tension was investigated with immunofluorescent heat maps of contractile protein myosin II (**Fig. 3-7**). Cells cultured on star shaped features demonstrated higher myosin II activity and is consistent with the biased osteogenic differentiation of cells cultured on this geometry. Taken together these studies underline the relationship between actomyosin contractility and its ability to regulate specific differentiation pathways.

3.1.2 Mechanisms of Topography Induced Mechanotransduction

While, the mechanisms responsible for topographically induced mechanotransduction are not well understood cell adhesion has been implicated as a major regulator.^[33] Focal adhesions are multi-protein complexes where cells attach to a substrate and are key components in all subsequent cellular response to surface topography.^[40] To date, studies indicate that integrin clustering and focal adhesion formation are strongly influenced by nanofeatures *in vitro* and that subsequent changes in focal adhesion density and length are linked to changes in stem cell function and differentiation.^[41]

Within focal adhesions, integrin transmembrane proteins bind to ECM ligands through specific amino acid motifs such as the Arg-Gly-Asp (RGD) sequence.^[42-44]

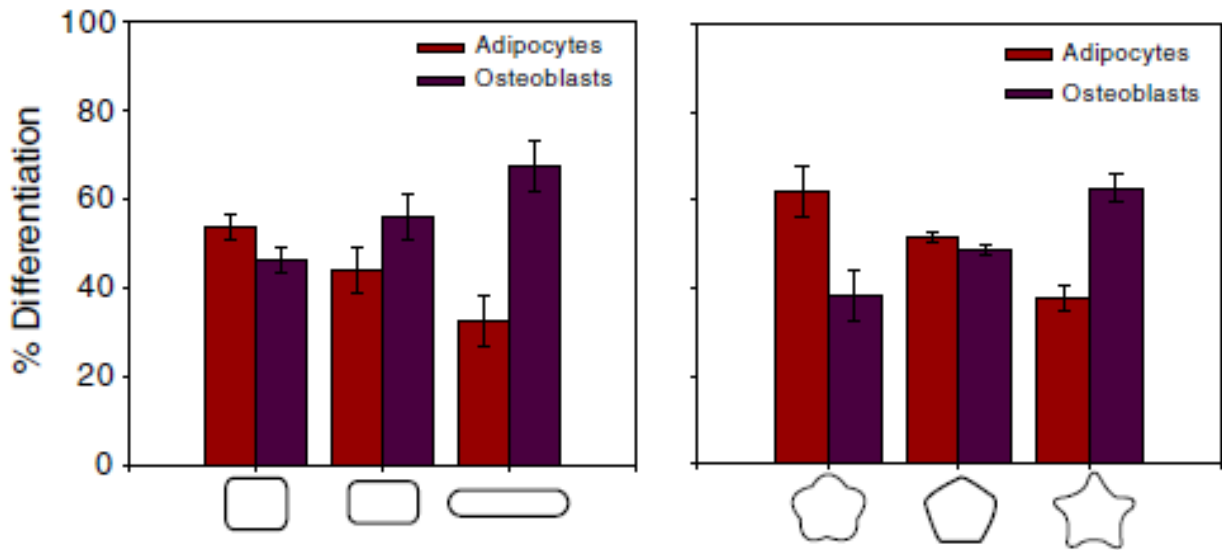


Fig. 3-6. Percentage of adipogenic or osteogenic differentiation of hMSCs captured on rectangles of varying aspect ratio or five point symmetric shapes of varying curvature.

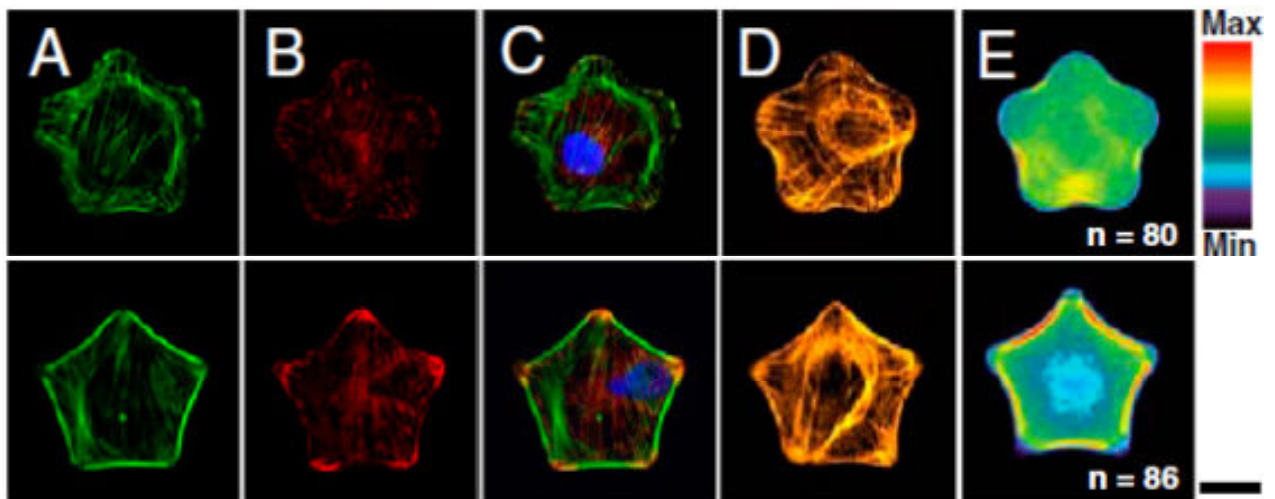


Fig. 3-7. A-C) Immunofluorescent images of cells captured on flower and star shapes stained for F-actin (green), vinculin (red), and nuclei (blue). D) Immunofluorescent images of cells on flower and star shapes stained for myosin IIa. E) Fluorescent heat maps of cells stained for myosin IIa as a quantitative measure of contractility. Scale bar is 20 μm .

Coupling integrins to the ECM induces conformational changes that reveal the opposing cytoplasmic tail which are then bound to the actin cytoskeleton. Integrin-dependent signaling is mediated by nonreceptor tyrosine kinases, most notably focal adhesion kinase (FAK).^[45] FAK localizes at focal contacts and influences cellular transcriptional events through adhesion-dependent phosphorylation of downstream signaling molecules. In particular, the extracellular signal-regulated kinase (ERK) signaling cascade is activated by focal adhesion elongation on patterned surfaces and has been shown to act as a mediator of cellular differentiation.^[46,47]

Focal adhesion formation and maturation can also alter stem cell phenotype through a direct mechanotransductive route where cytoskeletal tension is propagated directly to the nucleus of the cell.^[48] Internal tension is generated through the activation of Rho which occurs following initial integrin binding by phosphorylation to Rho-guanosine triphosphate (GTP).^[49] Rho-GTP then goes on to bind to a number of protein targets that leads to actin polymerization. Active Rho also binds to ROCK1 and ROCK2 which deactivate a subunit of myosin phosphatases which is primarily responsible for the deactivation of myosin II. In this way myosin II light chain activity is sustained, leading to increased cell contractility and cytoskeletal tension. The outer nuclear membrane is attached directly to the cytoskeleton through the linker of nucleoskeleton and cytoskeleton (LINC) complex.^[50] Moreover, DNA directly bound on the interior nuclear membrane at matrix attachment regions (MARs).^[51] Large differentials in cellular tension have been shown to result in the physical deformation of both the cytoskeleton and the nucleus and have been strongly correlated with changes in gene expression and cell differentiation.^[52] Nuclear deformation is thought to result in chromosomal repositioning

within the nucleus. Chromosomal redistribution has the potential to effect gene transcription if the chromosomes are forced from inactive-heterochromatic regions to more active-euchromatic regions of the nucleus. Moreover, the condensation or relaxation of DNA regions can suppress or enhance their susceptibility to soluble differentiation factors.

3.1.3 Dynamic Micro- and Nanotopography in Dictating Cellular Response

Until recently, cell-topography studies have been exclusively performed on static substrates. However, there are a growing number of groups synthesizing dynamic cell culture substrates to examine the response of cells to dynamic surfaces topographies. Takayama first demonstrated the application of dynamic topography to cultured cells using reversible poly(dimethylsiloxane) (PDMS) surfaces.^[53] Reversible wavy micro-features were fabricated by subjecting the PDMS surfaces to plasma oxidation and subsequently applying compressive stress to induce surface buckling. The study provided evidence that C2C12 myoblast cell morphology can be directed dynamically using surface array transitions. While these preliminary findings are innovative, the suitability of these materials for dynamic *in vitro* analysis is constrained by poor replication fidelity, batch variability, low feature resolution, and limited shape versatility.

An alternative approach to fabricating reversible surface features is by exploiting the unique properties of shape memory polymers (SMPs). Recently, Henderson reported the control of fibroblast cell alignment and microfilament organization using reversible 150 μm grooves embossed into NOA-63, a polyurethane-based thiol-ene crosslinked SMP (**Fig. 3-8**).^[54] The implementation of a T_g -based SMP resulted in a high degree of control over the activation of the surface shape memory effect.

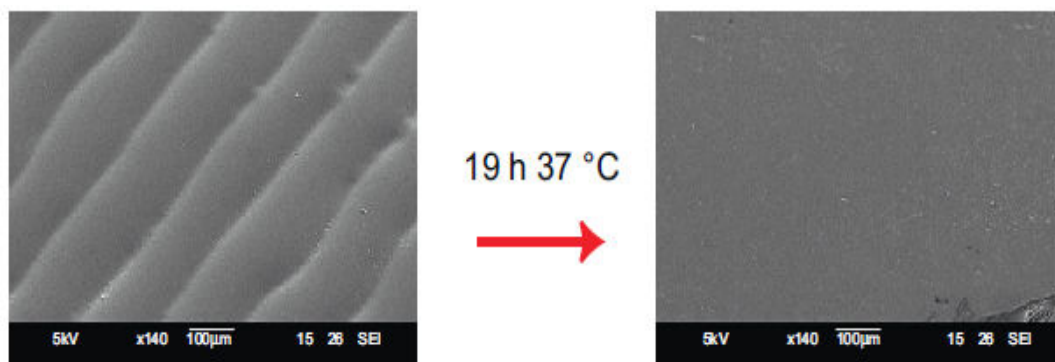


Fig. 3-8. SEM image of a surface transformation between temporary 150 µm grooves to a flat surface after 19 h of heating at 37 °C.

However, the large and irregular dimensions (12 x 150 μm) of the surface patterns limited the degree of control over fibroblast cell morphology (**Fig. 3-9**). Moreover, the surface topography changed sluggishly, requiring over 5 h to recover to the planar morphology at body temperature. This can be attributed in part to the high T_g (75 $^{\circ}\text{C}$) which is approximately 35 $^{\circ}\text{C}$ over the working temperature. Furthermore, the T_g is broadly distributed over a 60 $^{\circ}\text{C}$ range, making shape recovery difficult for chains with a T_g above physiological temperature. Developing strategies that enable strict regulation of the shape memory effect and precise control over surface geometry with sub-cellular resolution remains a great challenge for dynamic cell culture applications.

We were interested in addressing these limitations by engineering biocompatible shape memory surfaces that can accommodate diverse, well-defined, and biologically relevant surface transformations under physiological conditions. To this end, thermally-responsive PCL SMP surfaces were fabricated for the purpose of dynamically probing cell-topography interactions. The covalently crosslinked PCL networks presented in chapter two are promising candidates for dynamic cell culture applications. Specifically, SPCL-13k ($T_m = 36$ $^{\circ}\text{C}$) has a suitable T_m to activate the surface shape memory effect at physiological temperature. Specifically, our aim was to fabricate surfaces which demonstrate exceptional shape memory properties in biological media and are capable of executing rapid and uniform shape recovery at temperatures that will not result in heat shock or cell death. Furthermore, these materials must also be capable of accommodating shape transitions between a diverse range of surface geometries and dimensions.

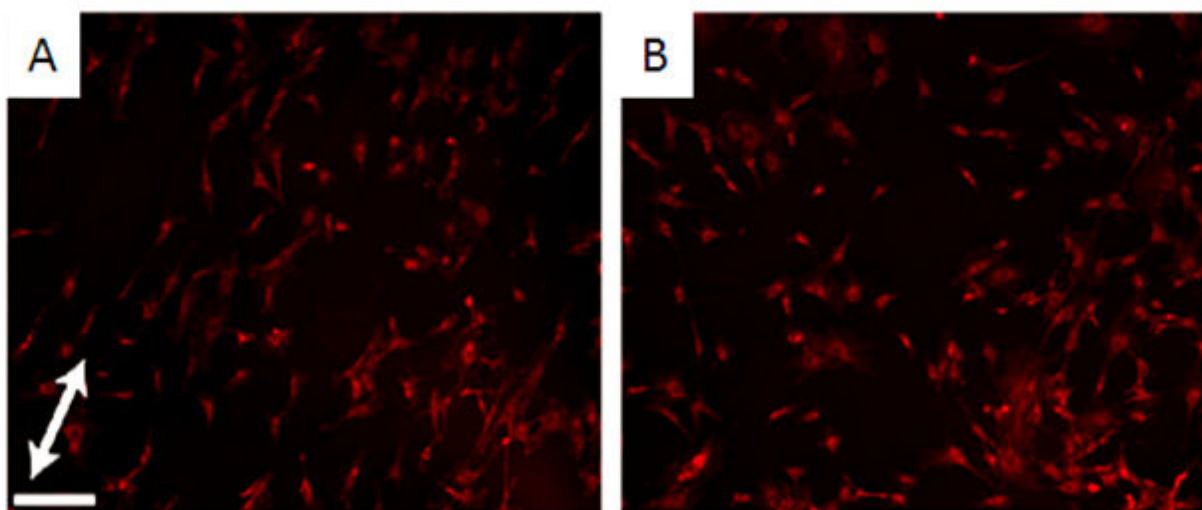


Fig. 3-9. Fluorescent images of human fibroblast cells cultured on A) $\sim 150\ \mu\text{m}$ wide temporary grooves and B) the recovered flat surface following thermal activation of the shape memory effect.

An equally important objective of this research was to examine the viability of the SMP surfaces as dynamic cell culture platforms by assessing surface properties, cytotoxicity, and the efficacy of the surface shape memory effect in dictating the morphology of adherent human mesenchymal stem cells.

3.2 Experimental Section

3.2.1 Fabrication of Shape Memory Polymer Surfaces. Perfluoropolyether (PFPE) and PDMS molds were prepared in house. PCL networks were prepared by casting molten PCL trimethacrylate precursor and photoinitiator diethoxyacetophenone (DEAP) (0.1 wt %) into a teflon mold. Subsequently, the molten PCL was irradiated with 30 mW/cm² UV light (365 nm) under N₂ atmosphere for 10 min. PFPE and PDMS replica molds were used as photo-curing templates or to emboss secondary surface patterns. Mechanical force was applied at 130 °C for 15 min followed by rapid cooling to -78 °C for an additional 60 min. The primary, secondary, and thermally recovered shapes (40 °C) were imaged using brightfield microscopy.

3.2.2 Surface Modification and Characterization: The PCL substrates were modified by oxygen plasma treatment in an AutoGlow oxygen plasma system by Glow Research. Oxygen pressure was maintained at 1.3 mbar while the PCL substrates were subjected to 30 W of power intensity for 1 min. Prior to cell seeding, films were sterilized with UV light for 10 min per side. The PCL films were conditioned first by incubating in PBS buffer (1X) for 2 h. Subsequently, the substrates were immersed in 1 mL of 0.005 mg/mL fibronectin solution for 1 h. Surface hydrophobicity was evaluated by static water contact

angle measurements. Contact angle was acquired using a KSV Instruments Cam 200 Optical goniometer using the sessile drop method.

3.2.3 Cytotoxicity and Immunofluorescent Staining: hMSCs were cultured on featureless PCL substrates for 24 h. The materials were then transferred to new 24 well plates and immersed in fresh MSCGM and WST-1 reagent. The cells were then incubated at 37 °C for 1h. Cell proliferation was detected using UV-vis spectroscopy. Wells with no substrate and ethanol-treated cells were used as controls. For immunofluorescent staining, samples were fixed using 4% p-formaldehyde, permeabilized with 0.1% TritonX-100 in phosphate buffered saline (PBS), and then blocked with 10% goat serum in PBS. F-actin was fluorescently labeled in fixed samples with Oregon Green 488 phalloidin (Molecular Probes, Eugene, OR), and the nucleus was counter stained with 4',6-diamidino-2-phenylindole (DAPI, Molecular Probes). Samples were imaged by confocal microscopy (Zeiss 510 inverted confocal microscope).

3.2.4 Human Mesenchymal Stem Cell Culture: Human MSCs were supplied by Dr. D. Prockop from Tulane Center for Gene Therapy at Tulane University, New Orleans, LA, USA. The hMSCs used in the experiments were at passages 3–6. hMSCs were cultured in complete culture media (CCM) comprising α -Minimum Essential Medium (α -MEM) supplemented with 16.5% (v/v) fetal bovine serum (FBS, Atlanta Biologicals, Inc., Lawrenceville, GA, USA), 2 mM L-glutamine (Gibco/Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco/Invitrogen, Carlsbad, CA,

USA). The cells were seeded at a density of 10,500 cells/cm² and placed in an incubator under 5% CO₂.

3.2.5 Dynamic cell culture: GFP-hMSCs were seeded on static planar and static channel arrays as the controls. GFP-hMSCs were also cultured on shape memory surfaces with a temporary shape of 3 x 5 channels and a planar primary shape. The cells were cultured at 28 °C for 1 day, subsequently the cells were subjected to a 40 °C heat treatment in hMCSGM for 1h. The cells were then allowed to equilibrate at 37 °C for 12 h.

3.2.6 Widefield image analysis of cell alignment: Images for quantification of GFP-hMSC alignment were acquired using a Nikon Eclipse TE2000-U fluorescence inverted microscope. Three images were collected from each of three replicate substrates – static planar, static channels, and SMP channels – at 28 °C, and 37 °C. Approximately 2,000 cells were analyzed for each condition using a custom Fiji Is Just ImageJ (FIJI) macro, implemented identically to each image by a tester blinded to the identity of each condition. Briefly, Bernsen's thresholding method was employed to define regions of high local contrast within each image, corresponding to the outline of each cell. Using FIJI's "Analyze Particles" command, ellipses were fit to these outlines, and an angle of deviation from the horizontal axis was measured for each, with 0° (90°) denoting a cell parallel (perpendicular) to the channel direction. Small (non-cell) particles were excluded with a size threshold. An average angle of deviation of 45° represents a random orientation with respect to the horizontal axis.

3.2.7 *Statistical analysis*: A global two-way ANOVA of cell alignment revealed significant main effects of temperature and substrate type, as well as of their interaction or the dependence of cell alignment on temperature as a function of substrate type ($p < 0.0001$ in each case). One-way ANOVAs with Bonferroni-corrected post-hoc testing was used to compare alignment between individual substrates at the same temperature conditions. Additionally, Student's t-tests were used to compare alignment between temperatures within each substrate condition.

3.3 Results and Discussion

3.3.1 Shape Memory Surface Fabrication and Characterization

SPCL-13k films were prepared using the method previously described in chapter two. Soft PFPE and PDMS replica molds of a silicon master were used in concert with specific thermomechanical cycles to program the primary and secondary surface arrays. The fabrication process is shown in **Fig. 3-10**.^[55,56] **Fig. 3-11** shows three surface array transformations between various secondary and recovered (40 °C in water) topographies. Primary shapes (a, d, and g) are also shown for visual comparison to recovered topographies (c, f, and i). The surfaces transformations are as follows: (b-c) 2 μm cubes to 3 x 1 μm hexnuts, (e-f) 7 x 14 μm cylinders to 10 x 1 μm boomerangs, and (h-i) 3 x 5 μm channels to a planar topography. The PCL surfaces demonstrated excellent replication fidelity, secondary shape retention, and primary shape recovery. Using this method a library of surface transformations can be achieved with exquisite control over surface feature size and geometry.

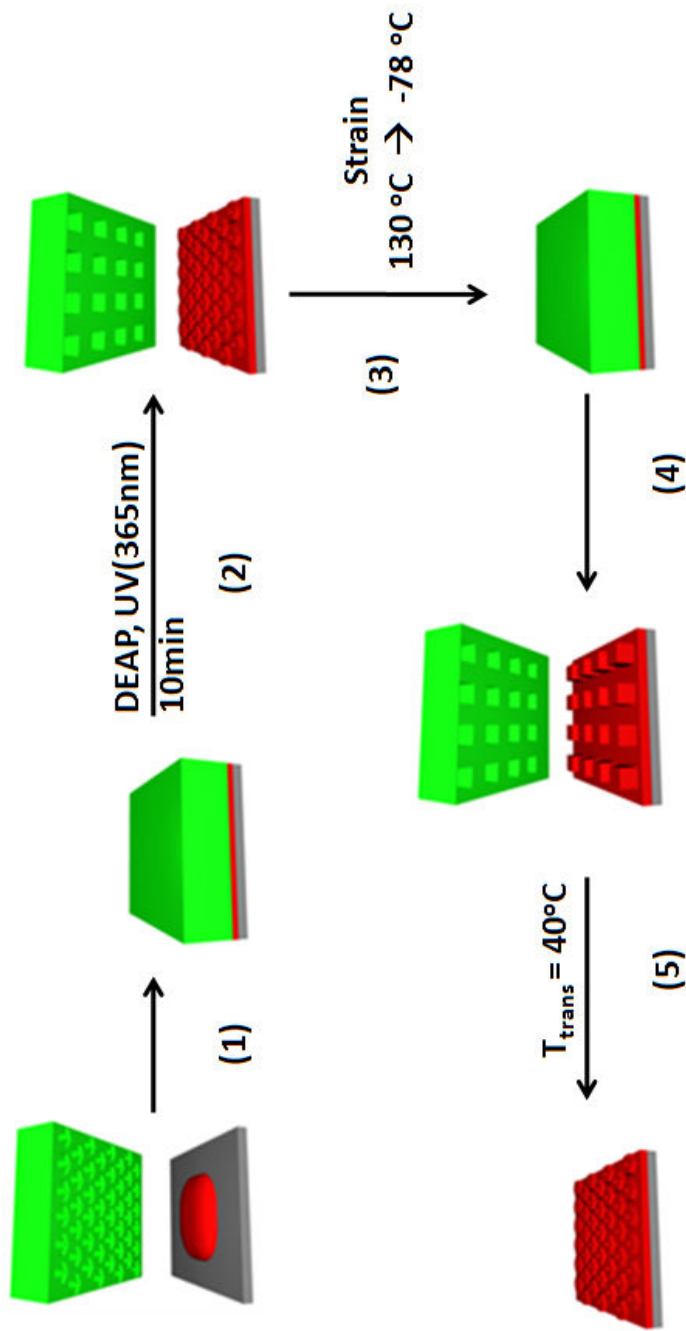


Fig. 3-10. Schematic representation of thermomechanical programming and recovery of shape memory surfaces. (1) The prepolymer in the melt was cast into a mold of the primary shape and (2) photo-cured using diethoxyacetophenone (DEAP) as the photo-radical initiator. (3) The primary shape was then mechanically deformed at 130 °C using a second replica mold and subsequently cooled to -78 °C while still under load. (4) To recover the primary shape, the compressive stress was removed and the polymer film was (5) immersed in water at 40 °C for 10 min.

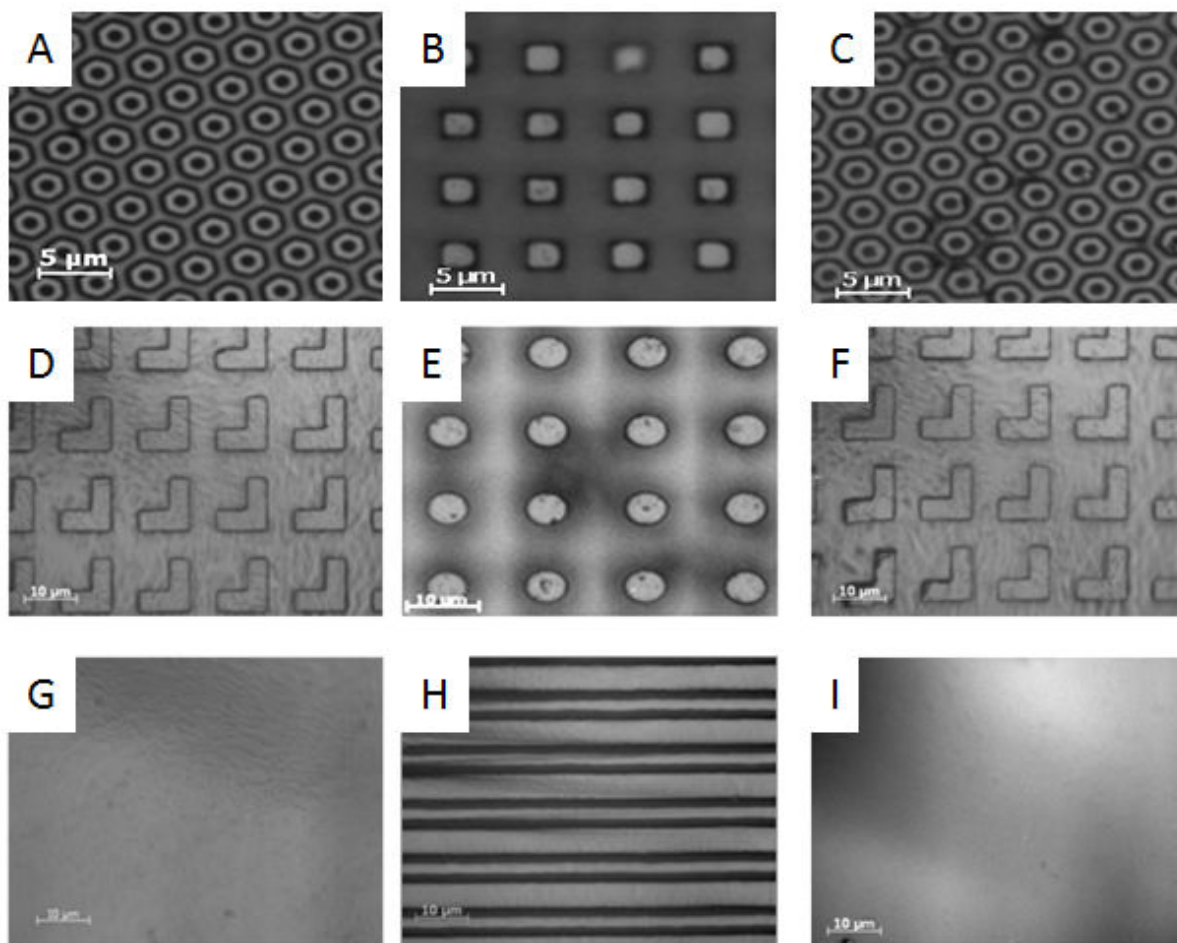


Fig. 3-11. Panning from left to right the primary, secondary and recovered shapes are shown. Brightfield images show surface transformations between various secondary and recovered (40 °C in water) topographies. Primary shapes are shown for visual comparison to recovered topographies (a, d, and g). The surface transformations are as follows: (b-c) 2 μm cubes to 3 x 1 μm hexnuts, (e-f) 7 x 14 μm cylinders to 10 x 1 μm boomerangs, and (h-i) 3 x 5 μm channels to planar topography. Scale bar is 5 μm (a-c) and 10 μm (d-i).

The fabrication technique can also be readily adapted to include submicron-topographies which have been shown to exert a more pronounced effect on cell behavior than micro-topography.^[13]

3.3.2 Surface Modification and Cytotoxicity

In addition to excellent thermal and mechanical properties, SMP biomaterials must also possess optimized surface properties for cell adhesion. To improve cell attachment the PCL films were oxygen plasma-treated to reduce the static contact angle from approximately 90° to 40° (**Fig. 3-12**). Oxygen plasma-treatment is an ideal method of post-polymerization surface modification because it effectively decreases hydrophobicity through the introduction of oxygen containing groups to the surface without disturbing the bulk thermal and mechanical properties.^[57] The improved wettability of oxygen plasma-treated polymers has been shown to enhance the adsorption of cell adhesion proteins such as fibronectin (Fn). Additionally, hydrophilic surfaces are known to favor the active conformational states of adhesion proteins which also leads to enhanced cell attachment.^[58] Thus, to further encourage cell adhesion, the oxygen plasma-treated films were coated with Fn.

Green fluorescence protein transduced-human mesenchymal stem cells (GFP-hMSCs) were cultured on untreated, oxygen plasma, and oxygen plasma-Fn modified planar PCL surfaces in addition to tissue culture poly(styrene) (TCPS) as the control (**Fig. 3-13**). Surface modification with Fn and human collagen I (hCOLI) in the absence of oxygen plasma treatment resulted in low cell densities which were similar to neat PCL.

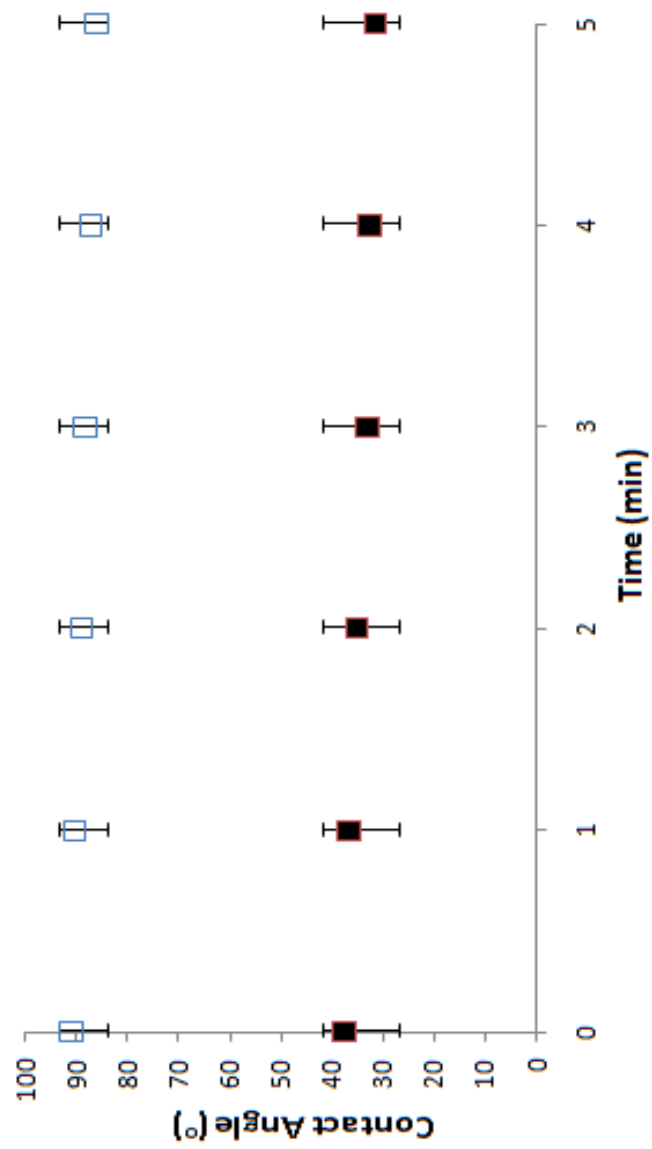


Fig. 3-12. Contact angle measurements for untreated (□) and oxygen plasma-modified (■) PCL planar surfaces.

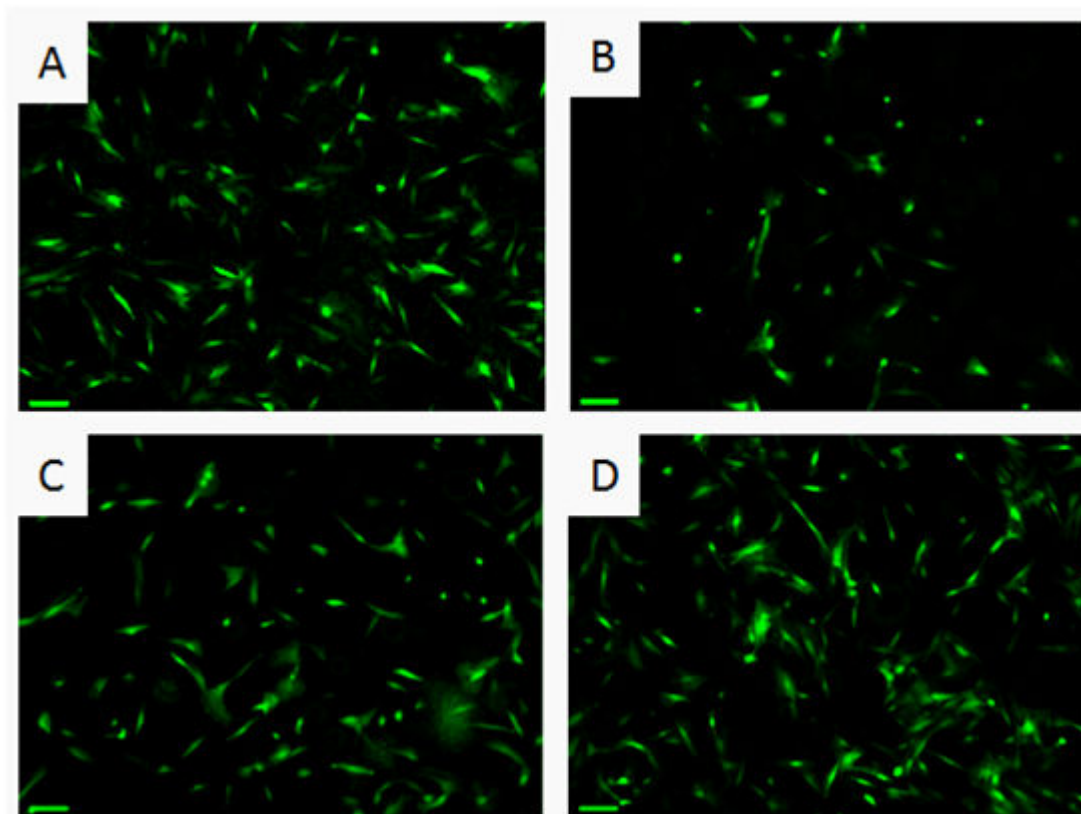


Fig. 3-13. a) Fluorescent images of GFP-hMSCs cultured at 28 °C for 1 day on A)TCPS, B) untreated, C) oxygen plasma-treated, and D) oxygen plasma-Fn treated planar PCL surfaces.

It was clearly shown that the oxygen plasma-treatment (**Fig. 13c**) substantially increased cell attachment over untreated (**Fig. 13b**) PCL surfaces. Additional Fn modification of oxygen plasma-treated surfaces (**Fig. 13d**) resulted in a more pronounced effect on cell attachment, with cell densities comparable to that of the TCPS control (**Fig. 13a**).

Cytotoxicity was evaluated by WST-1 cell proliferation assay. Surface modified PCL substrates demonstrated a much higher cell viability than untreated PCL materials. hMSCs cultured on untreated and oxygen plasma-Fn modified planar PCL surfaces demonstrated a 38% and 80% cell viability, respectively. In conjunction to cell viability, immunofluorescent staining of the actin cytoskeleton (488 phalloidin-Oregon Green, DAPI nuclear counterstaining) showed the hMSCs were able to establish normal, healthy cell morphology on static PCL planar surfaces and on 2 μm cubic arrays (**Fig. 14**).

3.3.3 Dynamic Control of Human Mesenchymal Stem Cell Morphology

Finally, to demonstrate the potential of PCL SMP surfaces in dictating cell morphology, GFP-hMSCs were cultured on dynamic PCL surfaces at 28 °C for 1 day, after which the cells were subjected to the surface shape memory effect at 40 °C for 1h and subsequently allowed to equilibrate at 37 °C for 12 h. Fluorescent microscopy revealed that cells cultured on static planar PCL control surfaces demonstrated a stellate shaped morphology before heat treatment (**Fig. 15a**). When subjected to the heat treatment the morphology of the cells remained stellate shaped (**Fig. 15b**). GFP-hMSCs cultured on static 3 x 5 μm channel control arrays demonstrated marked contact guidance (**Fig. 15c**). No change in cell morphology was observed when the aligned cells were heated (**Fig. 15d**).

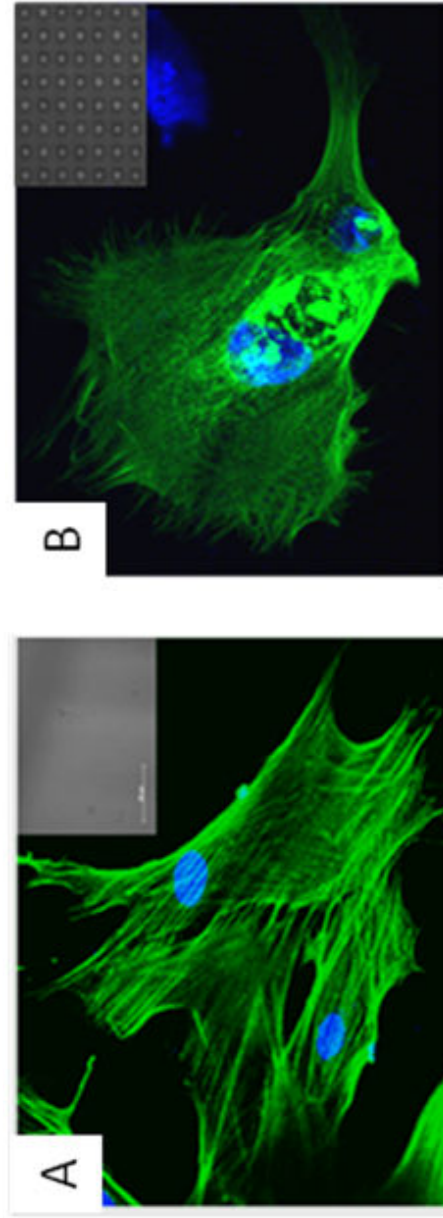


Fig 3-14. Immunofluorescent staining of hMSC actin (Green) cultured at 28 °C for 1 day on A) planar and on B) 2 μ m cubic array PCL surfaces. DAPI nuclei counter-staining shown in blue. Scale bar is 100 μ m.

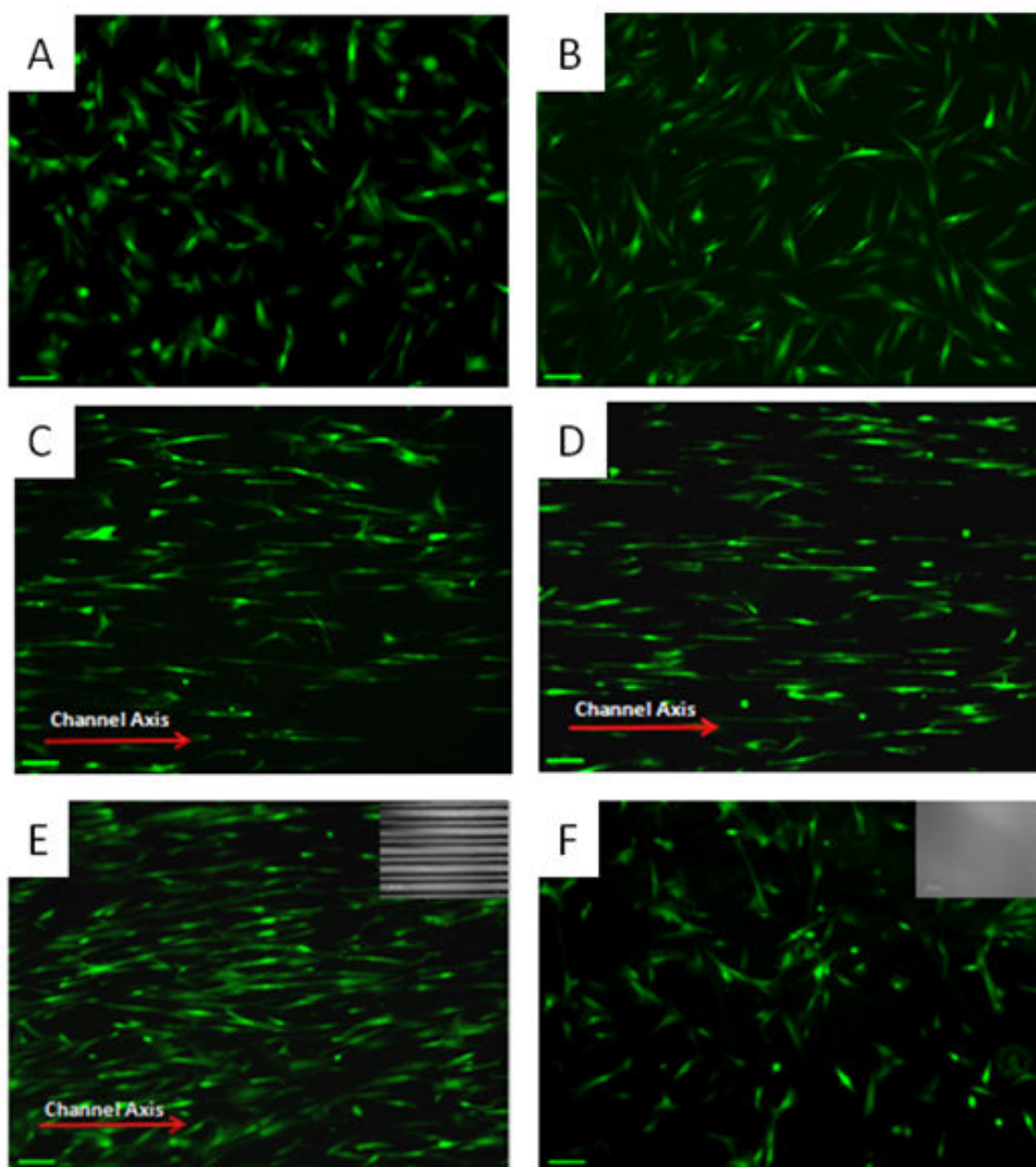


Fig. 3-15. GFP-hMSCs were cultured on static and dynamic PCL surfaces at 28 °C for 1 day and then subjected to 40 °C for 1 h. Subsequently, the cells were allowed to equilibrate for 12 h. Fluorescent images of GFP-hMSCs on static planar surfaces showed that the cells assumed a stellate shape A) before and B) after heat treatment. Images of cells cultured on static surfaces patterned with 3 x 5 μm channels showed that cell alignment was present C) before and D) after heat treatment. Cells cultured on E) temporary 3 x 5 μm channel SMP arrays demonstrated significant alignment along the channel axis. However, when the substrate was heated and the surface shifted to F) a

GFP-hMSCs cultured on temporary 3 x 5 μm channel arrays also demonstrated significant cell alignment (**Fig. 15e**). The surface shape memory effect was activated by culturing the adherent cells at 40 °C in hMSC growth media for 1 hour whereupon the secondary channel topography rapidly dissipated and the primary planar topography was recovered. After the cells were allowed to equilibrate for 12 h the hMSCs returned to a stellate shaped morphology (**Fig. 15f**).

Widefield image analysis was performed to quantify cell orientation and alignment (**Fig. 16**). Three images were collected from each of three replicates for static planar, static channels, and SMP surfaces (3 x 5 μm channels to planar topography) at 28 °C and 37 °C. The angle of deviation from the horizontal axis was measured for each, with 0° (90°) denoting a cell parallel (perpendicular) to the channel direction. An average angle of deviation of 45° represents a random orientation with respect to the horizontal axis. GFP-hMSCs cultured on static planar PCL surfaces at 28 °C demonstrated a completely random (45°) cell orientation. There was no statistical difference between cell orientations on static planar surfaces before and after the heat treatment. In contrast, cells cultured on static channel arrays at 28 °C demonstrated marked cell alignment corresponding to a 10° average angle deviation from the channel axis. Following the heat treatment and equilibration period, the average angle deviation from the channel axis rose slightly, indicating a reduction in cell alignment. These findings can be accounted for by heat induced cell death of hMSCs cultured on channel topographies which resulted in cell rounding and a minor loss in cell alignment. hMSCs cultured on a temporary channel topography at 28 °C showed no statistical difference with cells cultured on static channel surfaces at the same temperature.

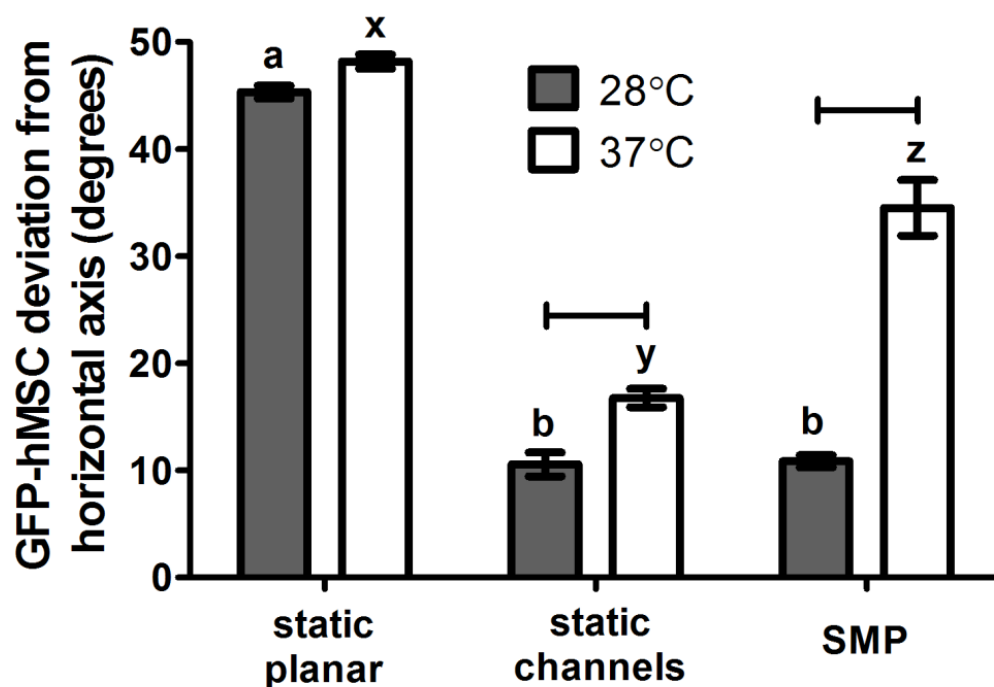


Fig. 3-16. Columns represent the average angle of deviation from the horizontal (channel) direction, with error bars depicting the standard error measurement for 3 images of 3 replicate substrates ($n = 9$). Letters a and b (x, y, and z) denote significant statistical differences between substrates at 28 °C (37 °C) by one-way ANOVA post-hoc testing ($p < 0.001$). Horizontal bars indicate significant differences of cell alignment on a given substrate between temperature conditions by Student's t-test ($p < 0.001$).

The surface transformation to a planar topography resulted in a significant increase in average angle deviation from the horizontal axis over the temporary channel topography. A completely random average cell orientation was not observed due to defects inflicted on the SMP surface during thermomechanical processing that inhibited complete shape recovery.

It has been shown that the morphology of GFP-hMSCs can be topographically dictated through the application of the surface shape memory effect between $3 \times 5 \mu\text{m}$ channels to a flat topography under physiological conditions. The SMP cell culture platforms described here provide a highly versatile and controlled means of probing cellular response to localized changes in topography. These materials possess feature resolution, sharpness, and variability that have not previously been reported in the literature for SMP surfaces. These findings may have far-reaching implications in investigating the effect of dynamic topography on cell adhesion, cytoskeletal organization, cell signalling, and mechanotransductive events.

3.4 Conclusions

In summary, the present study describes the synthesis, characterization, and application of thermally-responsive PCL SMP micro-arrays to dynamic cell culture. The PCL thermosets demonstrated excellent mechanical properties, a body temperature T_{trans} , and near quantitative R_f and R_r . Oxygen plasma-Fn modified SMP surfaces supported hMSC culture with good attachment efficiency, normal cell morphology, and minimal cytotoxicity. The hMSC morphology switched from highly aligned to stellate shaped in response to a surface transformation between a $3 \times 5 \mu\text{m}$ channel array and a planar

surface at 37 °C. This on-demand, surface directed change in cell morphology offers a novel means to study cell-topography interactions with unprecedented control over surface feature size and geometry and may represent a generally applicable method to investigate a wide variety of topography mediated changes in cell behavior.

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Chapter 4

Near-Infrared Activation of Gold Nanoshell/Polycaprolactone Shape Memory Polymer Nanocomposites

4.1. Introduction

Recently, several approaches have been developed which enable the remote activation of the shape memory effect. These systems have expanded the breath of SMP applications by overcoming some of the limitations traditionally associated with direct thermal activation. One such approach utilizes photo-responsive chemical moieties to dictate the shape memory effect.^[1,2] Seminal work by Langer and co-workers reported the synthesis of light activated SMP networks through the copolymerization ultraviolet (UV)-responsive cinnamic acid (CA) (1) groups with *n*-butylacrylate, hydroxyethyl methacrylate, and poly(propylene glycol) dimethacrylate.^[3] In the same report, the authors also synthesized networks from *n*-butylacrylate, poly(propylene glycol) dimethacrylate, and branched poly(ethylene glycol) terminated with UV-responsive cinnamylidene acetic acid (CAA) (2) groups. Upon irradiation with $\lambda > 260$ nm, the CA and CAA groups underwent a [2 x 2] cycloaddition and formed additional crosslinks that were used to reinforce the temporary shape of the polymer. Irradiating the sample with $\lambda < 260$ nm, cleaved the newly formed cyclobutane rings and facilitated the recovery of the primary shape (**Fig. 4-1**). SMP systems based on photo-responsive chemical moieties are highly novel; however, they also suffer from poor shape retention and slow shape recovery.

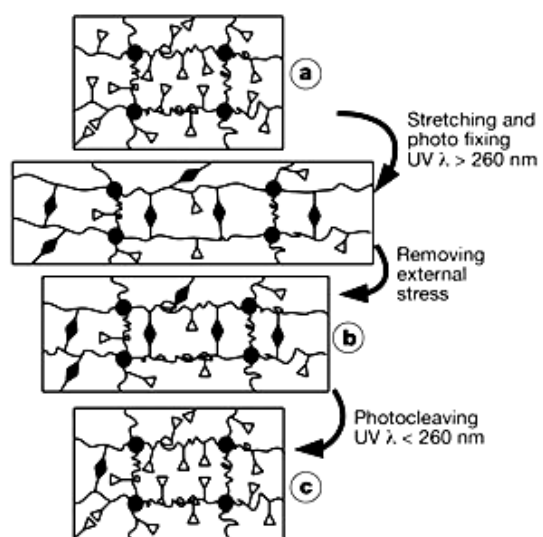
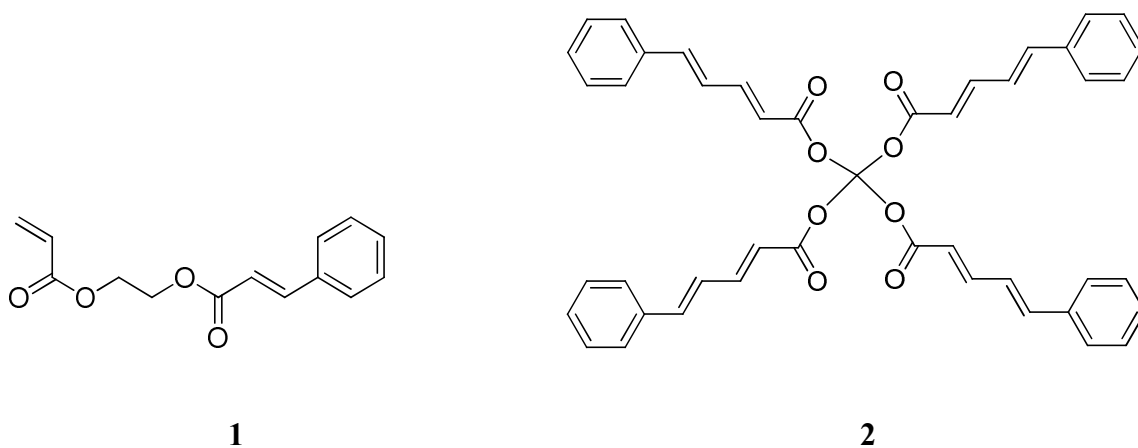


Fig. 4-1. Mechanism of UV-activated SMP systems containing CA or CAA.

SMP composites have also been investigated as a potential means of indirect activation. This approach utilizes the heat dissipated from the power loss of a magnetic filler to initiate the shape memory effect. Typical magnetic fillers include iron oxide particles, CNTs, and nickel-zinc ferrite particles.^[4-6] Recently, Lendlein and co-workers dispersed iron oxide nanoparticles (20-30 nm) into a silica matrix, which were subsequently embedded into poly(etherurethane) and polyester networks.^[7] The shape memory effect was triggered by inductive heating in an alternating magnetic field ($f = 258$ kHz, $H = 300$ kA·m⁻¹). Shape retention and recovery ranged from moderate to high at 68% and 91% recovery for the polyester and poly(etherurethane) networks, respectively at 10 wt% particle loading. Despite the improvement in shape memory properties over photo-activated systems, magnetically-driven SMP nanocomposites have been criticized for the high frequencies necessary to induce efficient shape recovery. Generally, frequencies between 50 and 100 kHz are considered safe for human tissue exposure.^[8]

A more promising triggering mechanism for the activation of biomedical shape memory devices is inductive heating using near-infrared (NIR) (700-1000 nm) absorbing dyes and nanoparticles. NIR absorbing fillers are highly attractive for safe and efficient transdermal SMP activation, as NIR absorbing chromophores are largely absent in most biological tissue.^[9-14] Small and co-workers used amorphous thermoplastic commercial PUs doped with indocyanine green and dithiolene platinum (Epolight 4121) dyes.^[15,16] The PU composites showed good shape memory properties at relatively low dye concentrations. However, the PU films possessed high T_{trans} values which are not suitable for biological applications. Recently, Burdick and co-workers developed a NIR activated SMP using poly(β -amino ester) and gold nanorod composites.^[17] Gold nanoparticles are ideal for

photothermal biomedical applications due to their biocompatibility, highly tunable optical properties, and unparalleled light-to-heat conversion efficiency.^[18] Moreover, the absorption cross-section at the resonance wavelength is several orders of magnitude larger than conventional photo-absorbing dyes and are known to be less susceptible to photo-bleaching.^[19] The surface plasmon resonance (SPR) wavelength of gold nanoparticles can be significantly red-shift from approximately 520 nm to 800 nm by increasing the particle aspect ratio. This exploratory work demonstrated that gold nanorods can be used to initiate the shape memory effect in amorphous poly(β -amino ester) networks.

We were interested in expanding upon these initial findings by establishing the potential of NIR absorbing gold nanoparticles in biodegradable semi-crystalline SMP materials. Melting temperature-mediated SMPs are known to possess more rapid and uniform shape memory transitions in comparison to amorphous materials and may provide improved properties for high performance applications such as drug delivery and dynamic cell culture.^[19,20] Few studies have investigated the structure-property relationships of light absorbing filler materials and semi-crystalline polymers.^[21] As such, several key underlying factors need to be addressed including, the effect of nanoparticle incorporation on the crystallization of PCL, the thermomechanical properties, and its broader implications for R_f , R_r , and recovery speed. Additionally, we sought to determine the appropriate combination of nanoparticle loading and laser power intensity that would produce sufficient heat to yield rapid shape recovery while mitigating temperatures that are potentially damaging to tissue. For this study PCL networks synthesized from branched PCL precursors were doped (≤ 1 wt%) with surface modified 150 nm gold nanoshells with a SPR of approximately 800 nm. The effect of nanoparticle loading on thermal properties were examined by DSC while

thermomechanical studies were studied by tensile analysis. Examples of NIR activated bulk and microscale shape memory are also described herein in addition to localized heat dissipation resulting from laser irradiation.

4.2. Experimental Section

4.2.1 Polymer synthesis and network fabrication. The synthesis of star-shaped PCL prepolymers was previously described in chapter two. Briefly, prepolymers were synthesized by bulk ROP of ϵ -caprolactone using tin octanoate and glycerol. The hydroxy-terminated oligo-PCL was refluxed with 2-isocyanatoethyl methacrylate and tin octanoate in anhydrous methylene chloride. PCL networks were prepared by adding a small aliquot of gold nanoshells dispersed in chloroform into the PCL trimethacrylate melt containing DEAP. The resulting mixture was cast into a Teflon mold and irradiated with 30 mW/cm² UV light (365 nm) under N₂ atmosphere for 10 min. Gold nanoshells were purchased from Nanobio Spectra and were imaged using a FEI Helios 600 nanolab dual beam system. Absorbance spectra were obtained on a UV-vis spectrometer in the spectral range of 250 to 1000 nm.

4.2.2 Thermal and Thermomechanical Characterization. Thermal characterization was performed on a TA instrument Q200 differential scanning calorimeter (DSC), under nitrogen atmosphere from -20 °C to 80 °C with heating and cooling rates of 5 °C/min and 10 °C/min, respectively. Mechanical properties were analyzed on an Instron tensile analyzer using a constant strain rate of 10 mm•min⁻¹ at 22 and 50 °C. The Young's modulus (E) was calculated using the initial linear portion of the stress/strain curve (0-5% strain).

Shape memory performance was analyzed by determining R_f and R_r . The equations for R_f and R_r are as follows:

$$R_f = \frac{\varepsilon_u}{\varepsilon_m} \cdot 100 \quad (1)$$

$$R_r = \frac{\varepsilon_u - \varepsilon_p}{\varepsilon_m - \varepsilon_p} \cdot 100 \quad (2)$$

R_f is defined as the fixed strain after unloading (ε_u) to the total strain induced during deformation (ε_m). R_r is defined as the ratio of the difference between the strain after unloading (ε_u) and the permanent strain after recovery (ε_p) to the difference between the total strain induced during deformation (ε_m) and the permanent strain after recovery (ε_p).

PCL molds (10 x 5 x 1 mm) were heated above the T_m and extended to a strain of approximately 100% using a custom bench clamp. The sample was then cooled to -78 °C using dry ice. Subsequently, the load was removed and the fixed strain was recorded to determine R_f . To measure shape recovery (R_r), a Coherent Chameleon Ultra II Ti:sapphire laser was used to irradiate samples at 800 nm for 35 s. The laser output beam was expanded to 15.69 mm in diameter and the power controlled between 1.558-0.582 W using a Pockels cell.

4.2.3 Heat Dissipation Characterization. The temperature change during irradiation was determined as a function of sample mass to water volume. Polymer samples (30-35.2 mg) were placed in direct contact with a thermocouple probe (Physitemp Thermalert TH-5) and

submerged in 2.0 mL of water, then irradiated for 120 s with temperature sampling every 10 seconds.

4.3 Results and Discussion

4.3.1 Gold Nanoshell/Poly(ϵ -caprolactone) Nanocomposite Fabrication

We previously reported the synthesis and characterization of PCL thermosets synthesized from photo-polymerizable star-shaped precursors in chapter two. The films demonstrated excellent mechanical properties, low cytotoxicity, and near quantitative macro- and microscale shape memory properties at physiological temperature. The PCL films were doped with 150 nm gold nanoshells with a SPR of approximately 800 nm (**Fig. 4-2**). The gold nanoshells are comprised of an amine functionalized silica nanoparticle core which acts as the site of reduction and subsequent nucleation and growth of gold from aqueous HAuCl_4 . The SPR wavelength can be readily tuned to the NIR by increasing the ratio of the core diameter to the shell thickness and is achieved most simply by controlling the HAuCl_4 solution feed ratio.

To make full use of the photothermal properties, the nanoshell particles must be well-dispersed within the PCL network. Nanoparticles are commonly surface modified to improve the compatibility of the nanoparticles with the polymer matrix.^[23,24] To enhance the hydrophobicity of the gold nanoshells, the nanoparticles were surface functionalized with a monolayer of 1-undecanol by immersing the nanoparticles in a 2 mM ethanol solution of 11-mercapto-1-undecanol for 12 h (**Fig. 4-3**). Prior to surface functionalization the nanoshells aggregated in organic media.

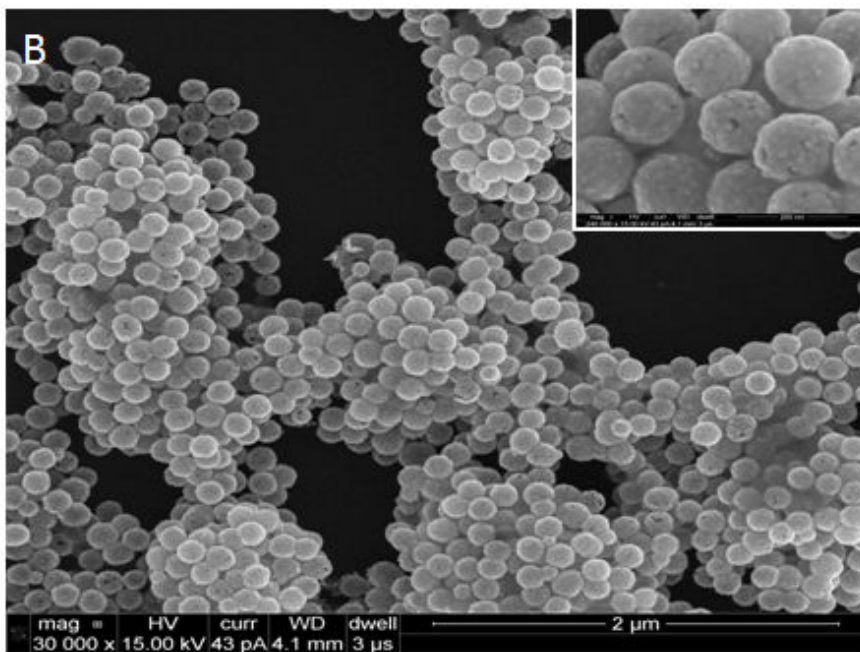
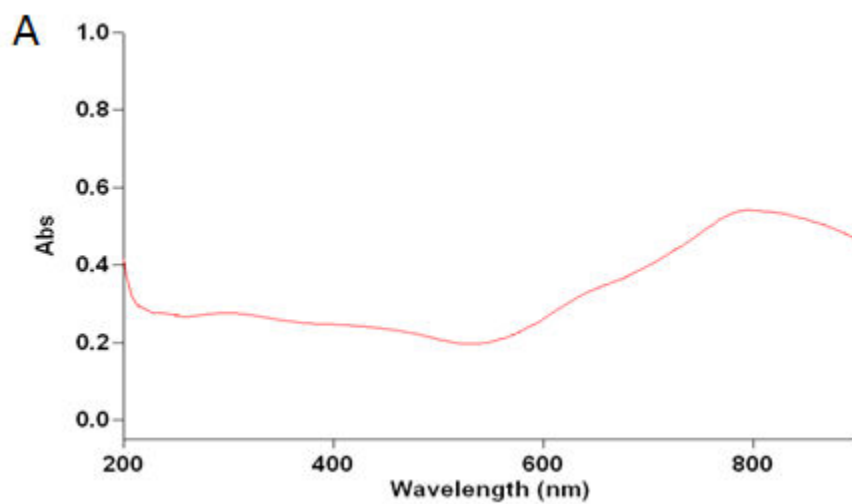


Fig. 4-2. A) Absorbance spectrum of 150 nm gold nanoshells from 200 to 900 nm. B) SEM image take at 30,000 magnification (Inset 240,000 x), scale bar is 2 μ m.

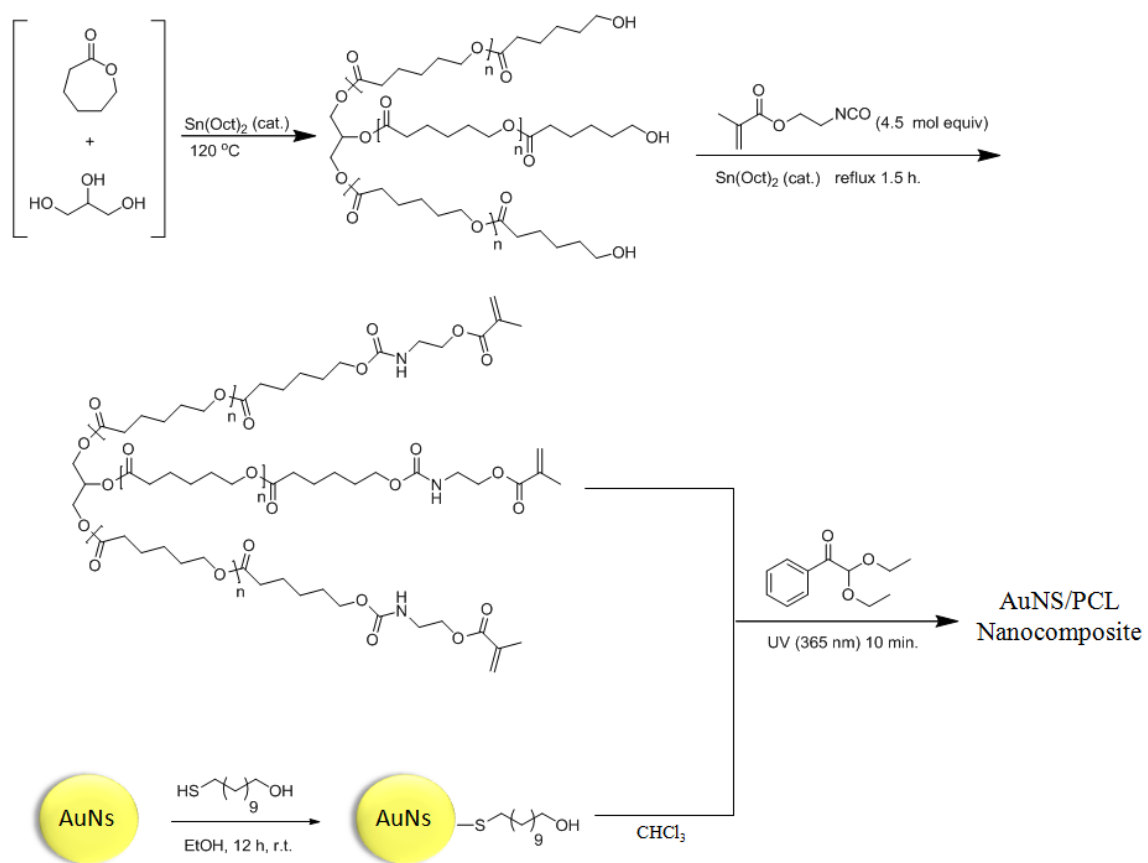


Fig. 4-3. The fabrication of gold nanoshell/PCL SMP nanocomposites.

After surface modification using the hydroxy-alkane thiol, the nanoparticles freely re-dispersed in chloroform after moderate sonication. A 100 μL aliquot of nanoshells dispersed in chloroform was added to the PCL precursor melt containing the photo-initiator DEAP.

The mixture was cast into a Teflon dogbone mold and irradiated at 365 nm for 10 min under N_2 atmosphere.

4.3.2 Thermal Characterization

To determine the effect of gold nanoshell weight fraction on T_m , PCL networks ($\langle M_n \rangle = 13,200 \text{ g mol}^{-1}$) were doped with gold nanoshells (0, 0.1, 0.5, 1.0 wt%) and analyzed by DSC in the temperature range of -60 to 80 $^{\circ}\text{C}$. Incorporation of nanoshells into the PCL matrix suppressed the formation of crystalline domains and resulted in a modest reduction in the T_m and χ_c with incremental nanoshell loading. The effect of nanoshell loading on T_m is shown in **Fig. 4-4**. All of the networks demonstrated exceptionally sharp thermal transitions at biologically relevant temperatures. The highest nanoshell loading (1.0 wt%) resulted in a T_m of 42 $^{\circ}\text{C}$, which is consistent with clinical hyperthermic treatments. Manipulation of the T_m was essentially a balance between prepolymer $\langle M_n \rangle$ and nanoshell loading. A larger prepolymer chain length was chosen to compensate for the loss in crystallinity and the associated reduction in T_m incurred by nanoparticle incorporation (**Table 1**). In all cases, the films exhibited sharp thermal transitions of approximately 8 $^{\circ}\text{C}$, which were measured from the onset of melting to the peak of the melting exotherm. Buckley and co-workers demonstrated that the width of the phase transition is tightly correlated with shape memory recovery speed.^[25,26]

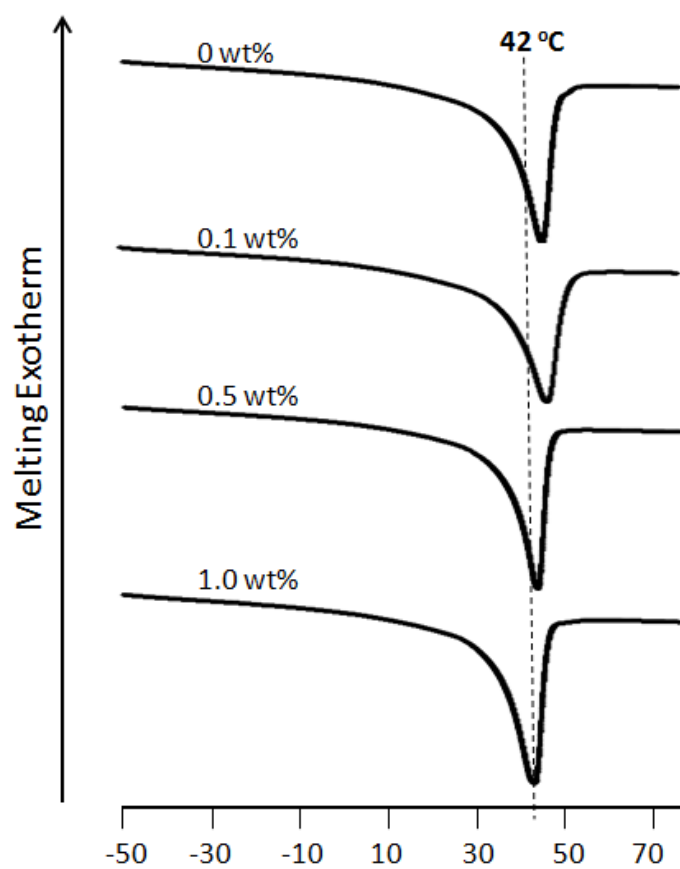


Fig. 4-4. Effect of nanoshell loading on the T_m of the gold nanoshell/PCL films.

By comparison, glassy polymers generally possess thermal transitions many times wider than semi-crystalline materials and therefore may be unsuitable for applications which require higher degrees of recovery speed and uniformity.

4.3.3 Mechanical Characterization

Having shown the effect of nanoparticle weight fraction on T_m , the mechanical properties of dogbone films were then studied at 22 and 50 °C by tensile analysis at a constant strain rate of 10 mm•min⁻¹. The mechanical properties of the neat and nanocomposite films at 25 and 50 °C are summarized in **Table 1**. At 25 °C, Young's modulus (E) decreased steadily with increasing nanoshell loading. Conversely, elongation at break (ϵ_R) increased with the addition of 0.1 wt% nanoshells and did not demonstrate any continued effect for larger nanoshell weight fractions. This loss in modulus and concurrent increase in extensibility is clearly due to the inhibitory effect of the gold nanoparticles on the formation of PCL crystallites which act as physical crosslinks and impart strength to the network. This phenomenon is supported by the reduction in T_m and χ_c of nanoshell loaded composite materials in comparison to pure PCL.

At 50 °C, all of the films are well above T_m and have been rendered completely amorphous. PCL films loaded with 0.1 wt% gold nanoshells exhibited a large reduction in E when compared to neat PCL samples. Additional gold nanoparticle loading to 0.5 wt% resulted in reduction in E and an increase in ϵ_R . At 1 wt% nanoshell loading, the mechanical properties reached a plateau and essentially did not change. The addition of rigid nanoparticles into SMP matrices is commonly used to improve recovery stress by increasing the rubbery modulus.^[28,29]

Table 4-1. Thermal and mechanical characterization of Gold/PCL nanocomposites

Sample	wt% AuNP	T_m (°C)	χ_c (%) ^a	25 °C		50 °C	
				E (MPa)	ϵ_r (%)	E (MPa)	ϵ_r (%)
1	0.0	45	26	232	42	14	120
2	0.1	46	25	185	97	8	107
3	0.5	44	24	180	97	6	147
4	1.0	42	24	98	167	4	170

[a] χ_c : degree of crystallinity. Ratio of the heat of fusion (ΔH_m) to a perfect PCL crystallite (135 Jg^{-1}).

However, here E decreased in the rubbery plateau as a function of gold nanoshell weight fraction. These findings are similar to previous reports in the literature for surface modified nanoparticle/polymer nanocomposites at low weight fractions.^[30,31] Crosby and co-workers attribute these effects to two possible mechanisms.^[32] The first is associated with the free volume created between network chains by nanoparticle surface ligands, resulting in a decrease in T_g and E . The second is related to the conformation of polymer chains neighboring surface modified nanoparticles which have weak or neutral enthalpic interactions with the matrix. In addition to improved polymer-nanoparticle compatibility, surface functionalization can also reduce chain entanglement and minimize enthalpic interactions with the matrix, leaving entropic effects as the significant factor. Polymer chains near the surface of nanoparticles experience a decrease in conformational entropy. Consequently, a polymer depletion zone develops around the nanoparticle due to an entropically-driven repulsion of chains from the nanoparticle surface. The low chain density around the particle results in an increase in chain mobility and an associated reduction in T_g and E . It is possible that these effects are responsible for the loss in mechanical strength observed with the addition of low weight fractions of gold nanoshells in the PCL networks.

We have shown that incorporation of nanoshells into the PCL matrix generally results in a decrease in E and increase in ϵ_R at temperatures both above and below T_m . However, at low weight fractions the influence of the nanoparticles on the mechanical properties in the semi-crystalline and rubbery regions is marginal and should not adversely affect R_f and R_r , as will be demonstrated in the follow section.

4.3.4 Shape Memory Characterization

Shape memory performance was evaluated by determining R_f and R_r . PCL films (10 x 3 x 1 mm) were heated to well above the T_m and stretched to a maximum strain of approximately 100%. Subsequently, the samples were cooled to -78 °C while still under mechanical load to induce crystallization. At 100% extension, pure PCL exhibited near quantitative R_f values which were typically greater than 99%. In all cases, the addition of gold nanoshells resulted in a slight reduction in R_f ($\geq 95\%$) due to a loss in crystallinity. The films were then irradiated with 800 nm light at varying flux values between 0.200-0.806 W/cm² power density and a constant beam diameter of 15.69 mm for a total of 35 s irradiation time. The beam was sufficiently expanded to fully irradiate the film with uniform light intensity. Pure PCL films showed negligible recovery over the range of beam intensities with only 1.4% recovery at the highest beam intensity. R_r for 0.1 wt% nanocomposites were only marginally better with 5% recovery at the equivalent power density and consequently were not tested at lower spectral densities. A power density of 0.3 W/cm² resulted in 2% R_r for 0.5 wt% loaded films. Increasing power density resulted in a sharp nonlinear rise in R_r and a final R_r of 92.7% at a power density of 0.806 W/cm². Similarly, at 0.2 W/cm², 1.0 wt% loaded films exhibited an R_r value of 4.9%, which quickly rose with increasing laser power to 97.9% at 0.8 W/cm². The relationship between power density, nanoshell content, and R_r are shown in **Fig. 4-5**.

To demonstrate the macroscale shape memory capabilities of the nanocomposites, 1 wt% PCL nanocomposite dogbones were packaged into a rolled secondary shape and subsequently irradiated with NIR light for 35 s to induce recovery (**Fig. 4-6**). The films showed excellent secondary shape retention and complete recovery upon irradiation.

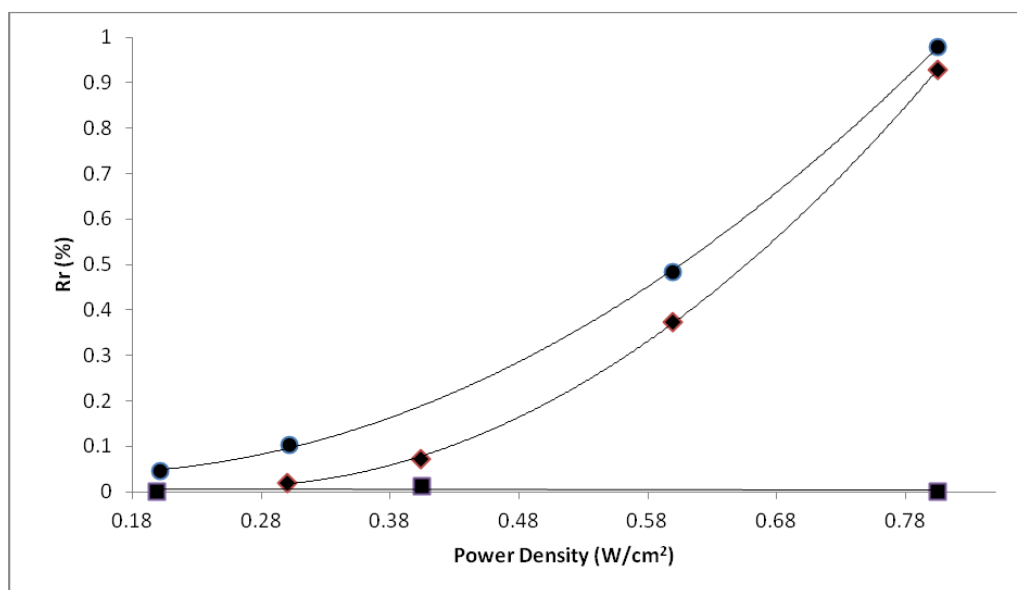


Fig. 4-5. R_r measurements as a function of increasing power density for 0 wt% (■), 0.5 wt% (◆), and 1 wt% (●) gold nanoshell/PCL composites.

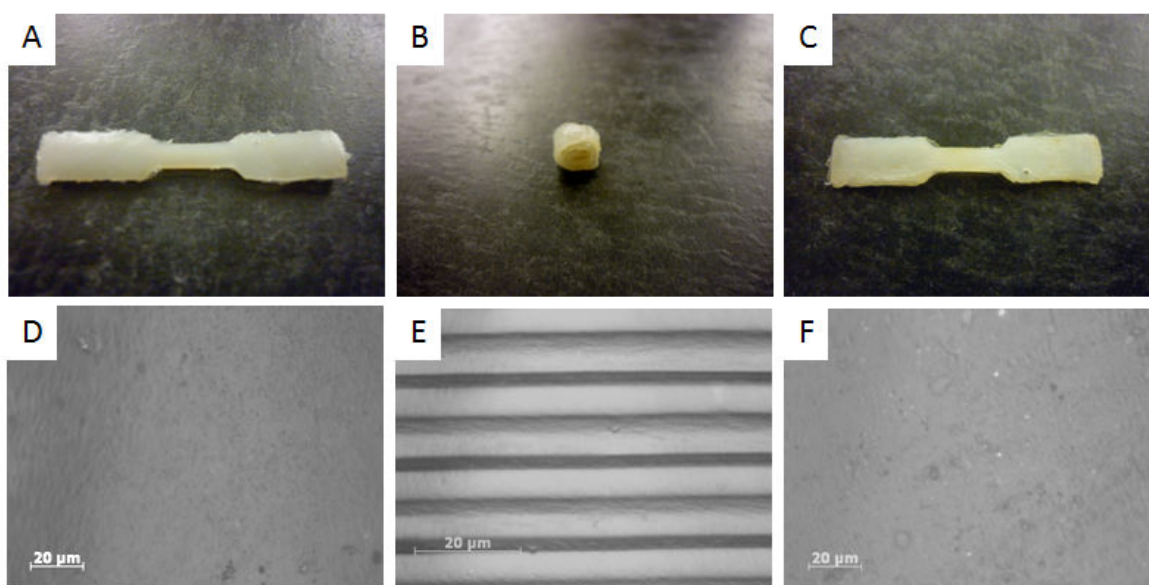


Fig. 4-6. Images of a 1wt% gold nanoshell/PCL nanocomposite in a A) primary dogbone mold shape and D) a permanent flat topography, B) a temporary rolled up shape, and E) a secondary 3 x 5 μm channel topography, and C) the recovered dogbone and F) planar surface morphologies after irradiation with 800 nm ($0.8 \text{ W}/\text{cm}^2$) for 35 s.

Shape memory microarrays were also fabricated from 1 wt% films using the double replica imprint lithography techniques previously described in chapter two. Films with a planar surface morphology were programmed with a 3 x 5 μm channel temporary shape. After irradiating the film with NIR light for 35 s the planar surface morphology was recovered completely.

These findings demonstrate that the gold nanoshell/PCL composites exhibit exceptionally sharp shape memory transitions in response to very low NIR incident power densities. Larger nanoparticle weight fractions modestly diminished R_f values and significantly increase R_r , particularly at higher power densities. Moreover, the reduction in rubbery modulus observed in tensile analysis did not have an effect on recovery speed as films with higher particle content generally recovered faster.

4.3.5 Heat Dissipation Characterization

To characterize the heat dissipation of the nanocomposites to the surrounding environment, local temperature changes were recorded using a thermocouple placed in direct contact with PCL films immersed in 2 mL of DI water. Films were irradiated with 800 nm light (0.8 W/cm^2) for a total of 180 s with temperature readings taken at 10 s intervals. All of the substrates generally showed a sharp increase in temperature during the first 40 s of irradiation before reaching a plateau. Pure PCL controls exhibited a 6.9°C increase in local temperature resulting in a final temperature of 28.6°C . Substrates with 0.5 and 1.0% nanoshell loading exhibited a 12.9 and 16.9°C increases in local temperature and a final temperature of 34.6 and 38.5°C , respectively.

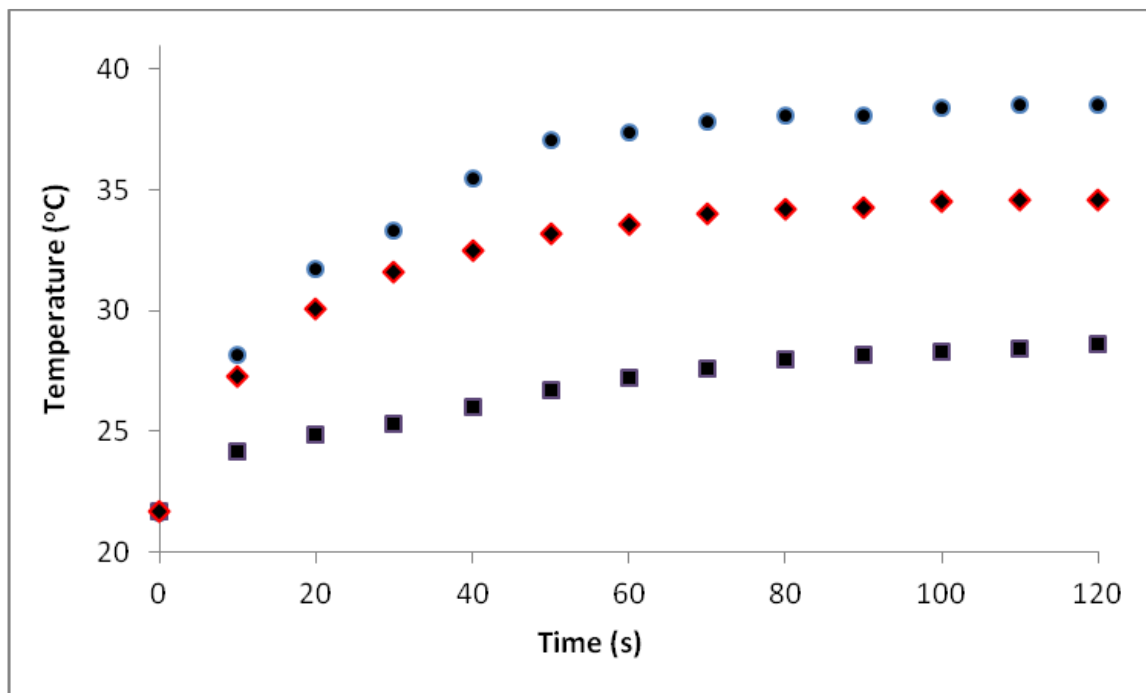


Fig. 4-7. Heat dissipation as a function of time for gold nanoshell/PCL nanocomposites irradiated at 800 nm (0.8 W/cm²) at 0 wt% (■), 0.5 wt% (◆), and 1 wt% (●) nanoshell loading.

There is clearly a large temperature differential between unloaded and loaded samples pointing to the high light to heat conversion efficiency of the nanoshells at low concentration. It is important to point out that the overall solution temperature remained below the T_m of the loaded polymers despite sufficient irradiation times to induce a shape change. The low heat conductivity of the polymer film therefore results in minimal dissipation to the surroundings, with the temperature plateau representing the equilibrium reached between internal film temperature and heat dissipation to the surroundings.

These results show that the heat dissipation of the gold nanoshells is sufficient to produce rapid and complete shape memory transformations at physiological temperature. Moreover, that the heat dissipated from the films during irradiation is biologically benign and remains below harmful temperatures for times well beyond the timeframe needed to complete the shape memory transition.

4.4 Conclusions

Herein, the fabrication and characterization of NIR activated gold nanoshell/PCL nanocomposites has been described. Incorporation of gold nanoshells into the PCL matrix marginally reduced the χ_c of the films and resulted in a decrease in T_m to within several degrees of body temperature. A loss in mechanical strength was also observed in the semi-crystalline regime with increasing nanoparticle weight fraction. At high temperature the rubbery modulus decreased as a function of nanoparticle loading. R_f values were very high and decreased slightly with nanoshell loading. Shape recovery was essentially only induced by NIR irradiation in nanoshell loaded films. Rapid and uniform shape recovery was observed at both macro-and microscale in response to low laser power densities at nanoshell

concentrations of 0.5 and 1.0 wt%. Heat dissipation resulting from NIR-induced shape recovery was shown to rise quickly with irradiation and taper off to a maximum near physiological temperature. We believe this new NIR activated SMP platform based on semi-crystalline PCL materials is of great interest for rapid and uniform photothermal shape memory activation and may be a promising alternative for transdermal biomedical applications.

4.6 References

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Chapter 5

Future Research Directions

5.1 Implications of Shape Memory Polymers in Cell Biology

Surface topography has been shown to be a powerful regulator of critical cellular processes and an important tool for investigating the fundamental mechanisms involved in cell-surface interactions. The approach described in chapter three departs from the conventional use of static surfaces by providing a practical means of applying active topographical stimuli to cultured cells. Beyond the use of these surfaces to examine focal adhesion and cytoskeletal re-organization, dynamic topographies also have the potential to address many other underlying biological questions, specifically in regards to mechanotransduction and disease progression.

5.1.1 Cytoskeletal Tension

As previously discussed, topographically directed cell adhesion can ultimately lead to changes in gene and protein expression. Moreover, surface feature regulation of Rho-associated kinase (ROCK) has strongly implicated cellular tension as a key intermediary of topographically induced mechanotransduction. Yet, quantification of the internal force generated by cells in response to cell attachment on micro-and nanoarrays has never been demonstrated in a dynamic fashion. A method designed to measure these real-time sub-cellular changes in tension would be very useful in developing a quantitative picture of the

relationship between specific surface morphology transformations and their associated effects on cell behavior.

Recently, Schwartz and co-workers reported a fluorescence resonance energy transfer (FRET)-based vinculin tension biosensor with single piconewton (pN) sensitivity.^[1] Vinculin is an intracellular protein comprised of a head domain (Vh) and a tail domain (Vt) which are separated by a flexible protein linker. Upon integrin-mediated attachment to the surface, Vh is actively recruited in a force dependent manner to the focal adhesion complex, while Vt binds to F-actin; essentially creating a direct link between the ECM and the internal cytoskeleton. Studies have demonstrated that the recruitment of vinculin to focal adhesions is critical to internal force generation and focal adhesion maturation as vinculin deficient cells typically display impaired spreading, cell migration, and exert reduced traction forces. A tension sensor module (TSMoD) was constructed by inserting an elastic repetitive amino acid domain between two fluorophores that undergo FRET (mTFP1-Donor and venus(A206K)-Acceptor) (**Fig. 5-1**). When the donor and acceptor moieties are separated by space, the donor emission is exclusively detected upon excitation. However, at distances of 1-10 nm, the interaction between the donor and acceptor results predominantly in the detection of the acceptor emission. The vinculin biosensor was completed by inserting the FRET module between Vh and Vt. Since FRET efficiency is highly sensitive to the distance between the fluorophores, the acceptor emission should decrease with increasing cellular tension. Modification of vinculin did not interfere with its recruitment to focal adhesions in vinculin tension sensor transfected cells. The tension across vinculin in a stable focal adhesion was determined to be approximately 2.5 pN.

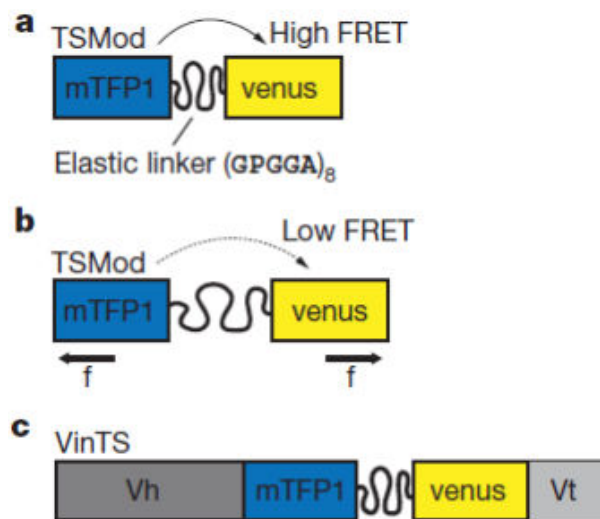


Fig. 5-1. Depiction of a tension sensor module (TSMOD) consisting of two fluorophores separated by an elastic protein linker where A) under low tension FRET efficiency increases and B) under high tension FRET efficiency decreases. C) TSMOD is then inserted between the head (Vh) and tail (Vt) domain of vinculin.

Cells transfected with the vinculin tension sensor could be used to monitor the real-time changes in cell tension in response to specific topographic shifts using time lapse confocal microscopy. In this way, both the distribution and magnitude of the internal tension could be related to surface morphology transformations between topographies of various sizes, shapes, and orientations. Similarly, these surfaces could be used to probe the effect of dynamic surfaces on other biological phenomena by fluorescence labeling. Two such ideas have been proposed: the shuttling of transcription factors from the cytosol to the nucleus and centromere re-positioning in response to topography induced changes in cell shape.

5.1.2 Cancer Biology

Another area of fundamental cell biology that could potentially benefit from these materials is cancer biology. Current oncogenesis models are largely based upon our understanding of genetic aberrations. However, there are many emerging studies pointing to an alternative epigenetic mechanism that links tumor development to abnormalities in the physical properties of the extracellular matrix (ECM).^[2] Recent experiments demonstrating the reversion of malignant tumors to more benign phenotypes or in some cases normal tissue when exposed to normal cell microenvironments have underlined the importance of the ECM in oncogenesis.^[3-6] These findings raise interesting questions about the reversibility of cancer and the possibility of new treatment modalities based on manipulating the tumor extracellular environment.

During tumor formation, localized ECM remodeling changes the mechanical properties of the cell microenvironment.^[7] Cells that are attached to these defects in the ECM are distorted and experience enhanced cellular tension making them more susceptible to

growth stimuli (**Fig. 5-2**). Enhanced cell proliferation is tightly coupled with ECM remodeling and expansion at the site of the lesion. However, if ECM remodeling in adult cells is not accompanied by basement membrane folding and extension, cells will stack and form dense cell masses. When the growth stimulus ceases, cells that are not attached to the substratum will induce apoptosis. However, if the growth stimulus is sustained, cells at the site of the defect can mutate into anchorage-independent cells and possibly lead to a palpable tumor mass. Large structural modifications in the form of gaps and thickening of the basement membrane are commonly observed in early stages of cancer formation.

Active surfaces could be engineered to transition from flat topographies to lesions of different dimensions and likewise could also be programmed to transition from a lesion back to a flat surface. Cultured cells would be subjected to the surface shape memory effect over an extended period of time and monitored for changes in cancer markers. The timescale of these experiments could be controlled by the heating rate and alternatively can be extended at a single temperature by using amorphous materials. Thus, these materials may offer a dynamic tissue engineering approach to studying cancer development.

5.2 Shape Memory Particles

A novel extension of shape memory surfaces is the development of shape memory particles. Particle shape is an important design parameter in nanoparticle mediated drug delivery. However, its precise role is not well understood. Non-spherical particles may demonstrate very unique degradation profiles as their particle shape will change over time. Particle flow and transport in the body will also be significantly different than their spherical counterparts and will be particularly important in filtering organs such as the liver or spleen.

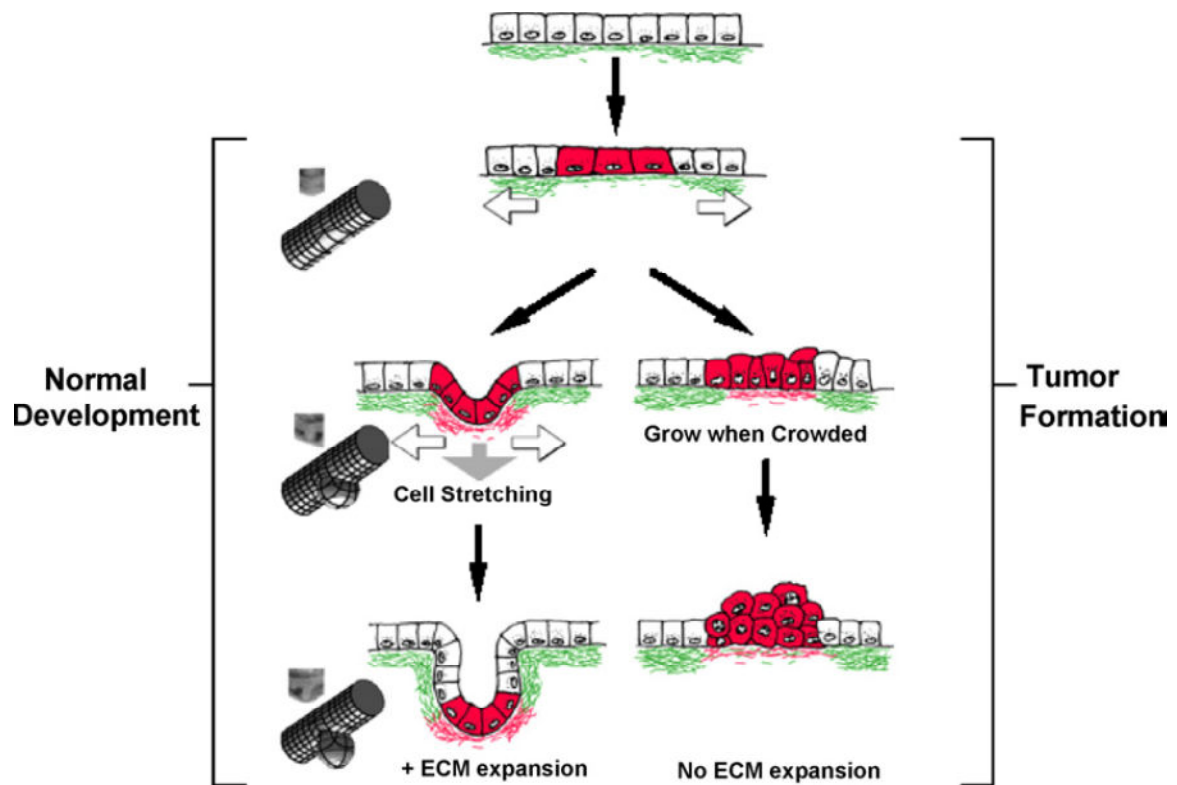


Fig. 5-2. (Left) During normal development ECM turnover results in local defects in the basement membrane (green). Adherent cells will distort their morphology due to the abnormal topography of the lesion, making them more susceptible to growth stimuli. Cell division is closely coupled with the deposition of new basement membrane (red) and ECM expansion leading to the formation of budding regions shown in micrographs of epithelial tissue on the far left. (Right) During tumor development ECM expansion is outpaced by cell proliferation resulting in a mass of unorganized tissue mass. It is thought that over time these cells can mutate into anchorage-independent cells and ultimately develop into a malignant carcinoma.

Ligand and protein adsorption and local presentation will also be highly influenced by the curvature of the particle. Moreover, there many intriguing questions surround the effects of particle shape on cell internalization and trafficking. If the particle is taken up, it is unclear how the intracellular vesicles will respond to non-spherical shapes and may have major effects on intracellular transport. Particle shape may also be critical in understanding phagocytosis and the body's histological response to abnormally shaped foreign materials. Shape memory particles are an extremely appealing way to probe many of the questions mentioned above by actively switching between various shapes and observing the real-time response of cells.

Particle fabrication is actively being pursued in our group using the top-down PRINTTM method founded by DeSimone and co-workers (**Fig. 5-3**).^[9] Initial success has also been demonstrated in particle deformation using methods similar to that pioneered by Mitragotri.^[10] Particles are embedded in a film of elastic polymer, which is then stretched and cooled. Subsequently, the deformed elastic film is solubilized, releasing the particles in the deformed state. Initial studies would be aimed at observing the effect of fluorescently labeled SMP particles on cell uptake and intracellular trafficking. Time-lapse fluorescent microscopy could be employed to observe how cell response coincides with changes in particle shape. Degradation profiles of SMP particles will also be of great interest as particles of different shapes will possess distinct degradation rates despite having the same chemical composition. Furthermore, the phase transition during shape switching is accompanied by a large reduction in Young's modulus and network porosity. Exploiting this change in physical properties may be favorable for controlled hyperthermic drug release.

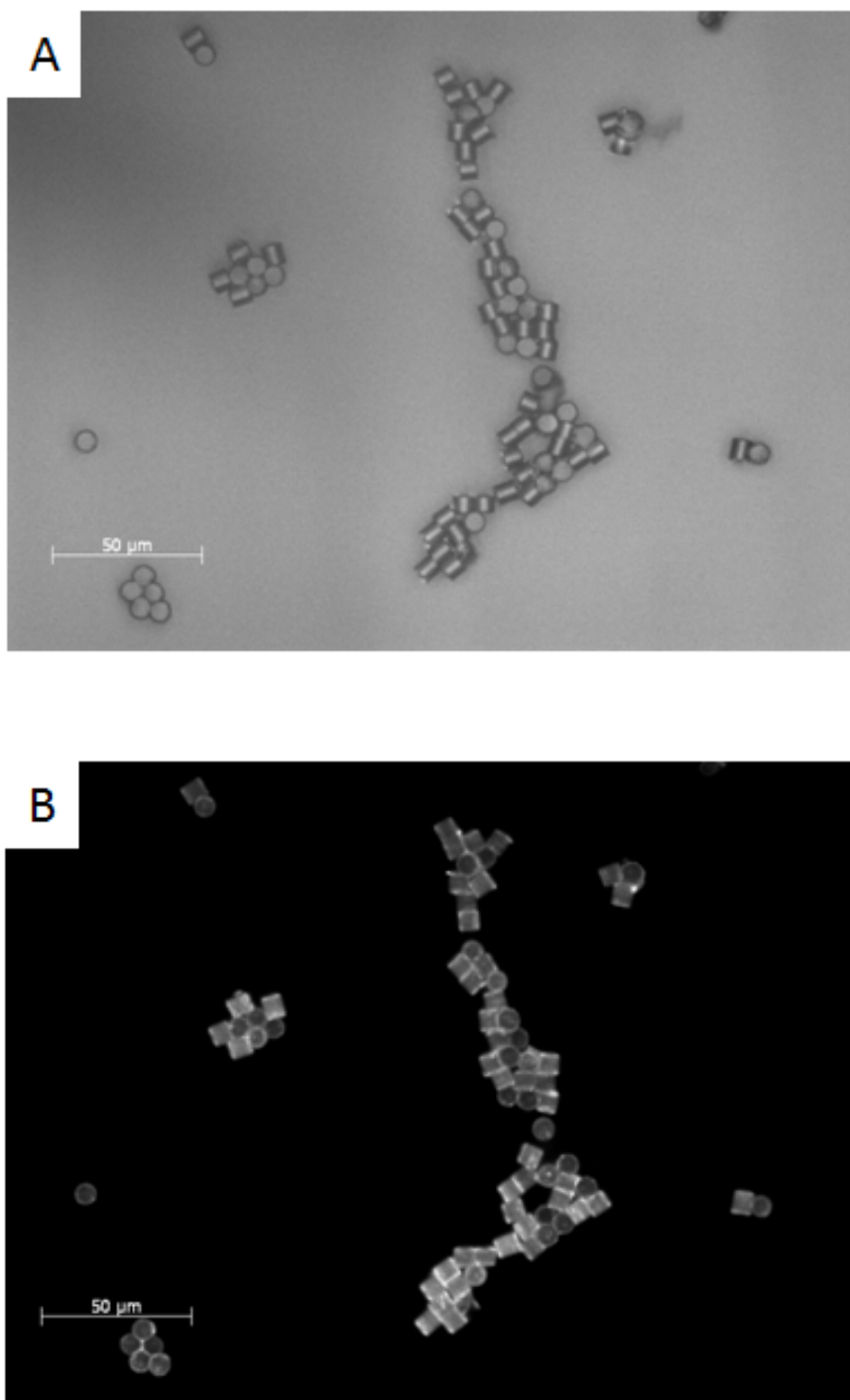


Figure 5-3. A) Brightfield and B) fluorescence images of 7x7 μm cylinder PCL particles synthesized from SPCL-13k. Scale bar is 50 μm .

5.3 Conclusions

Shape memory polymers have proven to be a unique enabling technology for many biomedical applications. Opportunities for continued growth lie in the development and optimization of the newest trends in shape memory including multi-shape memory, functionalized materials, unique triggering mechanisms, and drug delivery. Moreover, coupling shape memory polymers with fabrication techniques such as lithography or microfluidics may provide a novel means to new materials. New research should be geared towards the synthesis of novel materials and their creative implementation in biology and medicine.

Appendix A

Synthesis of Poly(dioxanone)-Poly(caprolactone) Copolymers

Introduction

One disadvantage of SMP systems based on linear PCL precursors seems to be the critical limiting $\langle M_n \rangle$ at which the network loses crystallinity. We have shown that decreasing $\langle M_n \rangle$ of the prepolymer also lowers χ_c and T_m . As the crystallinity diminishes, the ability of the film to support the stresses imparted by the embossed secondary shape also decreases and consequently the capacity for shape fixation greatly suffers. At $\langle M_n \rangle$ of 2,500 g mol^{-1} the resulting PCL films do not have enough crystallinity to support the secondary shapes. PCL elastomers prepared from high $\langle M_n \rangle$ prepolymers can retain secondary shapes and undergo shape recovery, but at a much higher T_m . However, this renders them unsuitable for cell culture applications which require a T_{trans} near 40 °C. To resolve this dilemma we synthesized branched 3-arm prepolymers which are described in chapter three. An alternative approach that was attempted using copolymers comprised of two crystalline segments, poly(*p*-dioxanone) (PDS) and PCL. PDS is a widely known biomaterial that is typically used in suture materials. The *p*-dioxanone monomer, which is not commercially available, can be prepared by terminal substitution of 2-(2-methoxyethoxy)acetic acid using hydrobromic acid at elevated temperatures, and subsequent ring closure through the addition of sodium bicarbonate. The *p*-dioxanone monomer can then be ring opened in the same fashion as PCL. Random copolymers were made by methacrylate end functionalization and subsequently photochemical copolymerization with PCLDM. It has been shown in literature that the presence of PDS strongly depresses the melting point of PCL. This phenomenon is related to their phase separation behavior and the nucleating effect of PDS on PCL.

Experimental Section

Materials. 2-(2-Methoxyethoxy)acetic acid (98 %), 1,2-dichloroethane, hydrobromic acid (50 %), tin octanoate, and ethylene glycol were purchased from Sigma Aldrich. Methylene chloride and methanol were purchased from Fisher Scientific. Acetic acid, methylene chloride, methanol, and acetone were purchased from Fisher Scientific. ϵ -Caprolactone was dried over CaH_2 for 24 h and distilled prior to use.

Synthesis of the p-Dioxanone Monomer. In a round bottom flask equipped with an addition funnel and reflux cooler hydrobromic acid and 2-(2-methoxyethoxy)acetic acid were added in a 3:2 ratio at 0 °C. Subsequently, the mixture was stirred at room temperature for 3h and then heated stepwise to 150 °C over a 2 h window and refluxed at this temperature for an additional 3 h. The reaction mixture was then cooled to room temperature and NaHCO_3 was added until the pH was adjusted to approximately three. The product was isolated by vacuum distillation at bp 76-81 °C at 0.1 mbar.

Synthesis of Hydroxy-Terminated Poly(dioxanone). PDS prepolymers were synthesized by bulk ROP of *p*-dioxanone using tin octanoate and ethylene glycol. Under nitrogen atmosphere, ethylene glycol, tin octanoate, and *p*-dioxanone were added to the reaction vessel and heated to 120 °C for 12 h, whereupon the reaction was quenched with acetic acid, and the product was precipitated in cold (-78 °C) methanol, isolated by vacuum filtration, and dried in a vacuum desiccator at 0.1 mmHg overnight.

Preparation of methacrylate end-functionalized polyesters. Polyol precursor was refluxed with 4.5 mol equivalents of 2-isocyanatoethyl methacrylate and 0.1 mol % tin octanoate in anhydrous methylene chloride for 2.5 h. The product was precipitated in cold (-78 °C) methanol, isolated by vacuum filtration, and dried in a vacuum desiccator at 0.1 mmHg overnight.

Preparation of chemically crosslinked polyester copolymer networks. PDS/PCL networks were prepared by casting molten methacrylate end-capped PDS and PCL precursors and photoinitiator diethoxyacetophenone (DEAP) (0.1 wt %) into a teflon mold. Subsequently, the prepolymer melt was irradiated with 30 mW/cm² UV light (365 nm) under N₂ atmosphere for 10 min.

Thermal and Thermomechanical Characterization. Thermal characterization was performed on a TA instrument Q200 differential scanning calorimeter (DSC), under nitrogen atmosphere from -20 °C to 80 °C with heating and cooling rates of 5 °C/min and 10 °C/min, respectively.

Results and Discussion

Poly(Dioxanone) and PDS/PCL Copolymer Synthesis

p-dioxanone monomer synthesis was carried out by nucleophilic substitution of the terminal methoxy group of 2-(2-methoxyethoxy)acetic acid using hydrobromic acid as the nucleophile resulting in the formation of a terminal hydroxy group. After the ether cleavage, NaHCO₃ was used to catalyze ring closure through esterification which furnished the *p*-

dioxanone monomer in approximately 55 % yield after purification. The synthetic route is summarized in **Fig. A1**. Formation of the monomer was confirmed by ^1H NMR spectrum (**Fig. A2**).

PDS diols were synthesized by ethylene glycol initiated ROP in the presence of catalytic quantities of tin octanoate (**Fig. A3**). The presence of hydroxy-end groups were confirmed by ^1H NMR signal $\delta = 3.66$ ppm (**Fig. A4**). ^1H NMR end group analysis showed the resulting diols were of relatively low $\langle M_n \rangle$ ranging between approximately 800 to 2000 g mol^{-1} . Polymerizations that proceeded for 24 and 48 h resulted in similar $\langle M_n \rangle$ values. The use of TBD as the catalyst in solution ROP typically yielded oligomers or diols of very low $\langle M_n \rangle$. Following purification and drying the diols were methacrylate end-functionalized using 2-isocynoatoethyl methacrylate. Methacrylation was indicated by the vinyl ^1H NMR peaks at $\delta = 5.62$ and 6.14 ppm, in addition to $\delta = 1.9$ and 3.51 ppm corresponding to the methyl protons of the methacrylate group (**Fig. A4**). Moreover, the hydroxy proton peak at 3.7 ppm disappears following endcapping.

Finally, the melt of the methacrylate end-functionalized PDS and SPCL-13k precursors were cast in the melt and mixed with varying weight percentages of SPCL13k_TMA (0, 25, 50, 75 wt %) and photopolymerized. The resulting films ultimately did not demonstrate crystallinity. Suppression of crystallite formation was due to the short chain length of the PDS precursors which dramatically increased the crosslink density. While PDS has been demonstrated as a nucleating agent for PCL, the nature of the film was completely dominated by the film microstructure. In the future PDS precursors of longer chain length should be investigated.

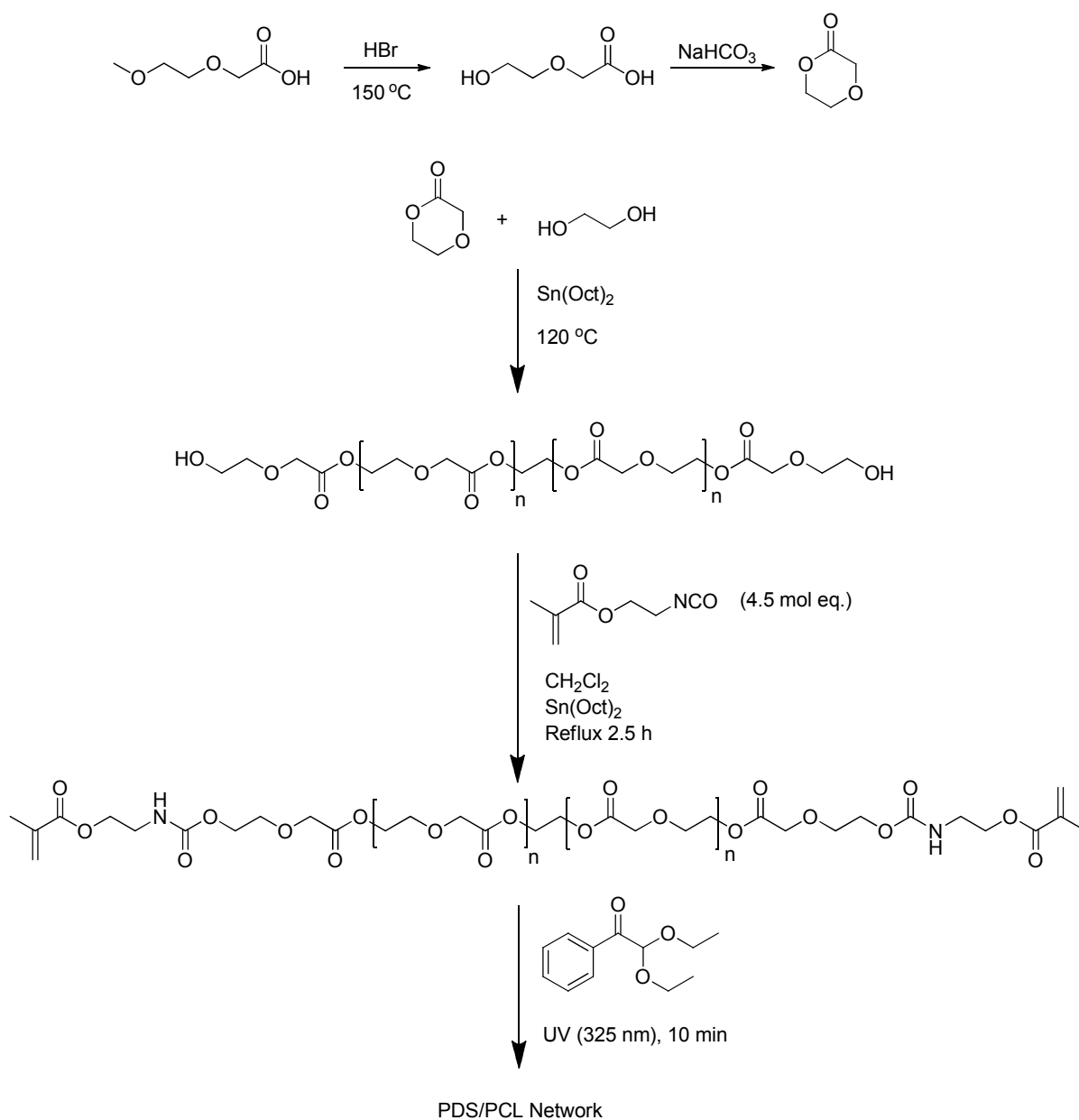


Fig. A1. Synthetic route to PDS/PCL copolymer films.

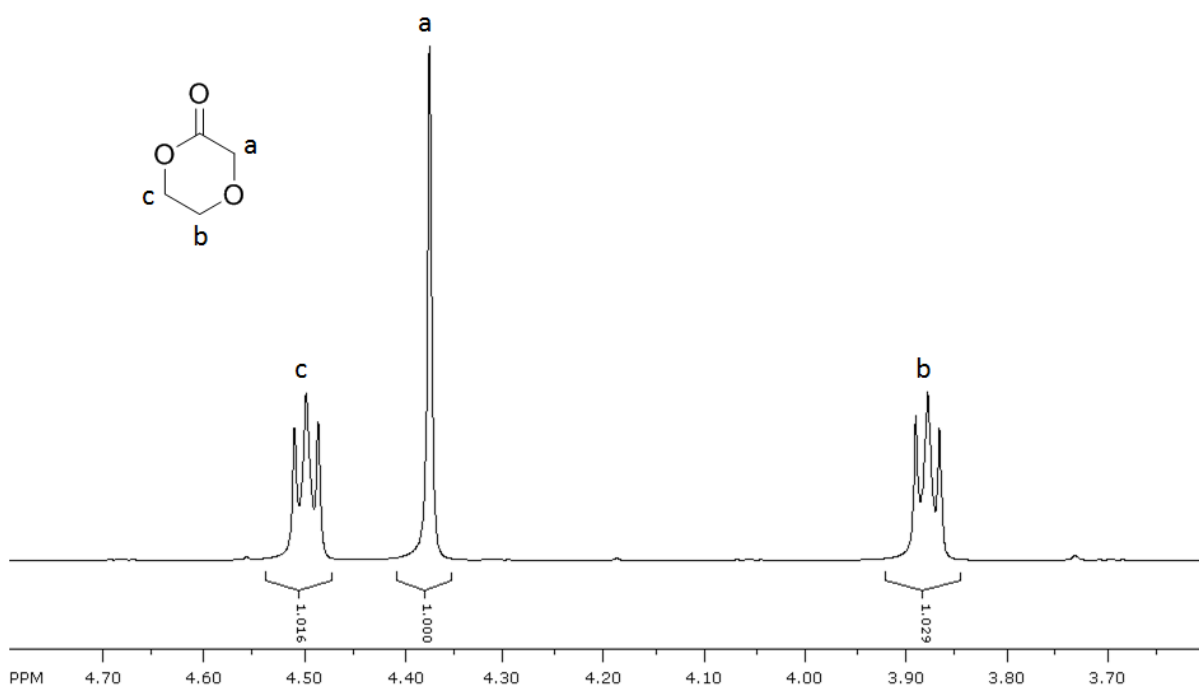


Fig. A2. ^1H NMR (CDCl_3) of purified *p*-dioxanone monomer.

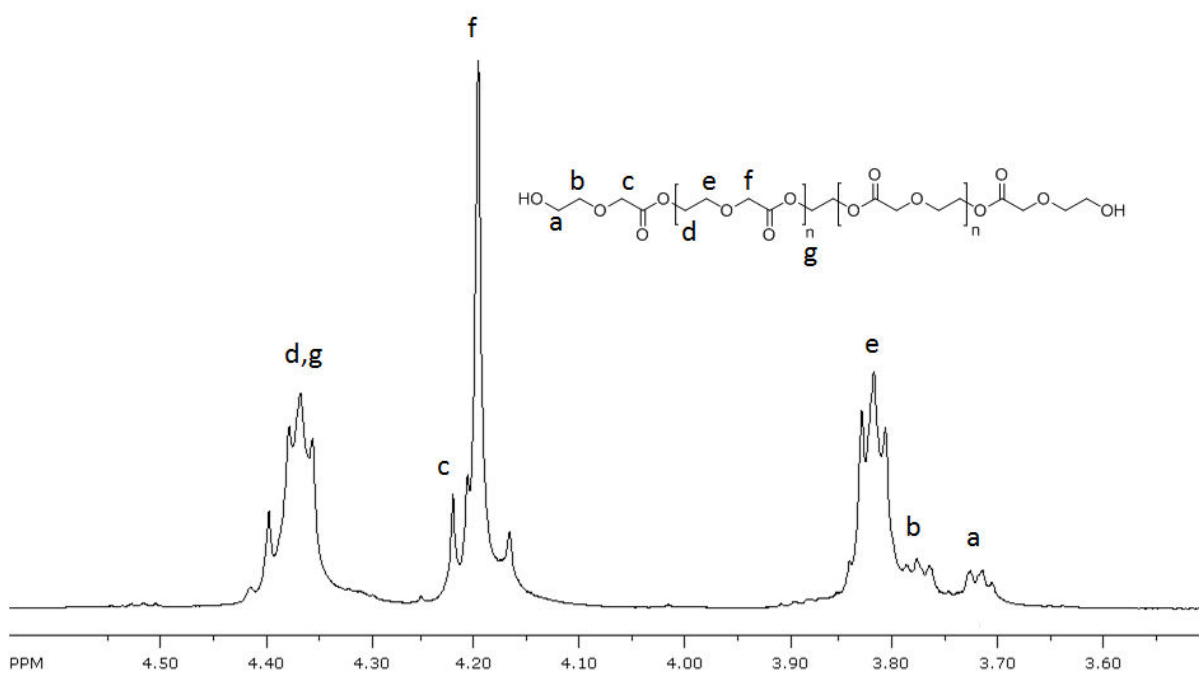


Fig. A3. ^1H NMR (CDCl_3) of PDS diol.

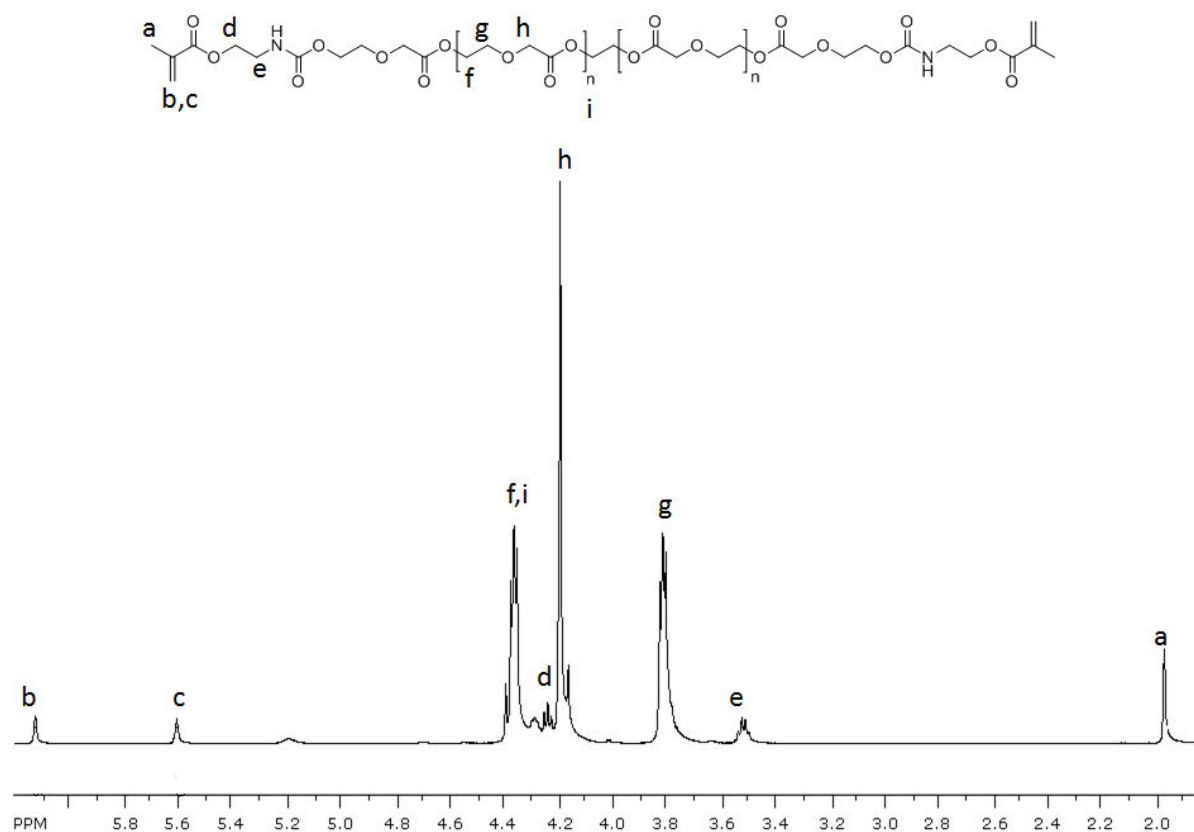


Fig. A4. ^1H NMR (CDCl_3) of PDS dimethacrylate.

Appendix B

Shape Memory Polymers with Tunable Payload Release

Introduction

Current SMP drug release strategies rely on the incorporation of the active payload during crosslinking or in post-curing by swelling in a good solvent. These methods have been able to produce functional SMP materials with a single immutable rate of drug elution for one payload. Precise control over elution rates would be highly favorable to ensure the success of SMP implants and could potentially be extended to the controlled, simultaneous release of multiple payloads. To our knowledge, the ability to deliver multiple payloads at controlled rates has never been explored in SMPs.

While advanced bolus drug release strategies have been explored in tissue engineering, these methods have not been extended to SMP scaffolds. For example, Fu and co-workers developed functional drug-carrier nanoparticle/hydrogel scaffolds with the capacity to deliver multiple drugs at distinct time intervals to address some of the limitations and shortcomings of functional vascular scaffolds containing only one drug payload.^[1] In early stages re-endothelialization was encouraged by the release of vascular endothelial growth factor 165 (VEGF₁₆₅) while paclitaxel was administered during later periods to prevent vascular intimal hyperplasia. Moreover, there is a great demand for the time resolved delivery of multiple payloads. Mooney and co-workers showed that the simultaneous delivery of bone morphogenic protein 2 (BMP-2) and transforming growth factor β 3 (TGF- β 3) from degradable hydrogels resulted in significant bone formation than when administered individually.^[2]

We were interested in coupling the delivery of both hydrophobic (Rhodamine B) and hydrophilic (pyranine) payloads with the ability to insert SMPs in a minimally invasive fashion through the copolymerization of PCL with monomethacrylated PEG.

Experimental Section

Materials. ϵ -Caprolactone (99%), tin octanoate, anhydrous glycerol, monomethacrylated PEG monoether, pyranine, and rhodamine B were purchased from Sigma-Aldrich. Acetic acid, methylene chloride, methanol, and acetone were purchased from Fisher Scientific. ϵ -Caprolactone was dried over CaH_2 for 24 h and distilled prior to use. PBS buffer was prepared in house.

Preparation of chemically crosslinked PCL/PEG networks. PCL/PEG networks were prepared by casting molten methacrylate end-capped PCL, PEG, and photoinitiator diethoxyacetophenone (DEAP) (0.1 wt %) into a teflon mold. Subsequently, the prepolymer melt was irradiated with 30 mW/cm^2 UV light (365 nm) under N_2 atmosphere for 10 min.

Characterization. 4 mg samples were placed in 2 mL of pH = 7.4 PBS at 37 °C for 30 days. Aliquots of each sample were taken at regular time intervals and replenished with equal quantities of fresh PBS. Aliquots were then analyzed by UV-vis spectroscopy at and absorbance wavelength of 554 and 455 nm for rhodamine B and pyranine, respectively.

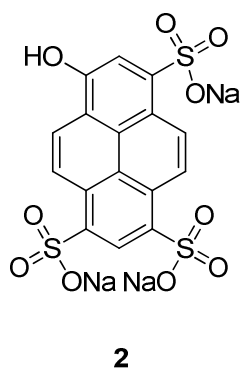
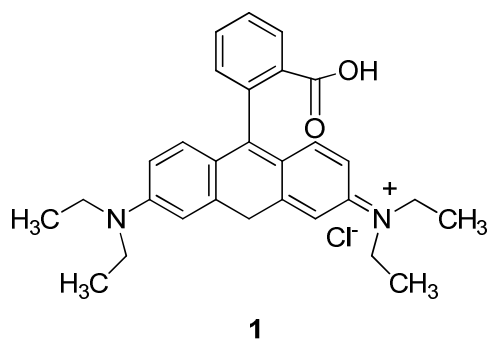
Results and Discussion

One weight percent rhodamine B (RhoB) (1) and pyranine (2) dye were mixed into the melt of monomethacrylated PEG (0, 25, 50 wt% based on PCL) and SPCL-13k. The RhoB and pyranine mixtures were photochemically polymerized and yielded bright red and green polymer films, respectively. Calibration plots for rhodamine B and pyranine were collected by analyzing the UV-vis absorption spectra of standardized samples at 554 nm and 455 nm, respectively (**Fig. B1, B2**). The relationship between absorbance and concentration for RhoB (B1) and pyranine (B2) are shown below.

$$A = 93699 [\text{RhoB}] - 0.0588 \quad (\text{B1})$$

$$A = 15325 [\text{Pyranine}] - 0.0088 \quad (\text{B2})$$

Sampling the absorbance of 4 mg strips of dye/polymer materials of varying PEG content revealed that higher PEG content resulted in faster elution rates of each dye payload. In the case of Rhodamine, 0 wt% PEG led to essentially no dye release over the trial period. Incorporation of 25 and 50 wt% PEG resulted in a burst release after approximately 3 days and then a slow continuous elution for the remainder of the 30 day elution time. Burst release was substantially higher for 50 wt% PEG when compared to lower PEG weight fractions. Similarly, 0 wt% PEG resulted in very slow elution rates for pyranine, while 25 and 50 wt% PEG resulted in a burst release and then slow and continuous release. The burst effect was especially pronounced for 50 wt% PEG loaded pyranine films which essentially release the entire dye payload in 9.5 h.



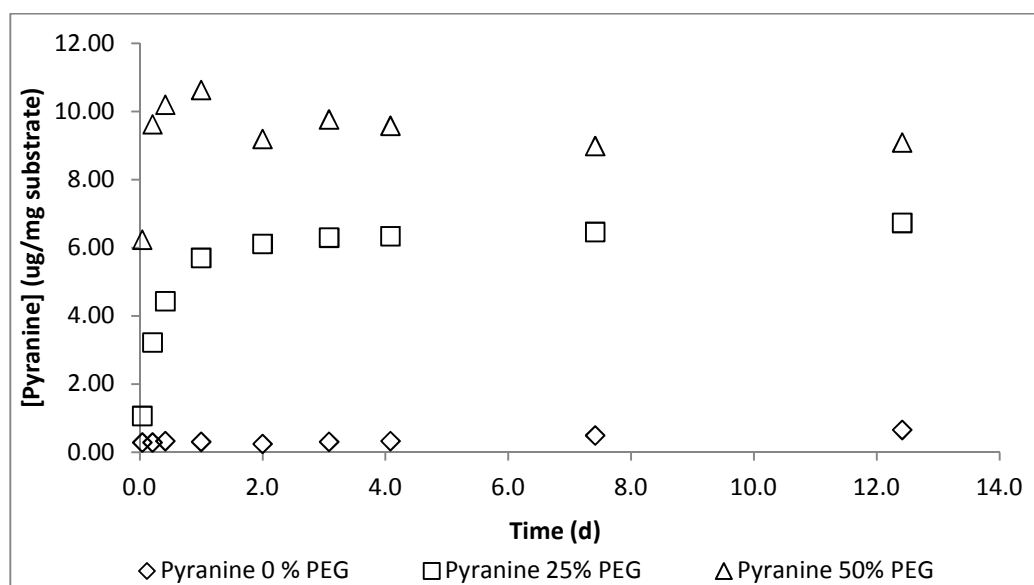
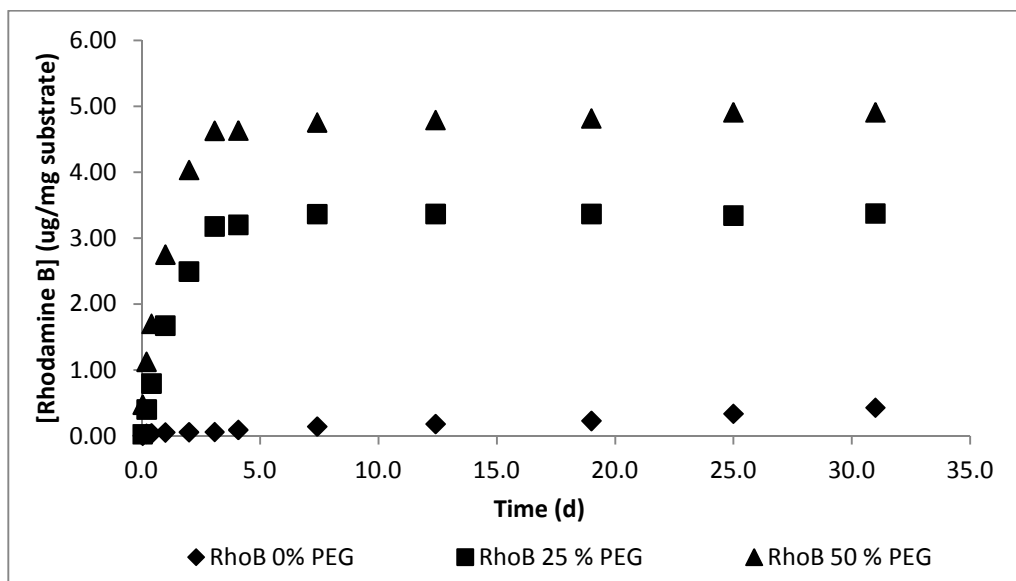


Fig. B1. Elution rates ($\mu\text{g}/\text{mg}$ PCL substrate) for PCL films copolymerized with varying weight fractions of monomethacrylated PEG and 1 wt% dye payload (Rhodamine B (above); Pyranine (Below)).

We showed that the burst behavior and μg of dye released per day can be controlled by manipulating the hydrophilicity of the SMP network. We also wanted to show that the shape memory effect was not lost due to the addition of PEG and dye to the network. DSC analysis shows that the T_m and χ_c change very little in the presence of the mono-functional PEG and payload. Copolymerization with mono-functionalized PEG increased the hydrophilicity of the network while avoiding an increase in crosslink density and the adverse effects on T_m , that would otherwise be observed using di-functionalized PEG. Moreover, the low weight fraction of the dyes did not exert an effect on T_m . We therefore conclude that the shape memory effect remains intact when mono-functionalized PEG and dyes are included into the network.

A multi-drug eluting stent or scaffold could be fabricated from these materials by incorporating spatially independent regions of PEG/PCL copolymer of different PEG weight fractions. The rate will be determined by the hydrophobicity of the payload and the weight fraction of the PEG polymer. Here, we have shown a method that could potentially be used to control the elution rates of multi-drug payloads.

Supporting Information

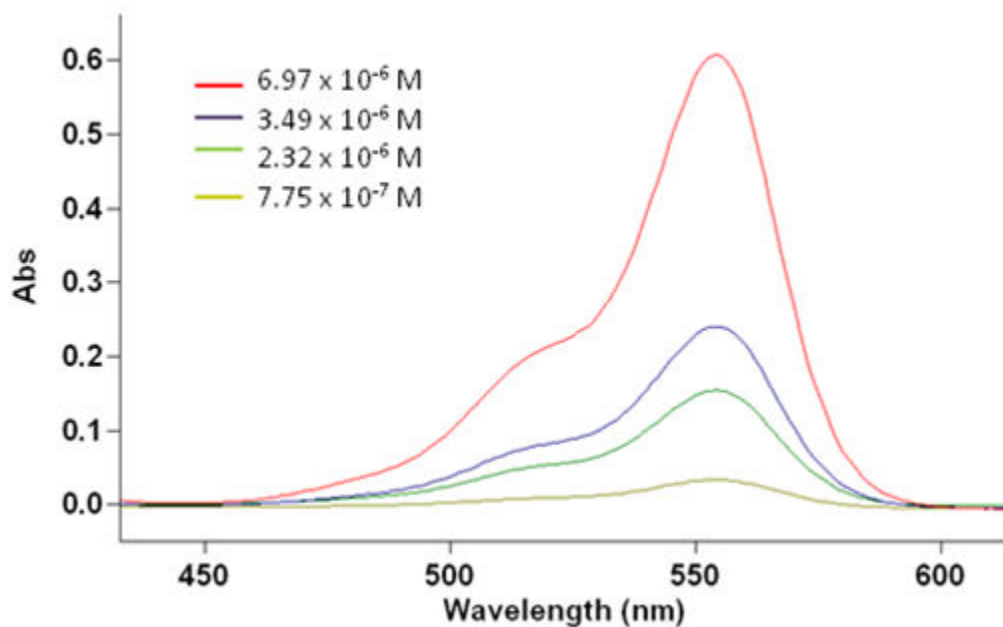
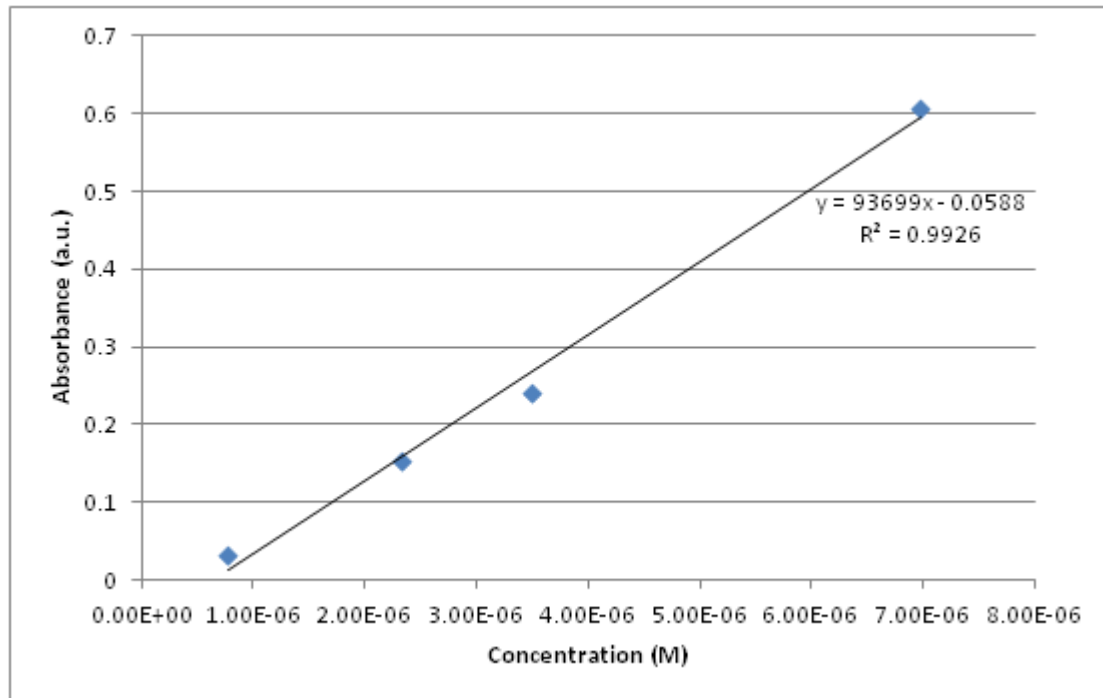


Fig. B2. Absorbance of standardized Rhodamine B solutions in PBS (above) and the corresponding Uv-vis spectrum (below).

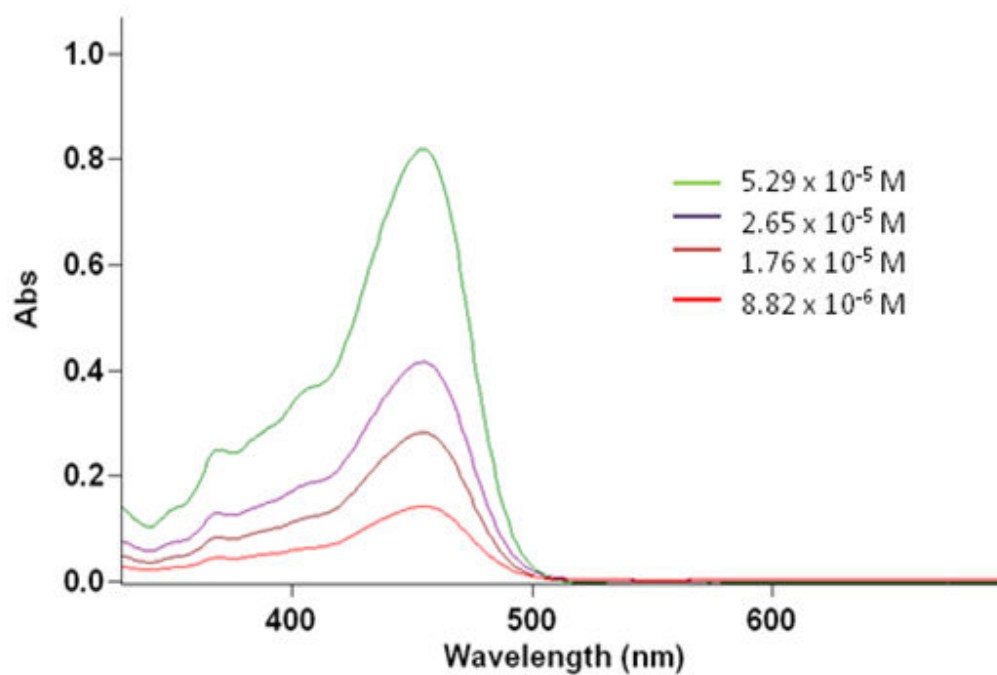
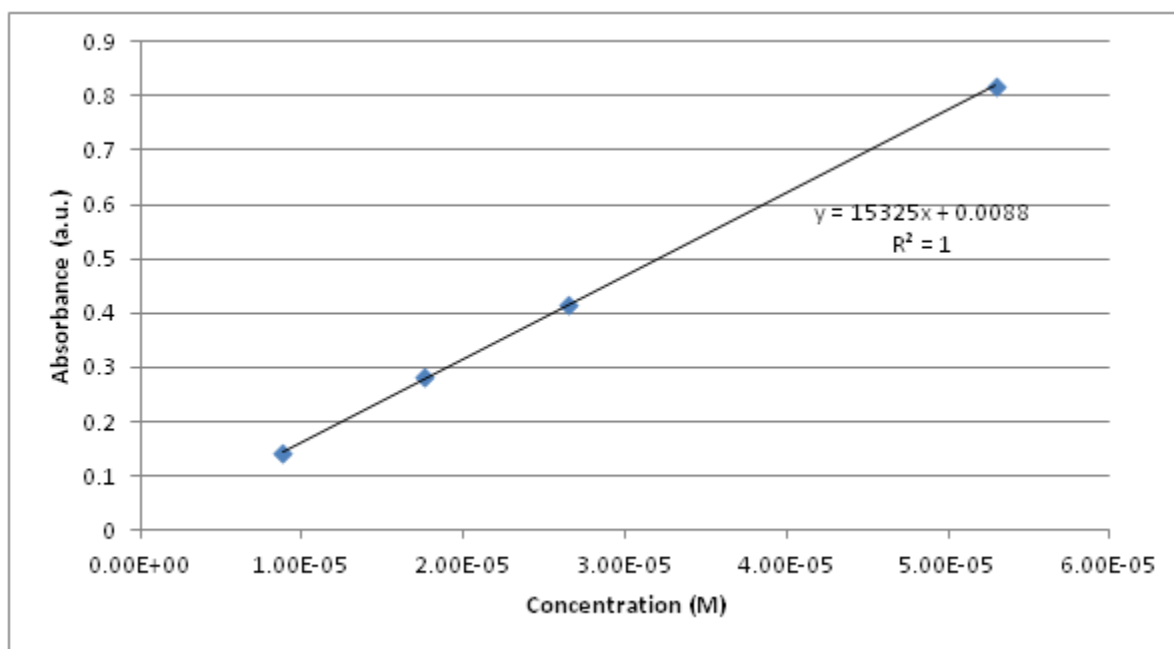


Fig. B3. Absorbance of standardized Rhodamine B solutions in PBS (above) and the corresponding Uv-vis spectrum (below).

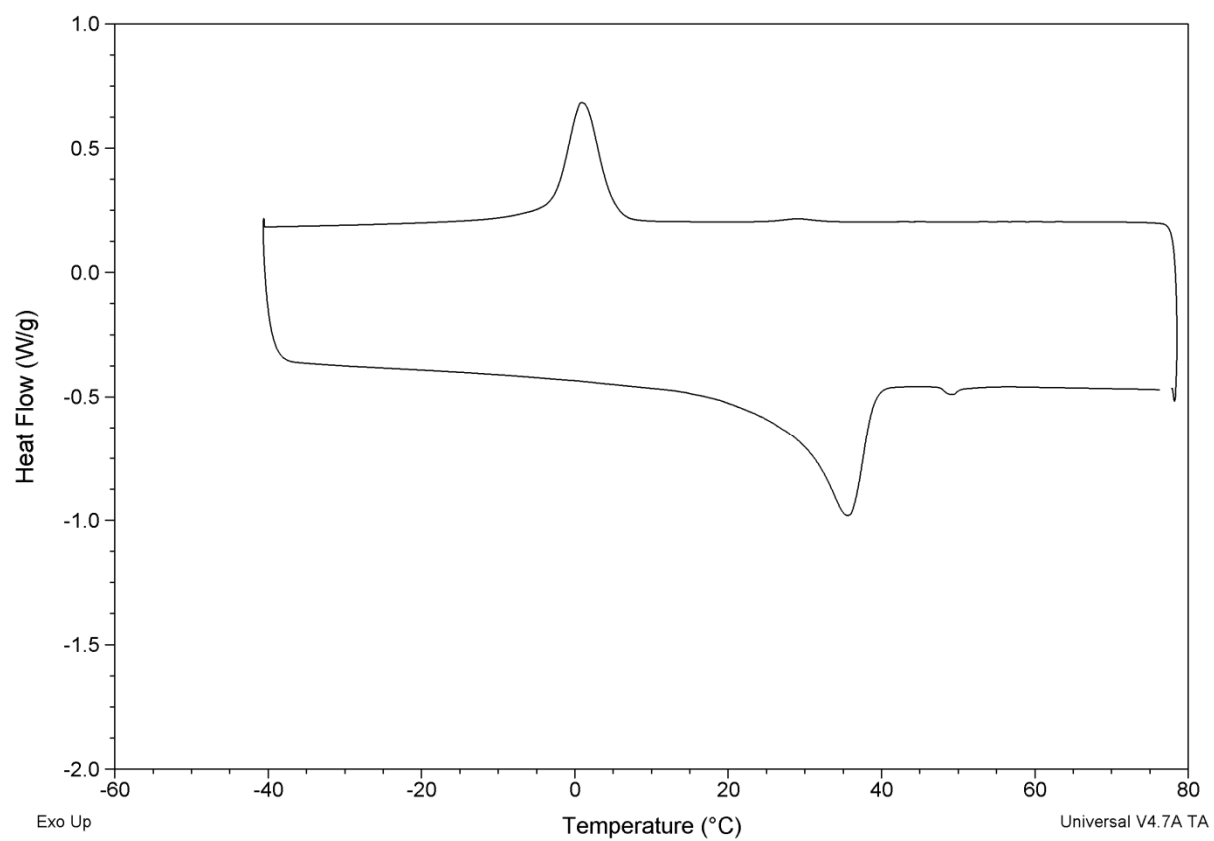


Fig. B4. DSC of neat PCL film loaded with 1 wt% Rhodamine B.

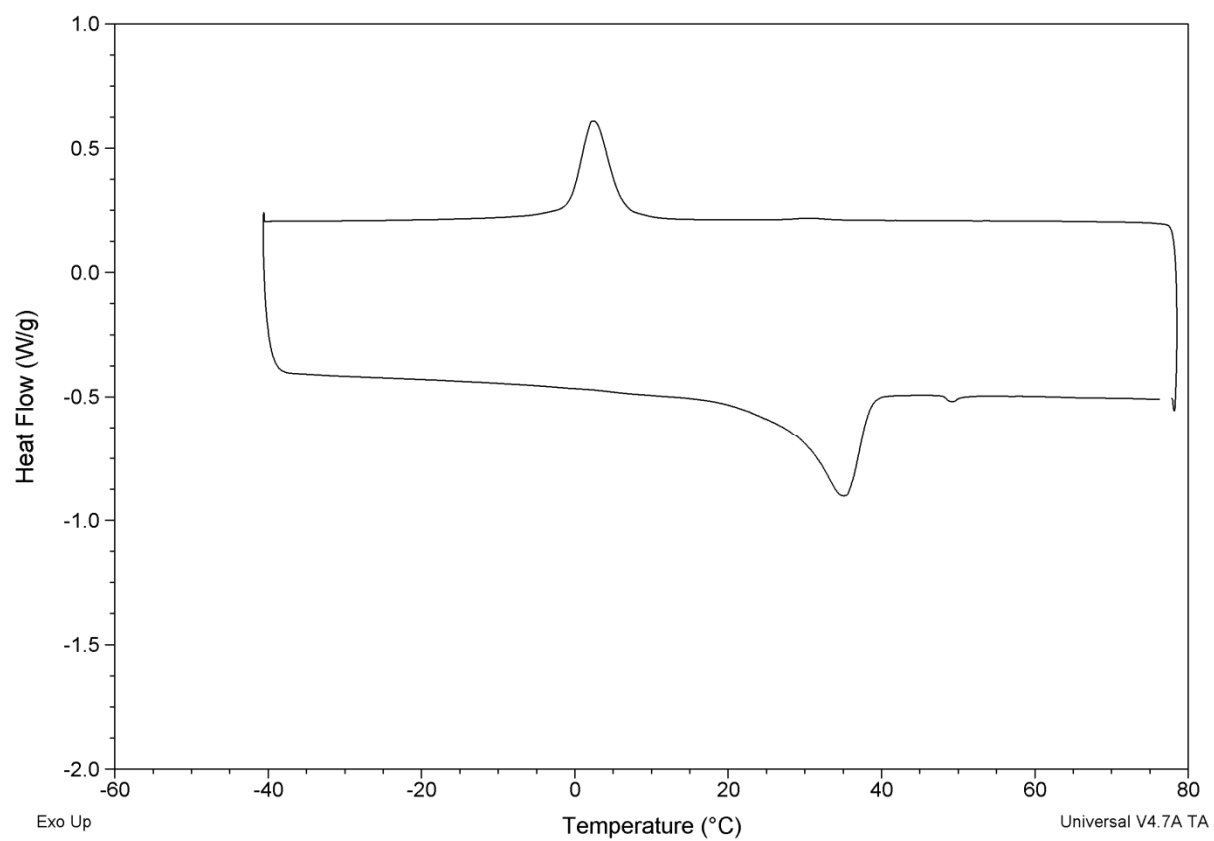


Fig. B5. DSC of PCL film with 25 wt% PEG, loaded with 1 wt% Rhodamine B.

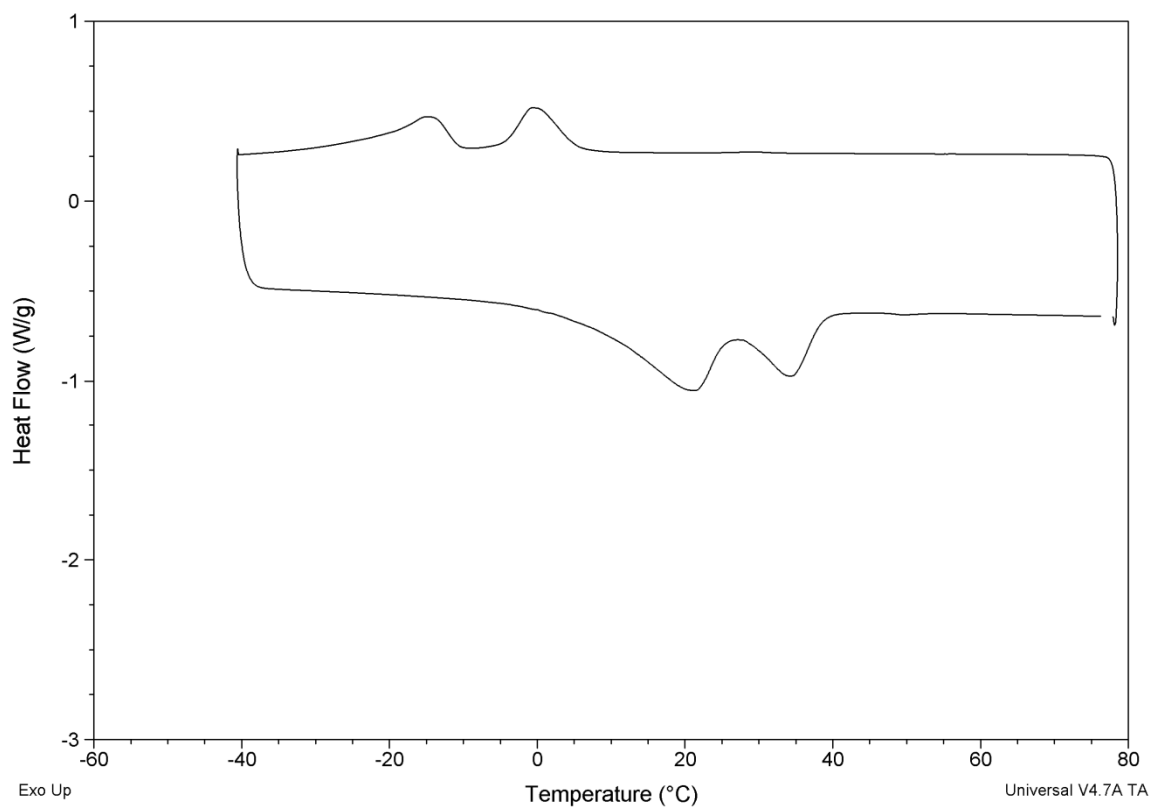


Fig. B6. DSC of PCL film with 50 wt% PEG, loaded with 1 wt% Rhodamine B.

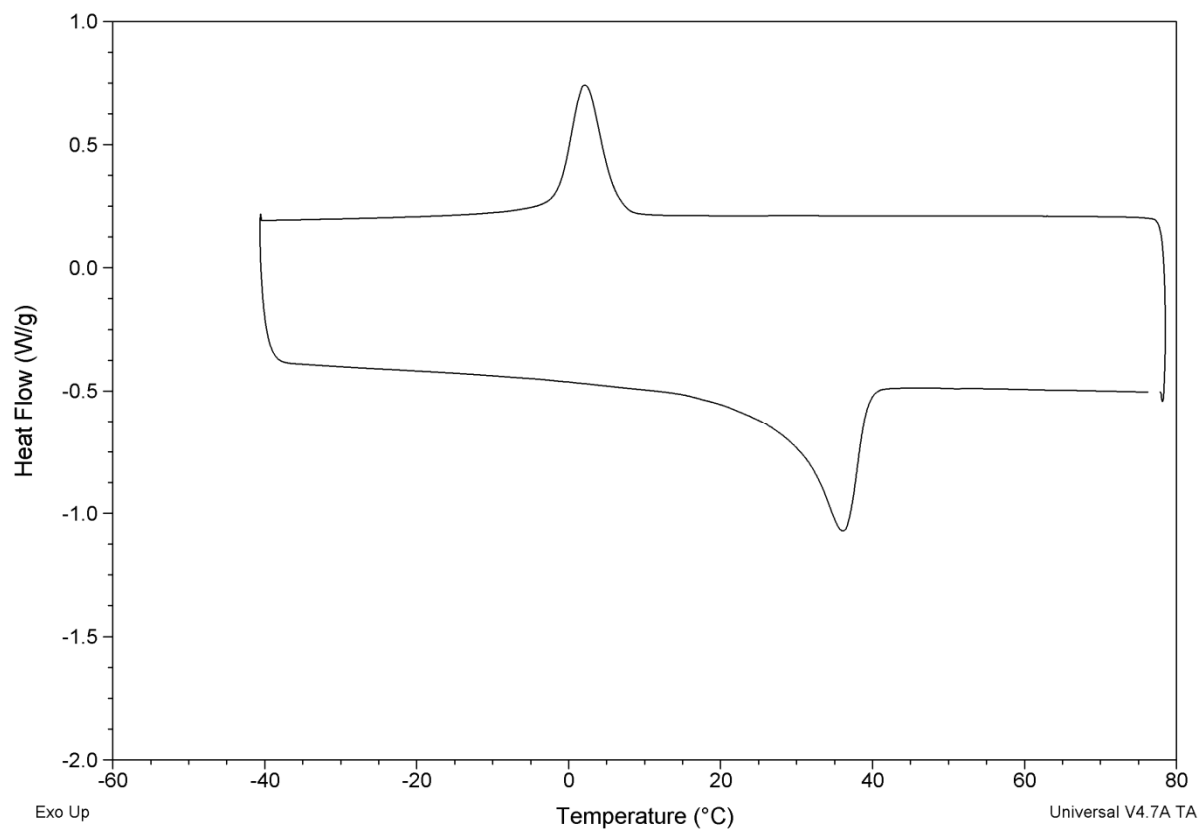


Fig. B7. DSC of neat PCL film loaded with 1 wt% pyranine.

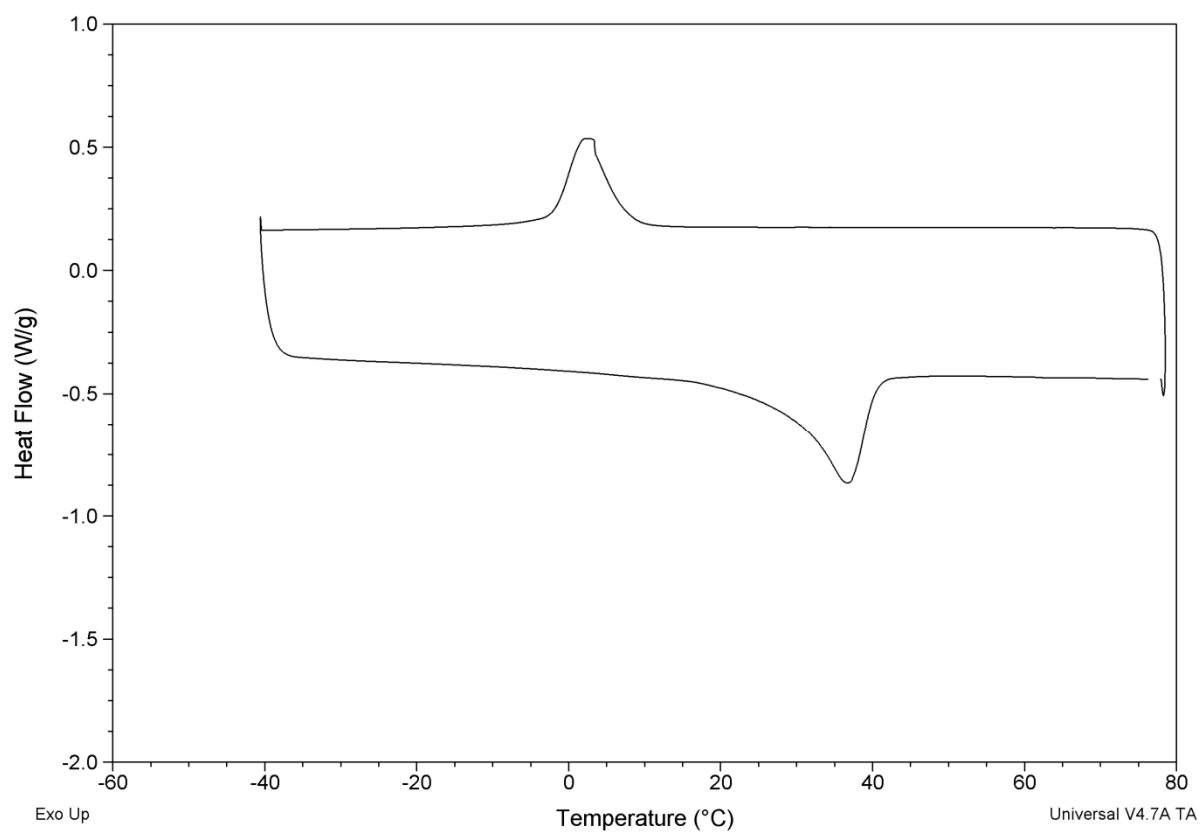


Fig. B8. DSC of neat PCL film with 25 wt% PEG, loaded with 1 wt% pyranine.

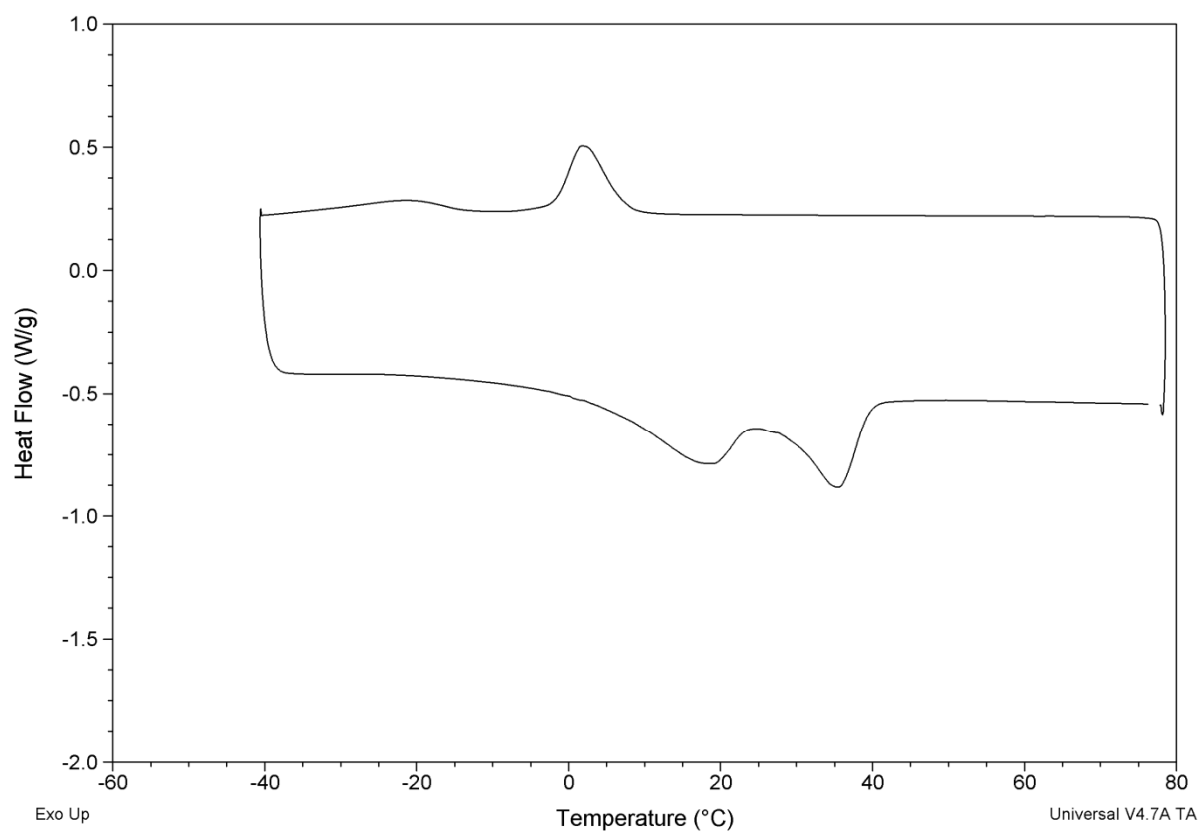


Fig. B9. DSC of neat PCL film with 50 wt% PEG, loaded with 1 wt% pyranine.

References

1. L. Wei, J. Lin, C. Cai, Z. Fang, W. Fu, Drug-Carrier/Hydrogel Scaffold for Controlled Growth of Cells. *Eur. J. Pharm. Biopharm.* **2011**, 78, 346-354.
2. T.P. Richardson, M.C. Peters, A.B. Ennett, D.J. Mooney, Polymeric Systems for Dual Growth Factor Delivery. *Nature* **2001**, 19, 1029-1034.

Appendix C

Supporting Information: Chapter 2

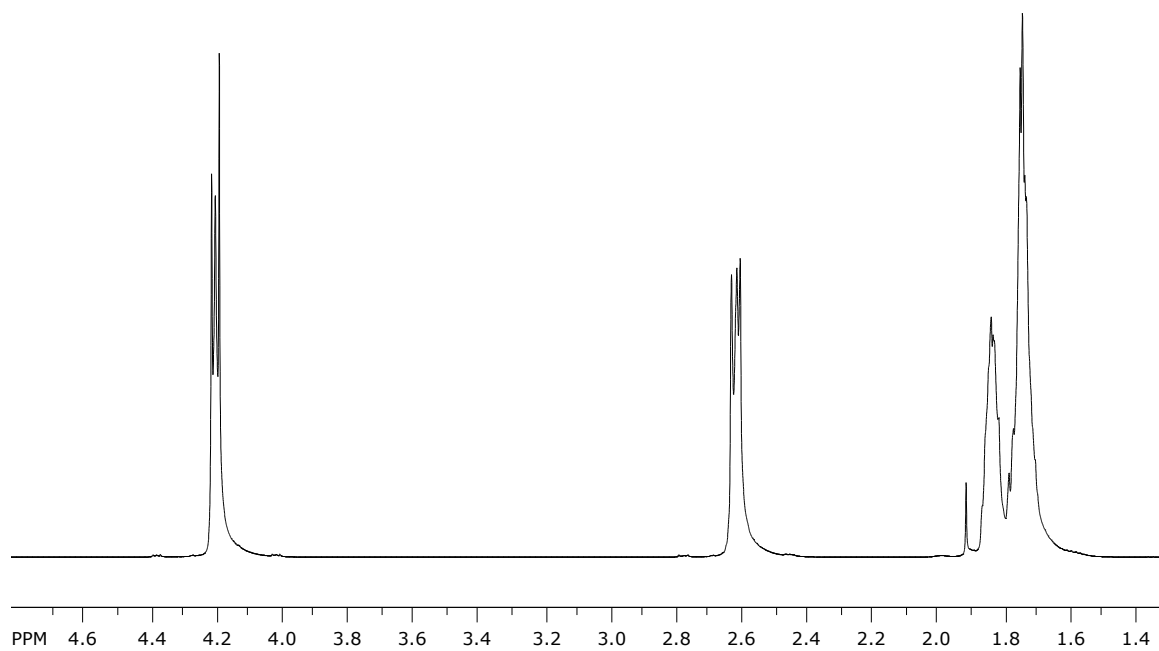


Fig. C1. ^1H NMR (CDCl_3) of ϵ -caprolactone monomer.

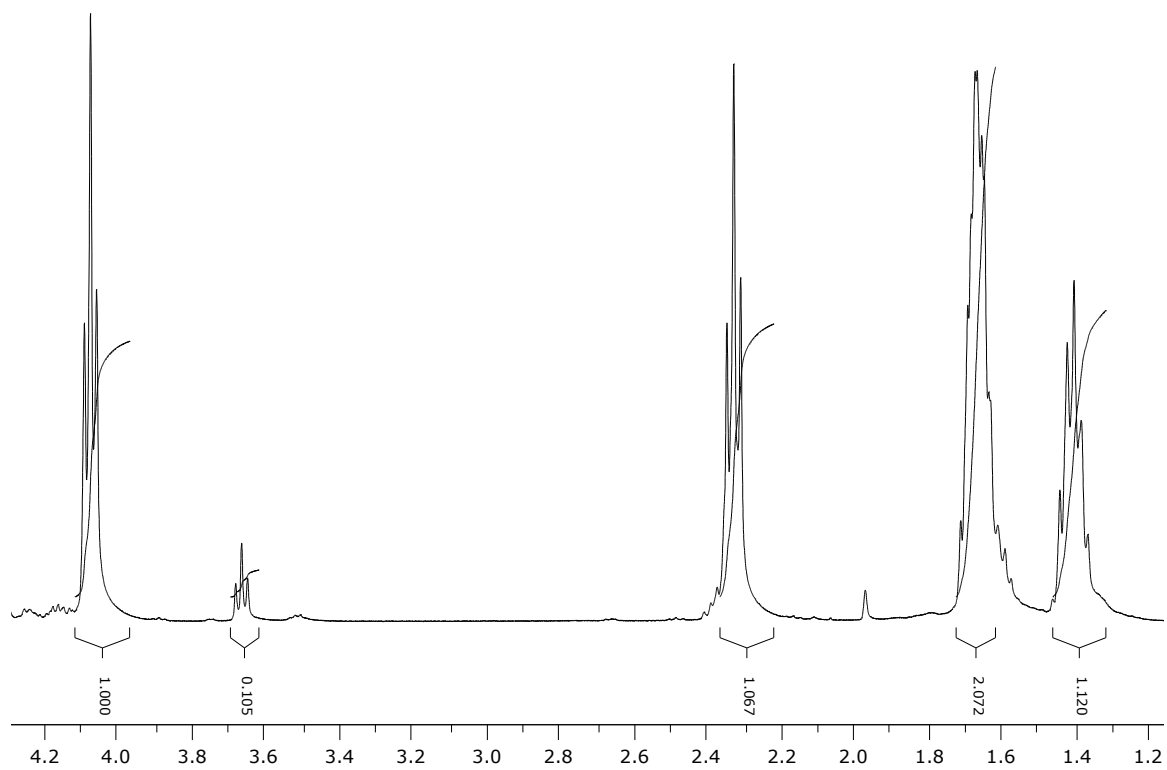


Fig. C2. ^1H NMR (CDCl_3) of 2,500 gmol^{-1} PCL diol.

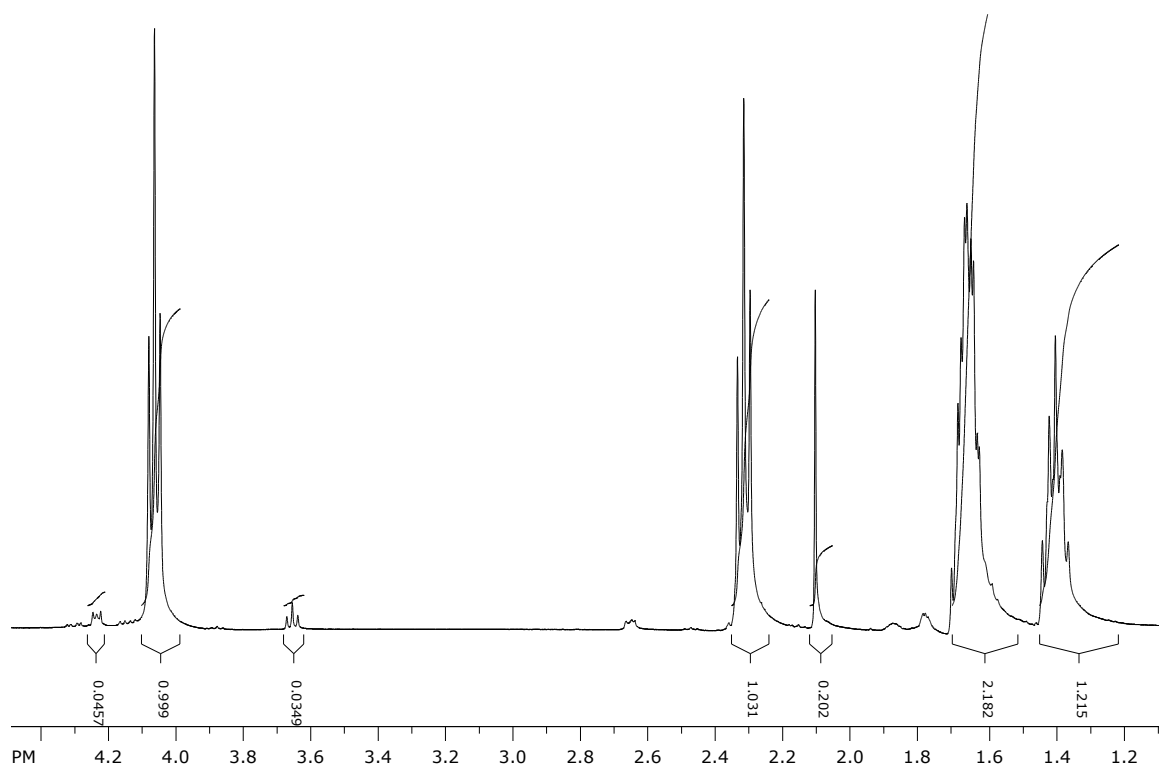


Fig. C3. ^1H NMR (CDCl_3) of $6,900\text{ gmol}^{-1}$ PCL diol.

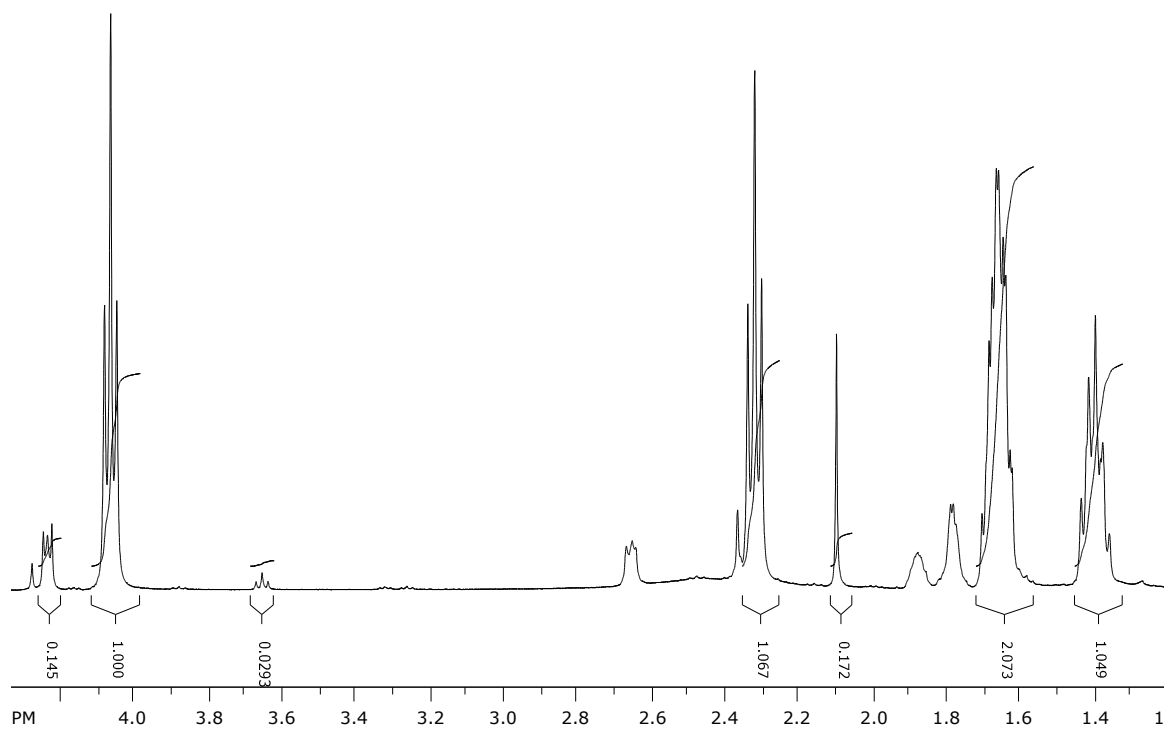


Fig. C4. ^1H NMR (CDCl_3) of $7,700\text{ gmol}^{-1}$ PCL diol.

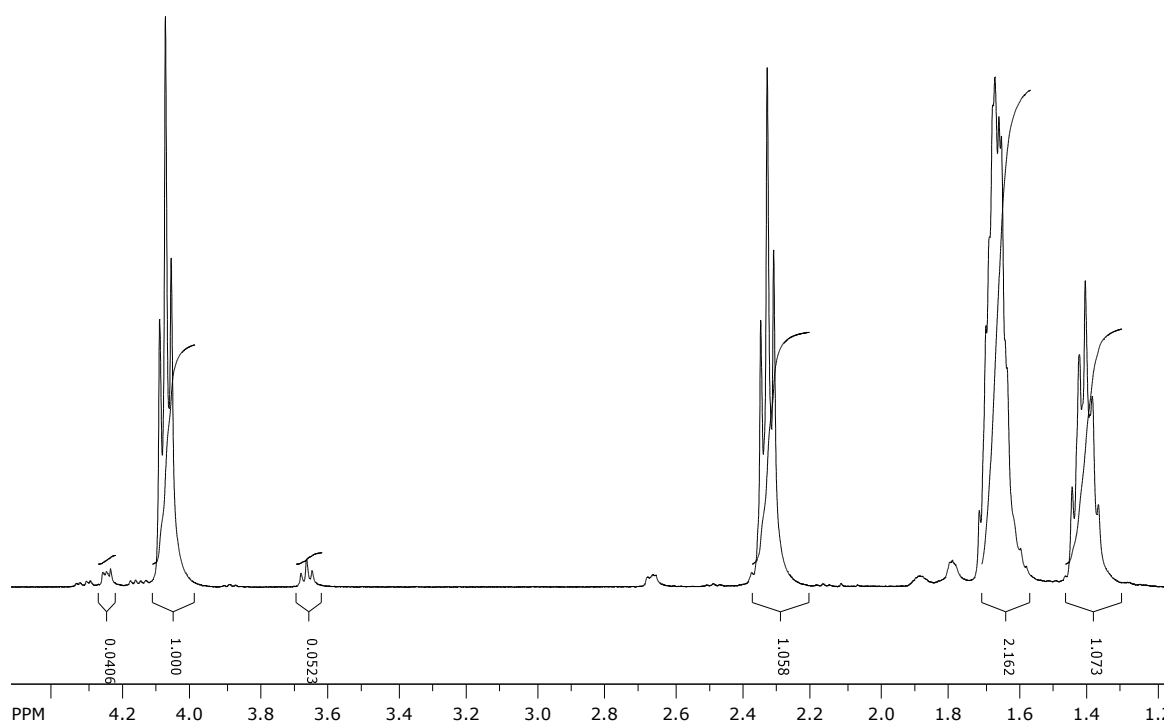


Fig. C5. ^1H NMR (CDCl_3) of $7,000\text{ gmol}^{-1}$ PCL triol.

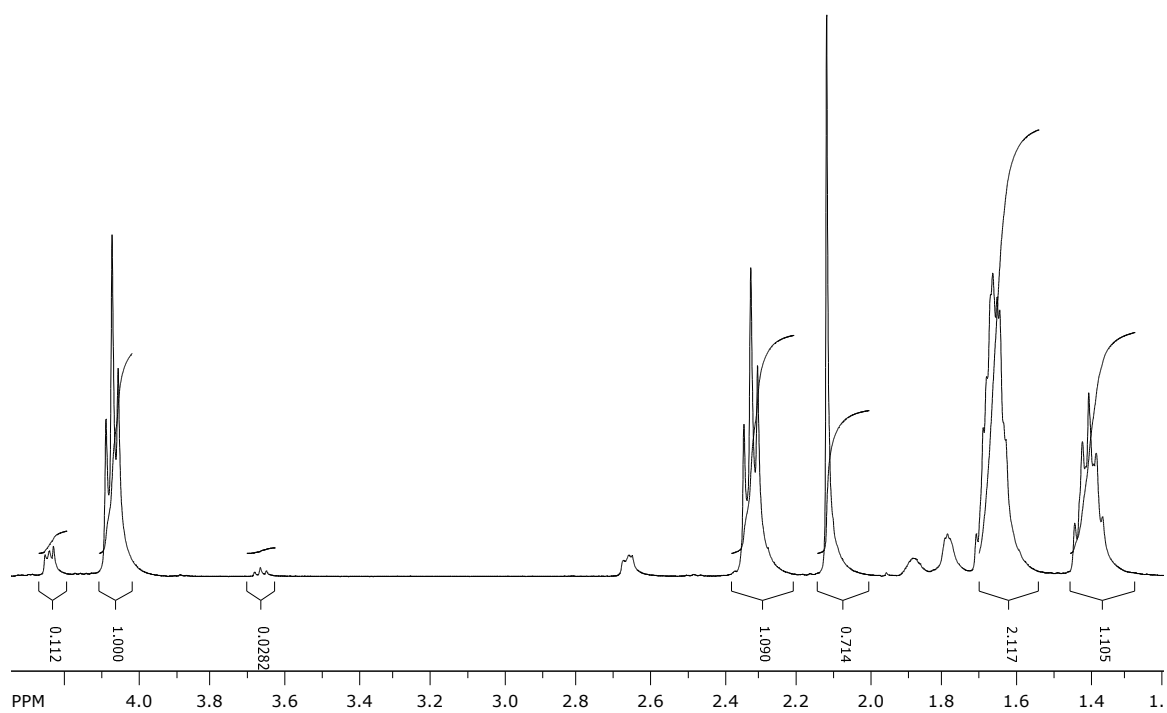


Fig. C6. ^1H NMR (CDCl_3) of $12,500\text{ gmol}^{-1}$ PCL triol.

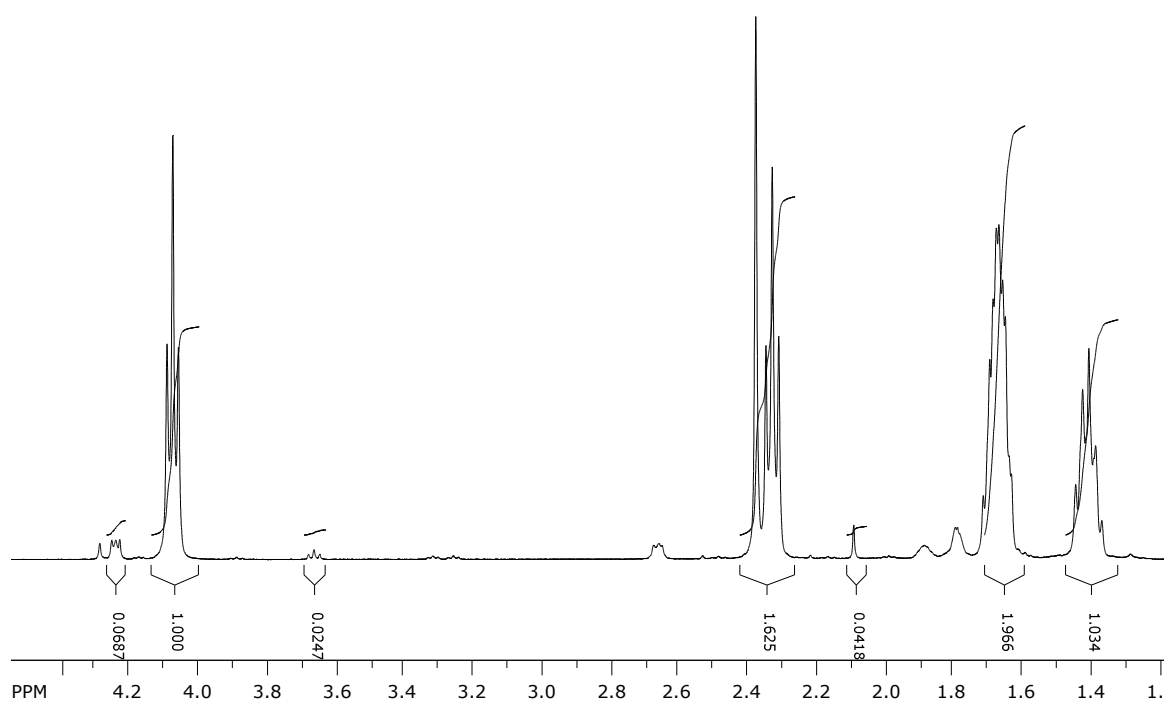


Fig. C7. ^1H NMR (CDCl_3) of $14,300\text{ gmol}^{-1}$ PCL triol.

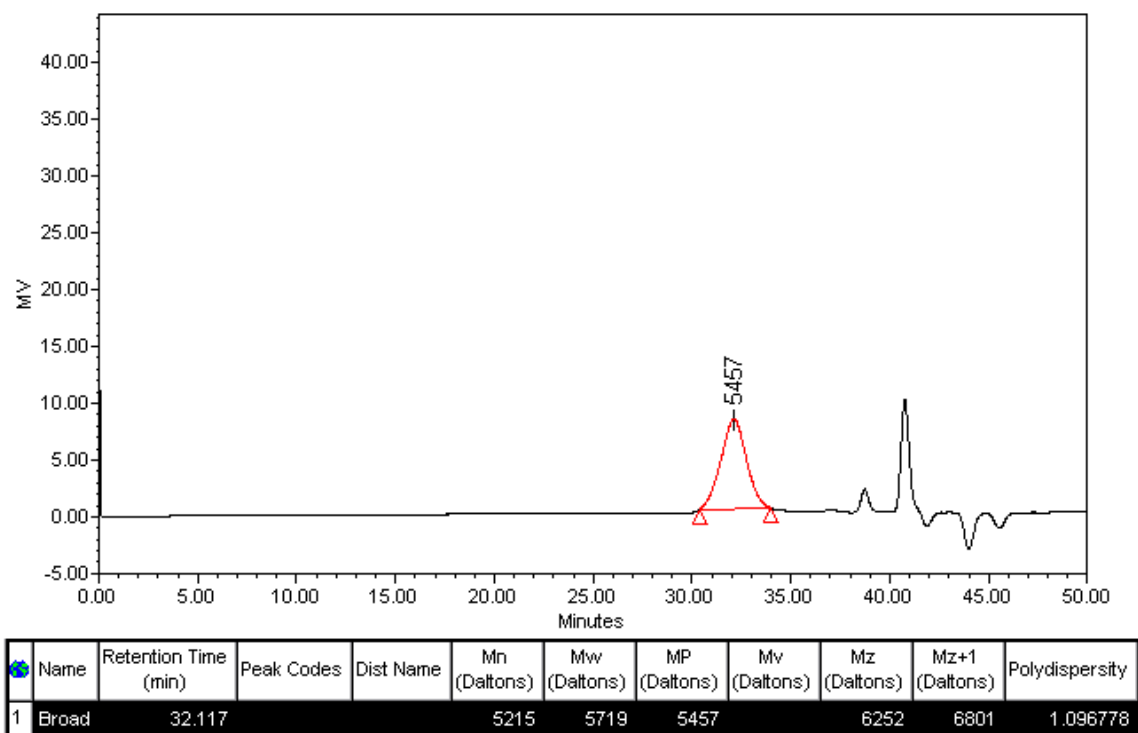


Fig. C8. GPC of 2,500 g mol^{-1} PCL diol.

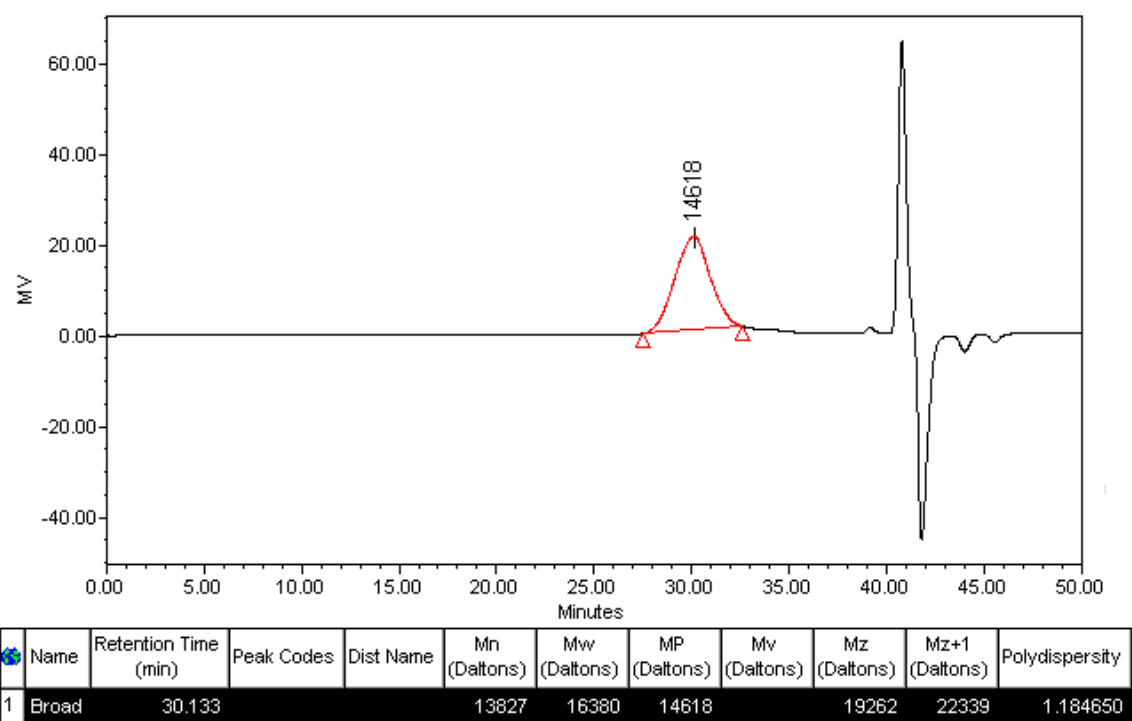


Fig. C9. GPC of 6,900 gmol⁻¹ PCL diol.

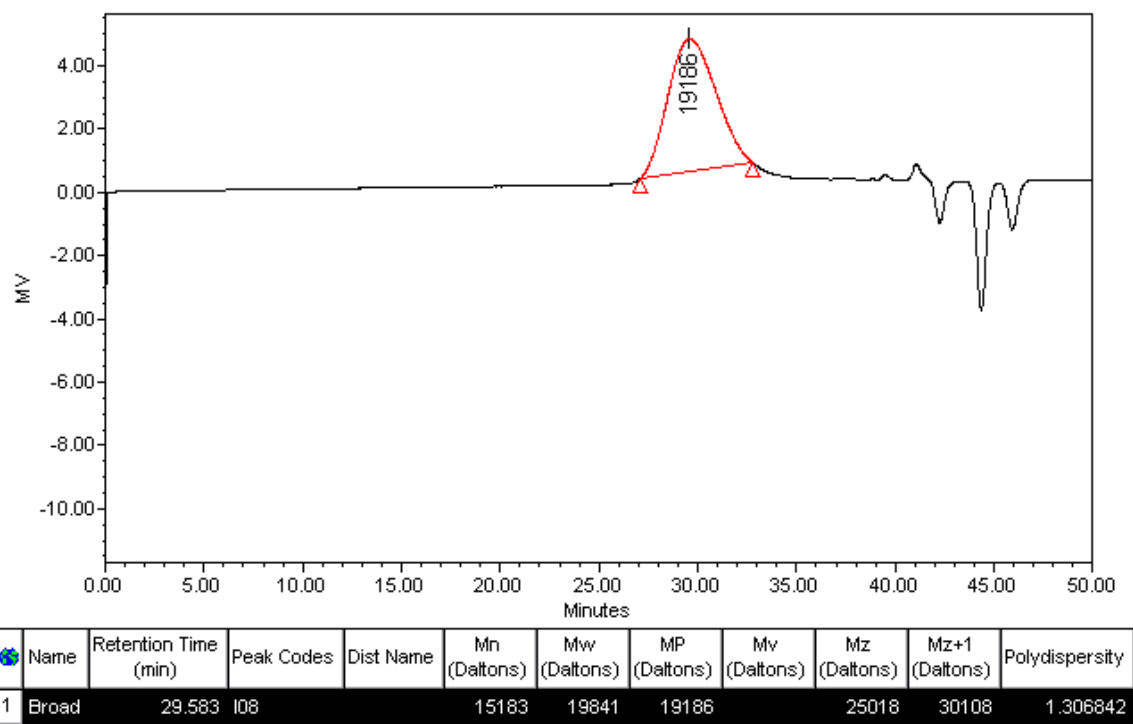


Fig. C10. GPC of 7,700 gmol⁻¹ PCL diol.

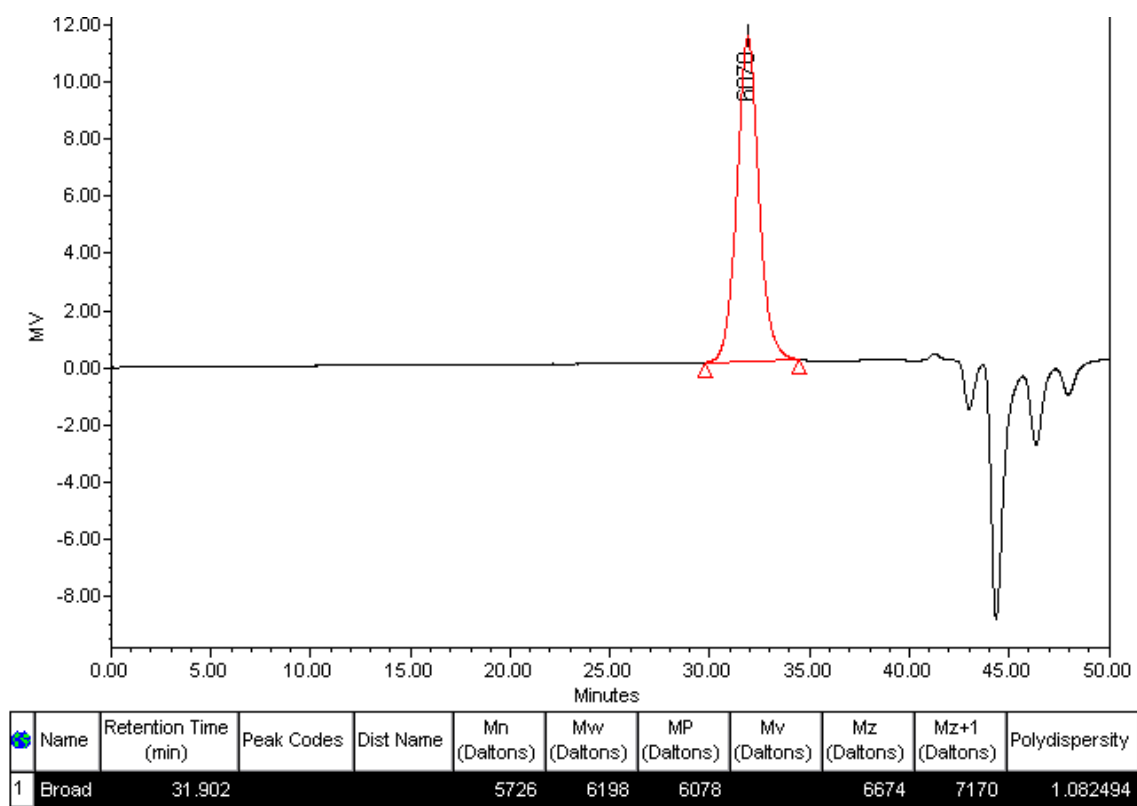


Fig. C11. GPC of 7,000 gmol⁻¹ PCL triol.

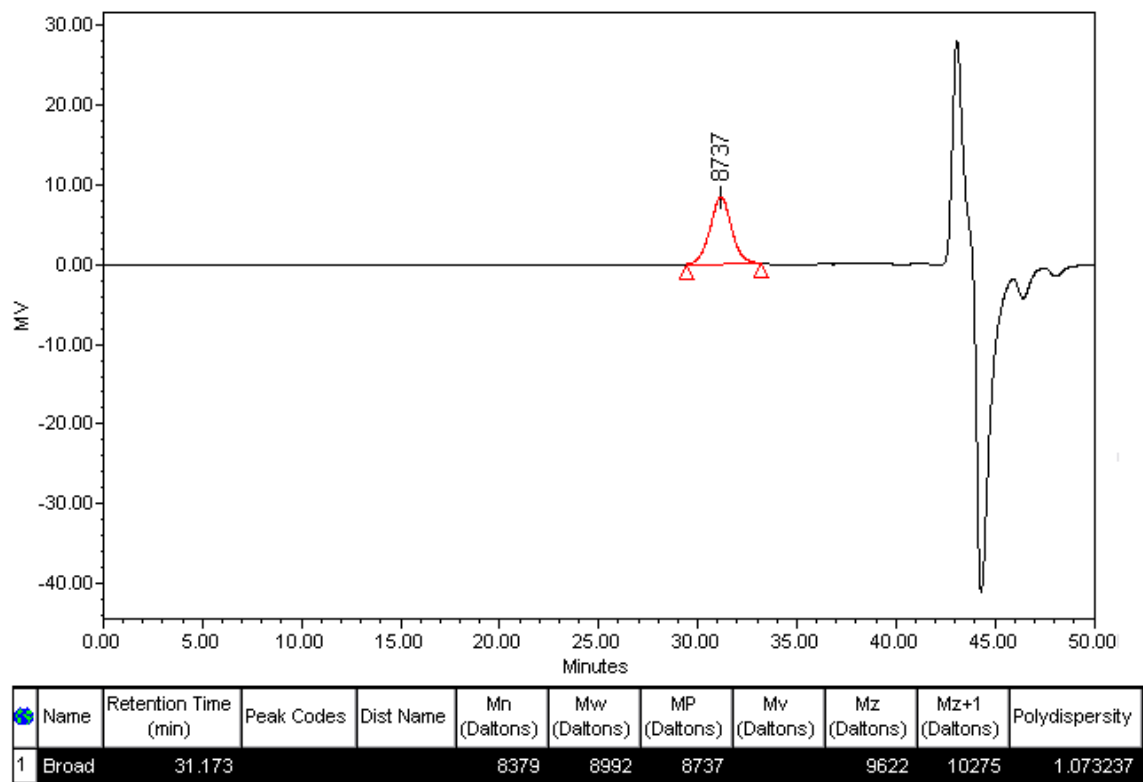


Fig. C12. GPC of 12,500 gmol⁻¹ PCL triol.

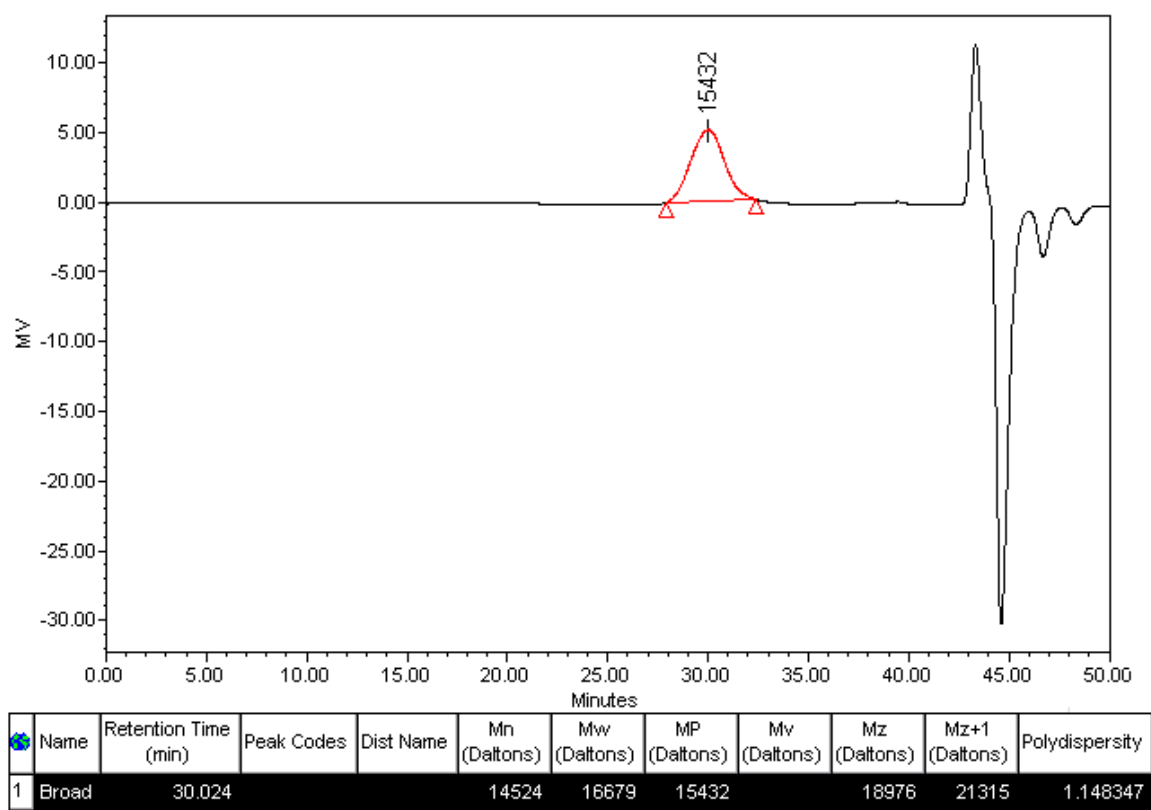


Fig. C13. GPC of 14,300 gmol⁻¹ PCL triol.

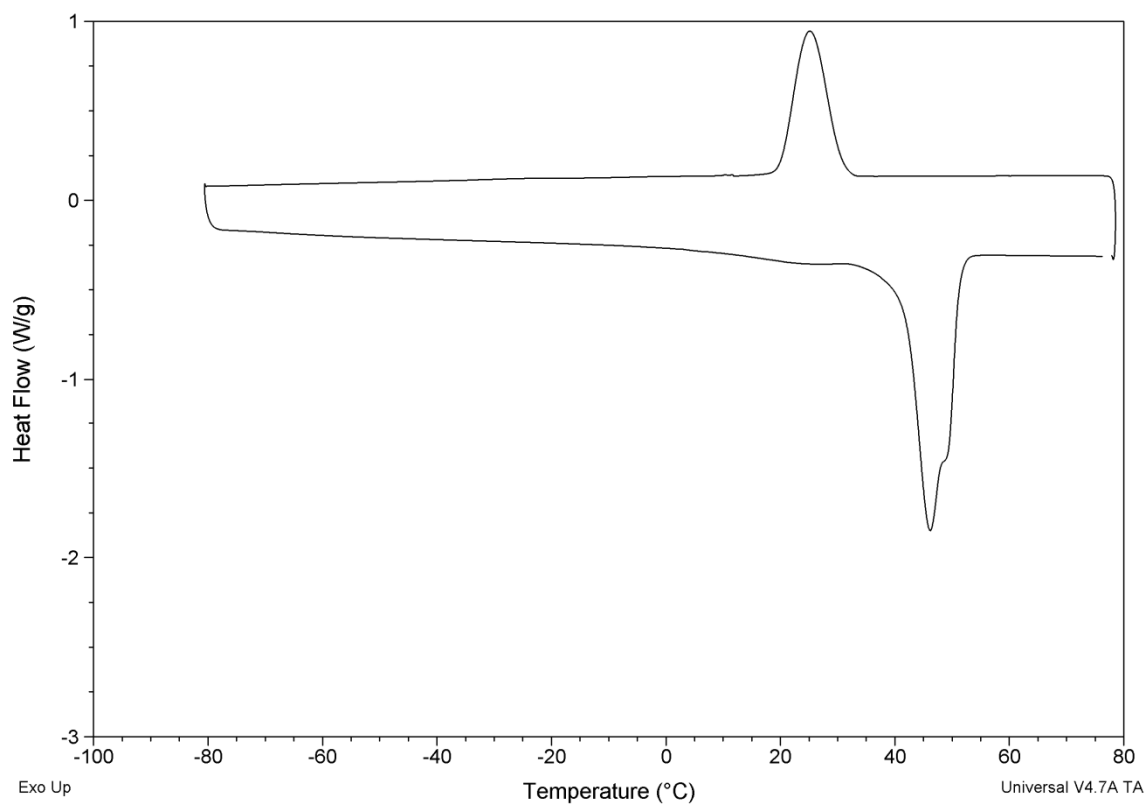


Fig. C14. DSC of 7,000 gmol⁻¹ PCL triol.

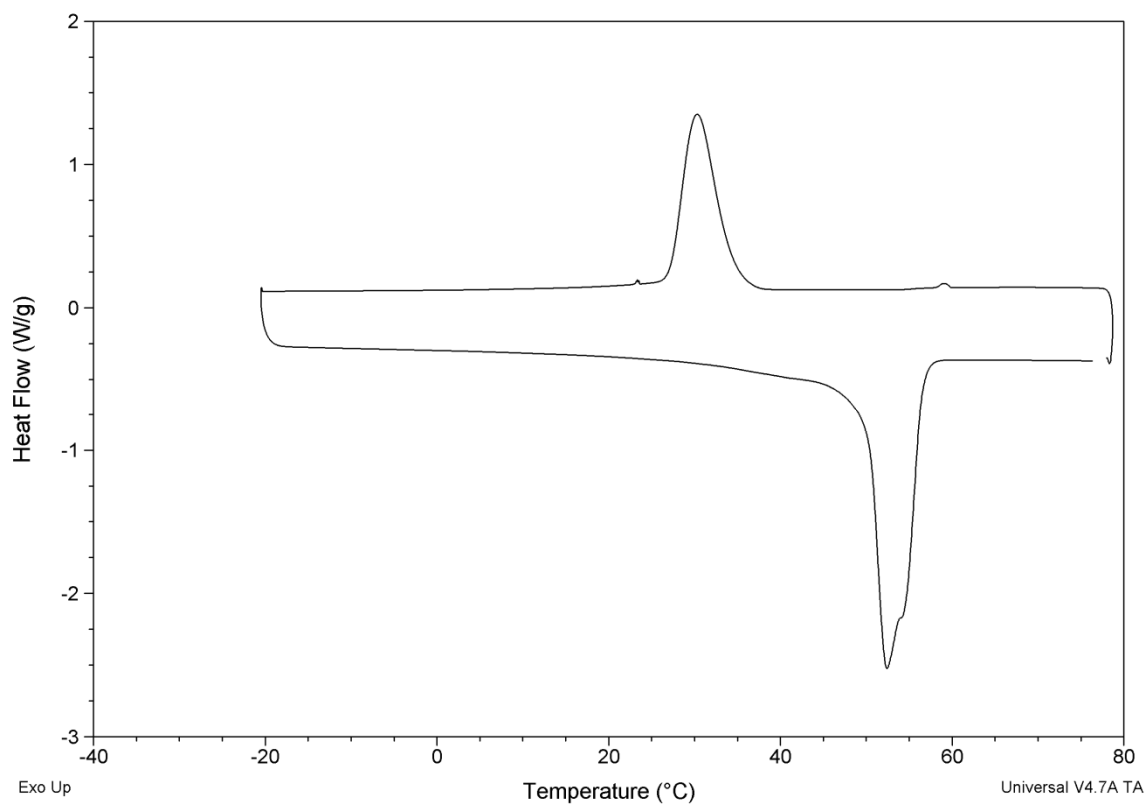


Fig. C15. DSC of 12,500 gmol⁻¹ PCL triol.

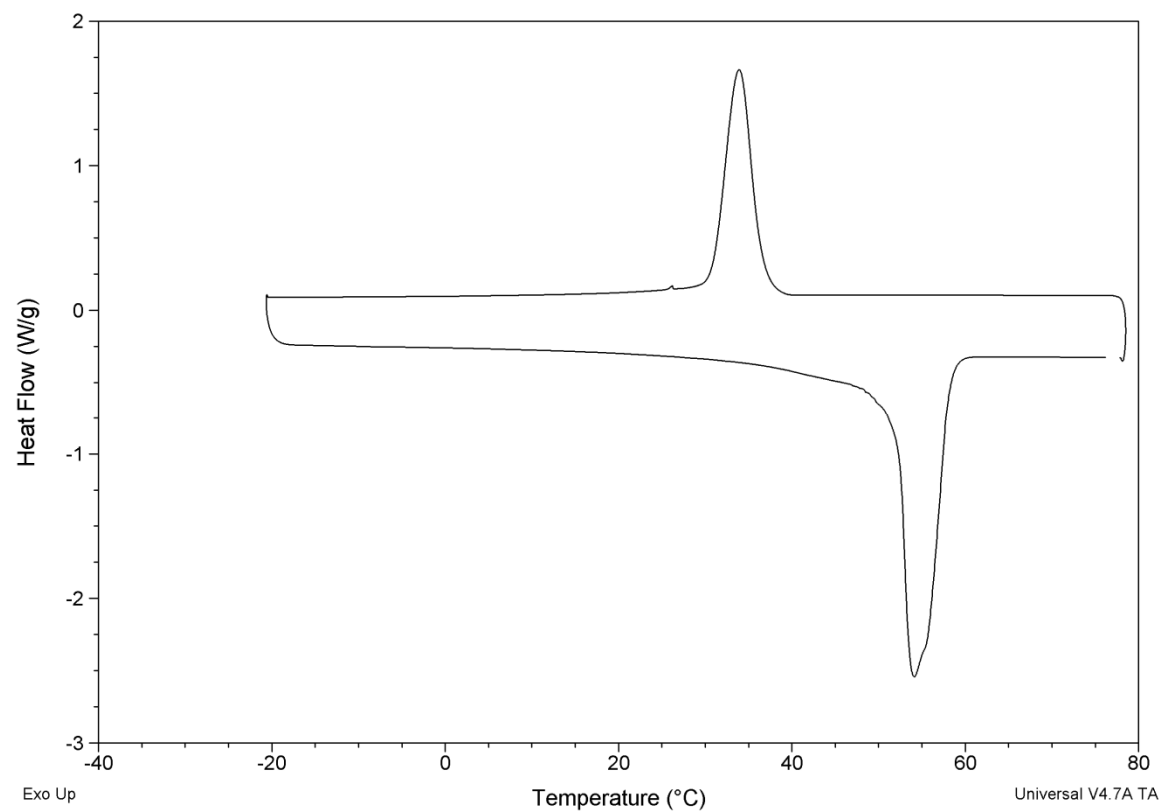


Fig. C16. DSC of 14,300 gmol⁻¹ PCL triol.

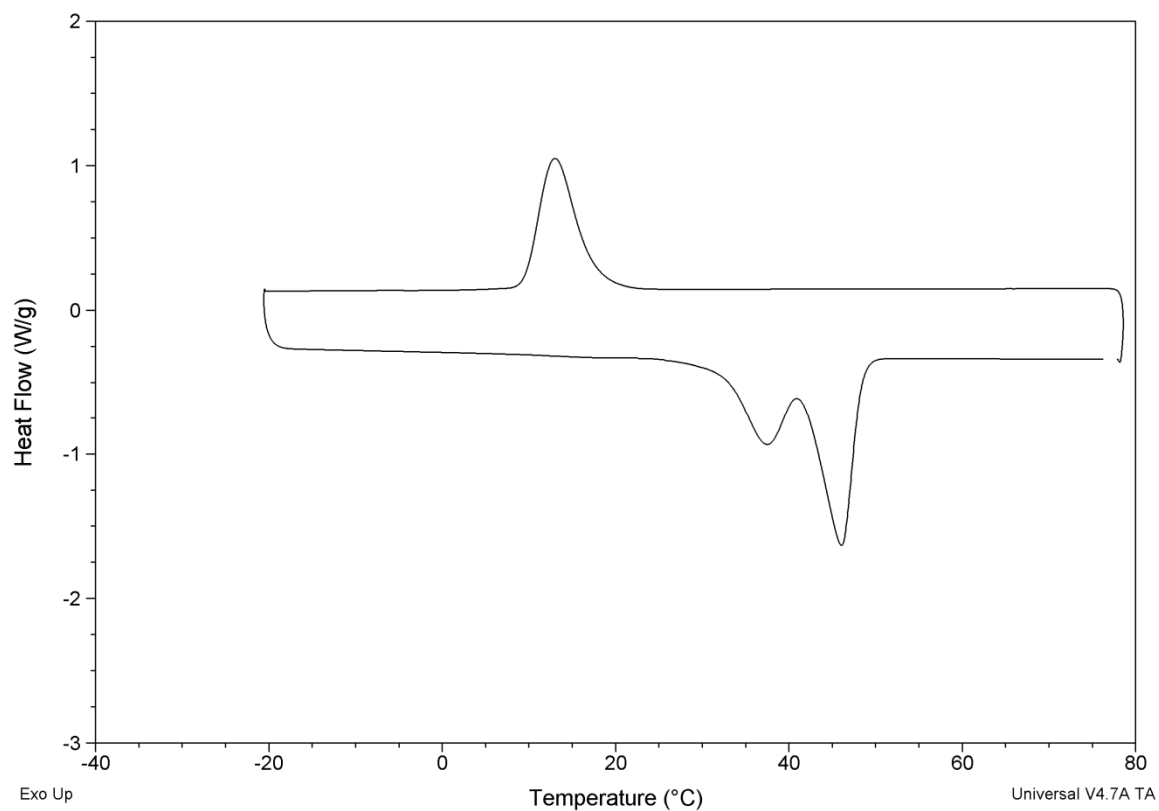


Fig. C17. DSC of 7,000 gmol⁻¹ PCL trimethacrylate.

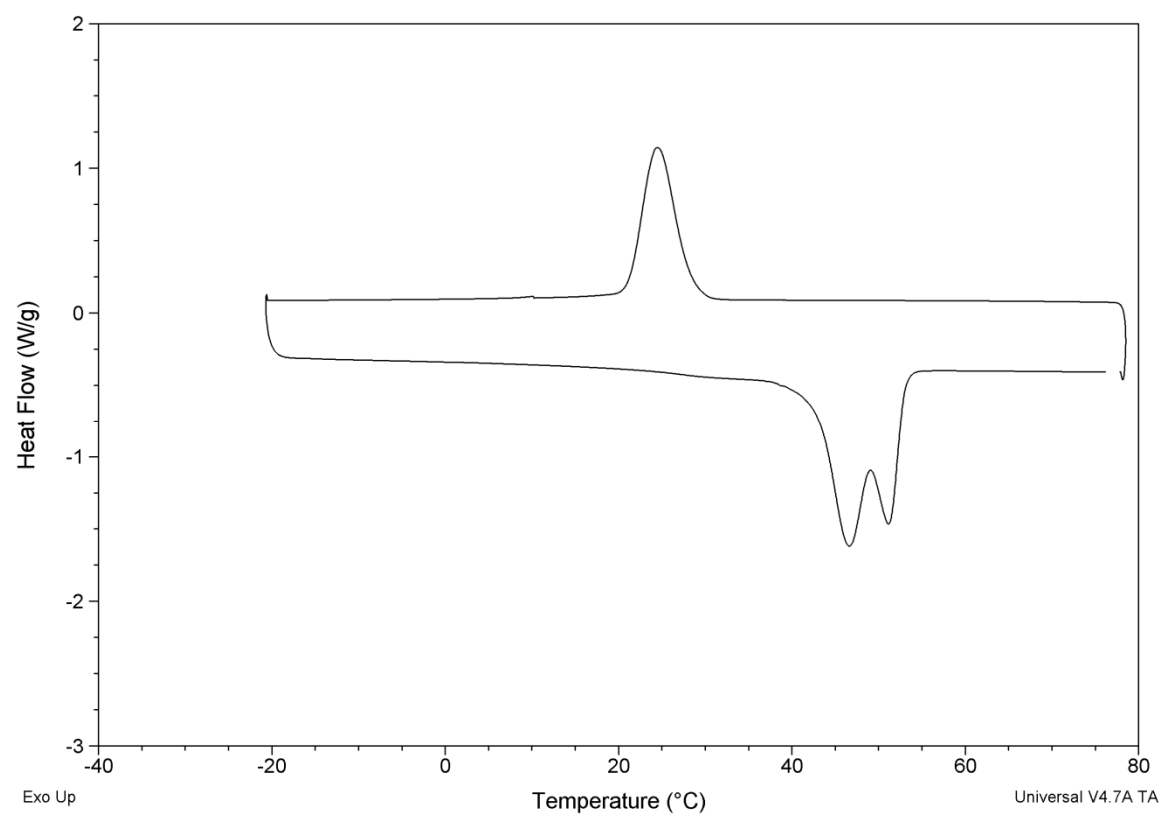


Fig. C18. DSC of 12,500 gmol⁻¹ PCL trimethacrylate.

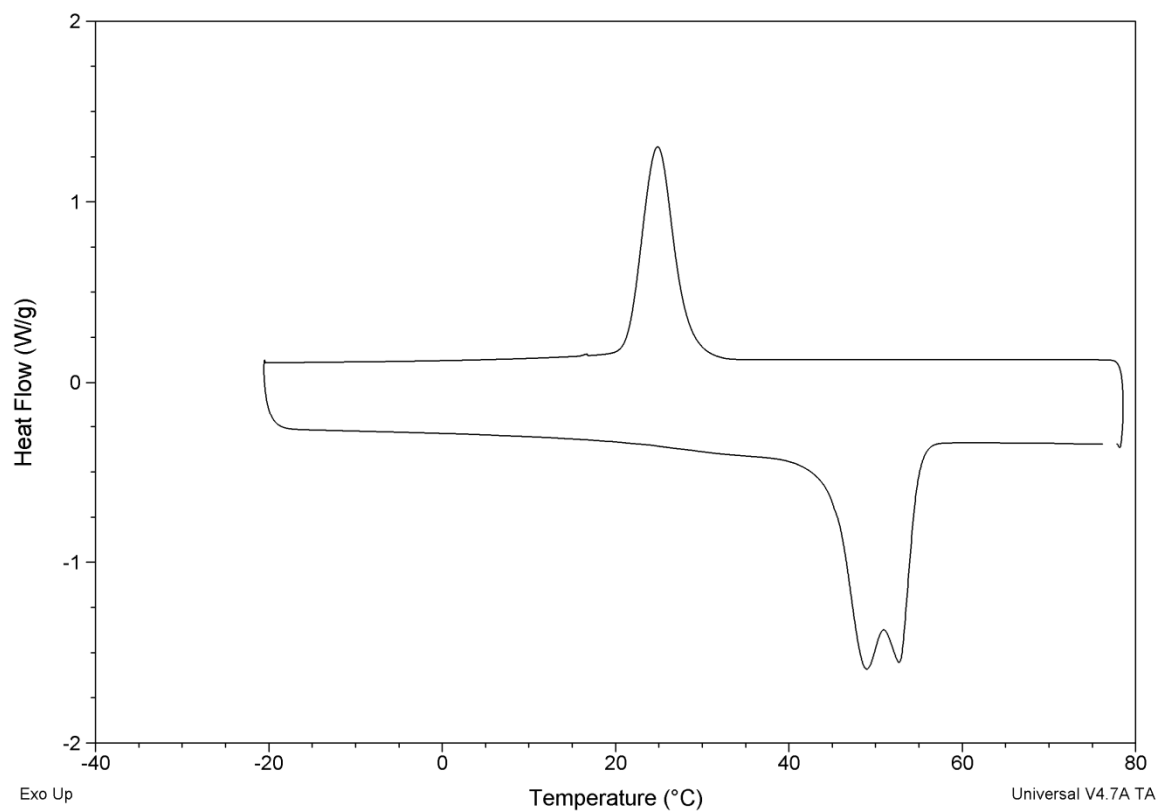


Fig. C19. DSC of $14,300 \text{ g mol}^{-1}$ PCL trimethacrylate.

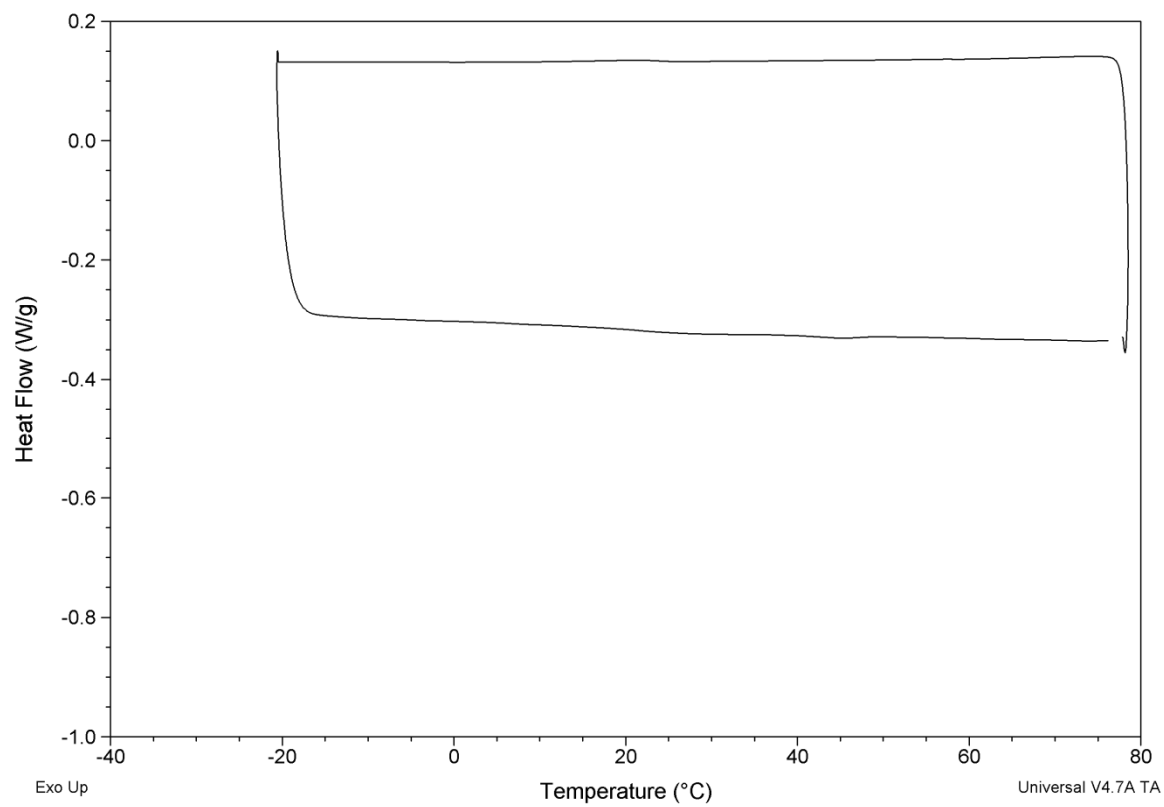


Fig. C20. DSC of 7,000 gmol⁻¹ PCL network.

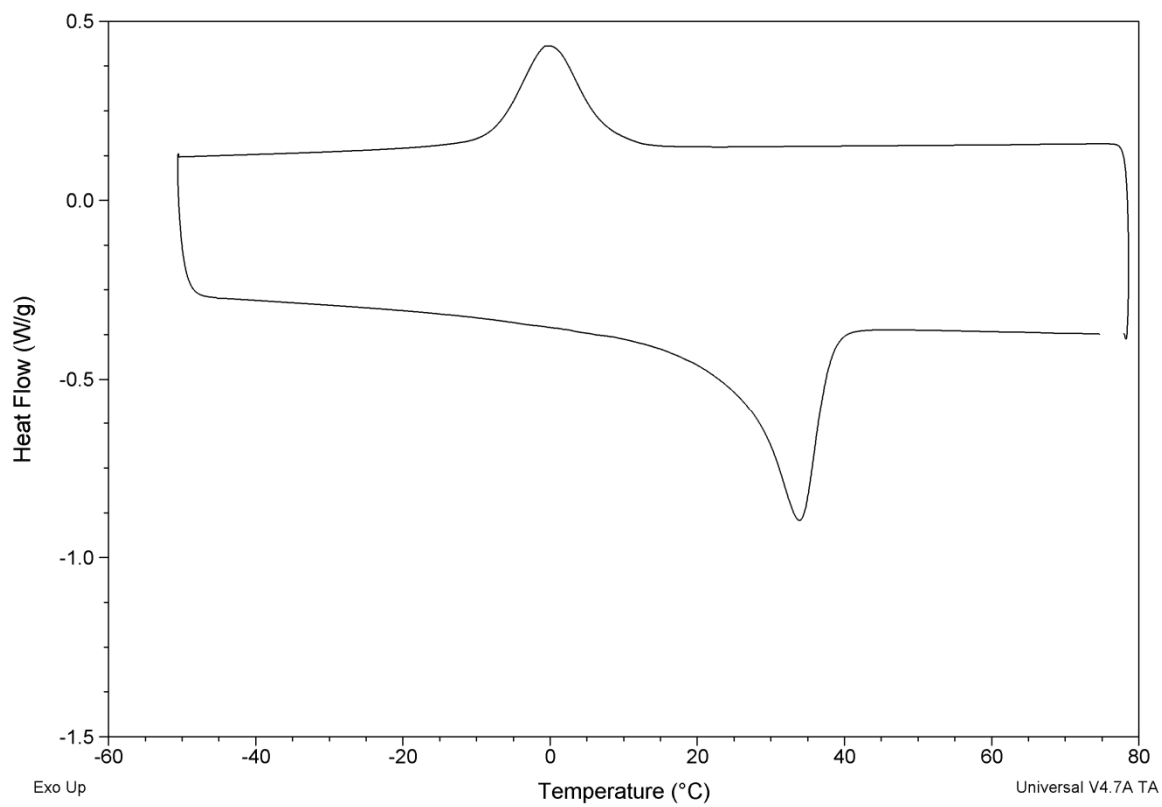


Fig. C21. DSC of 12,500 gmol⁻¹ PCL network.

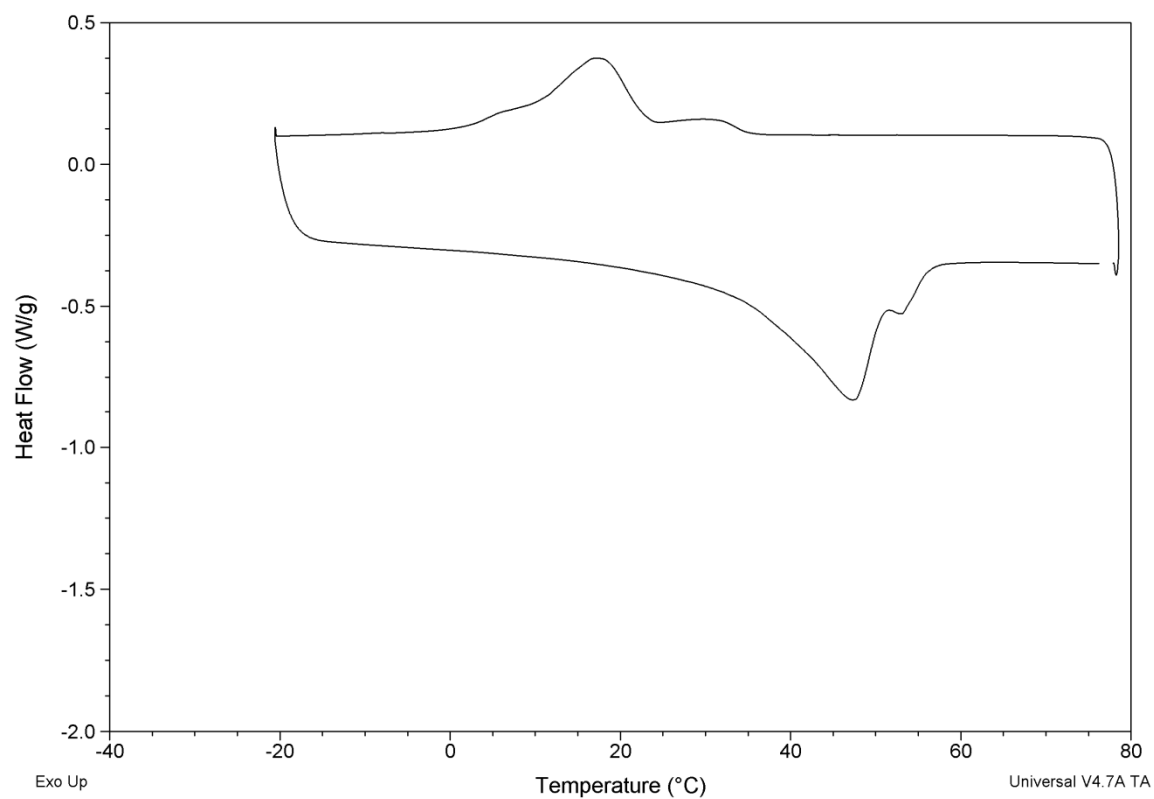


Fig. C22. DSC of 14,300 gmol⁻¹ PCL network.

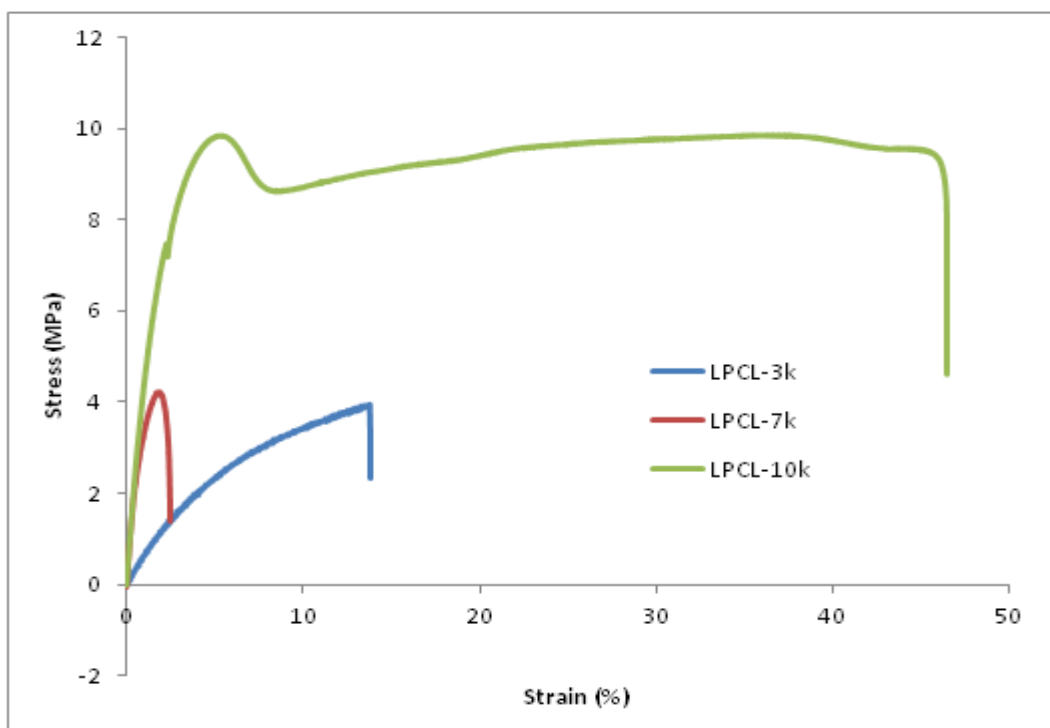


Fig. C23. Stress-strain behavior of PCL networks synthesized from linear prepolymers of vary $\langle M_n \rangle$ at 22 °C.

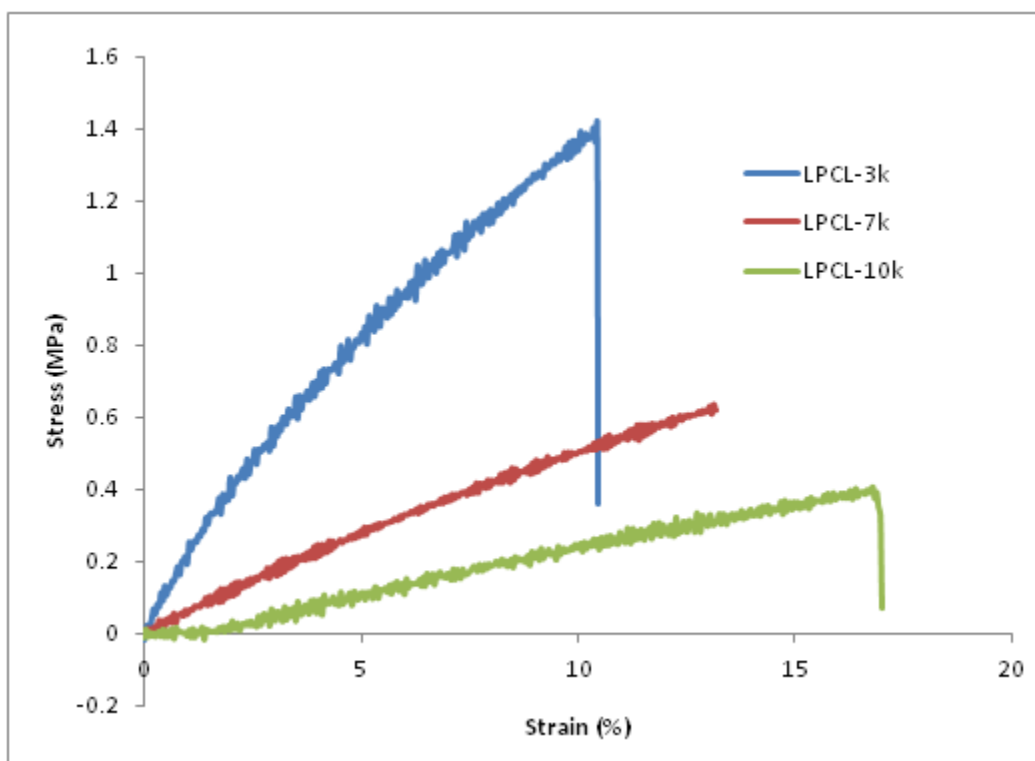


Fig. C24. Stress-strain behavior of PCL networks synthesized from linear prepolymers of vary $\langle M_n \rangle$ at 60 °C.

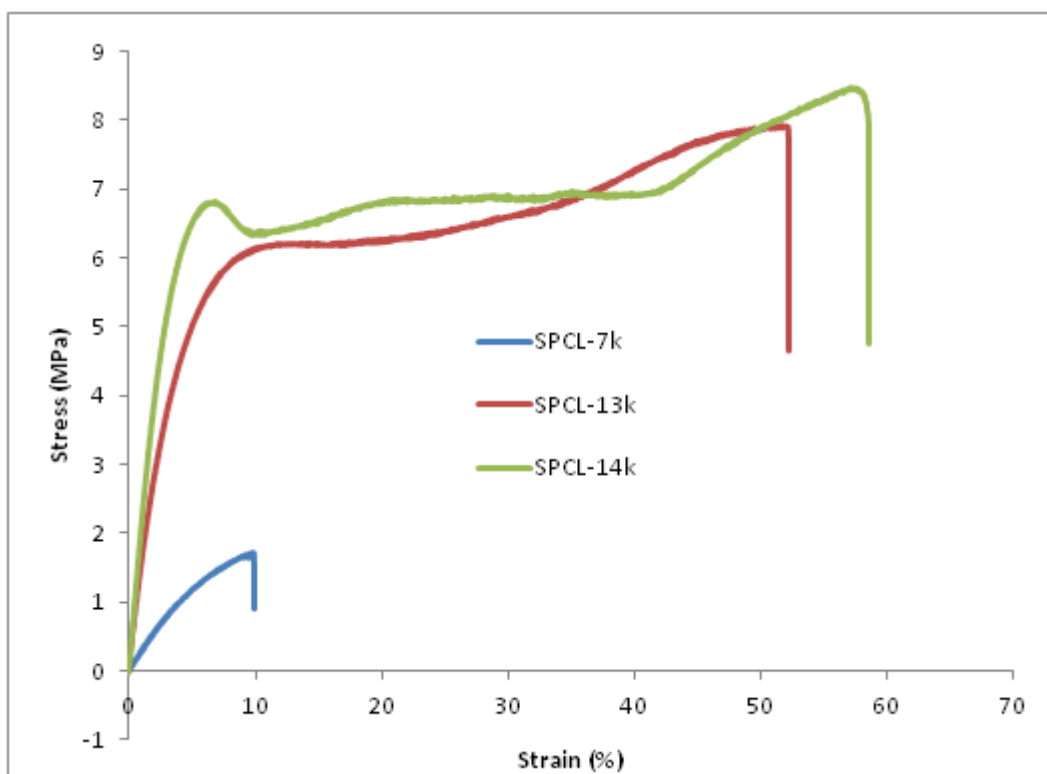


Fig. C25. Stress-strain behavior of PCL networks synthesized from branched prepolymers of vary $\langle M_n \rangle$ at 22 °C.

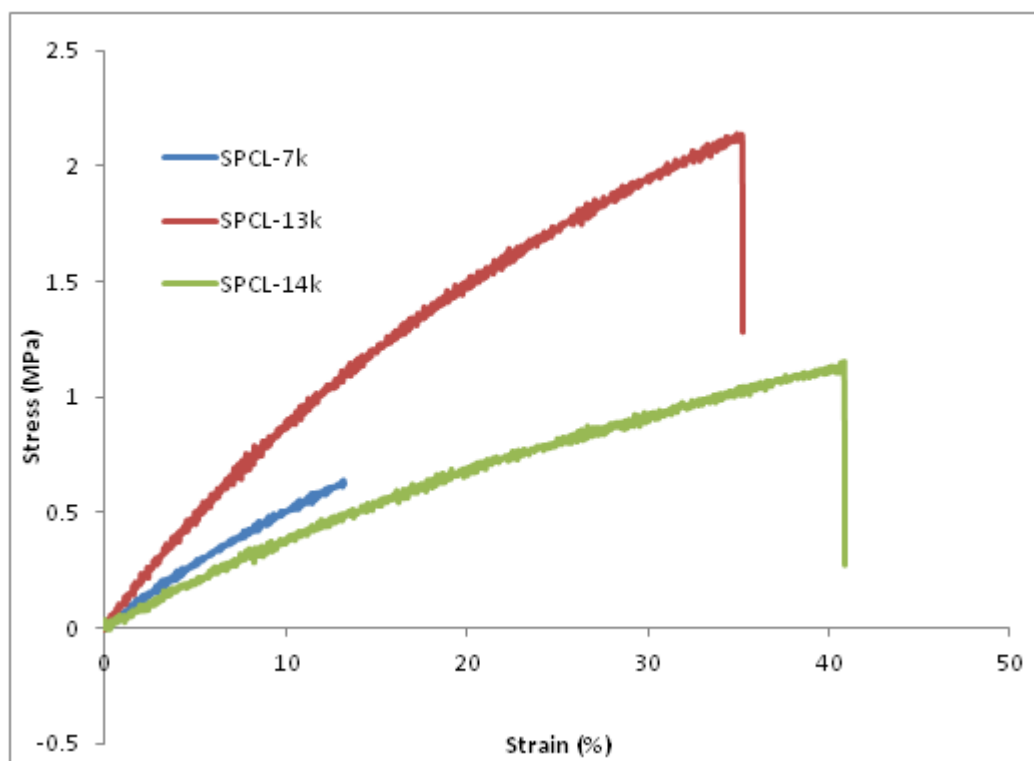


Fig. C26. Stress-strain behavior of PCL networks synthesized from branched prepolymers of vary $\langle M_n \rangle$ at 60 °C.

Appendix D

Supporting Information: Chapter 3

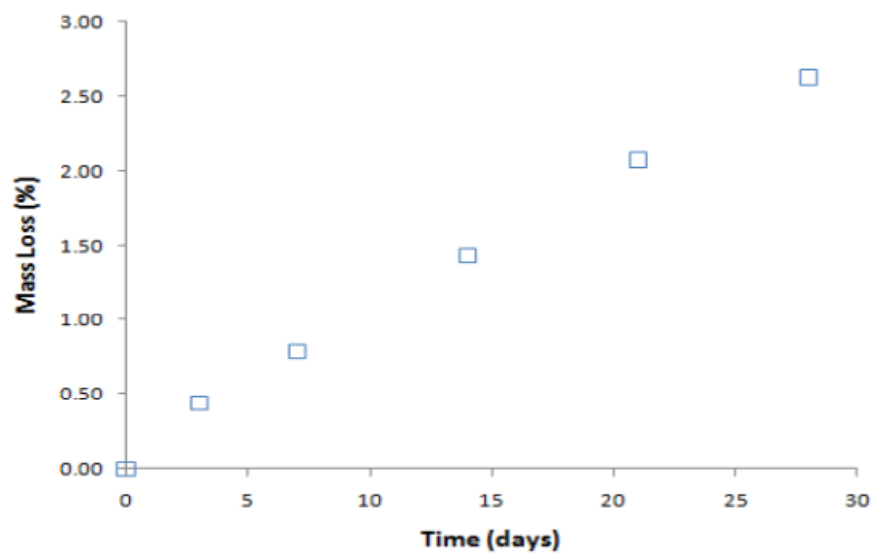


Fig. D1. Mass loss of SPCL-13k in PBS at 37 °C.

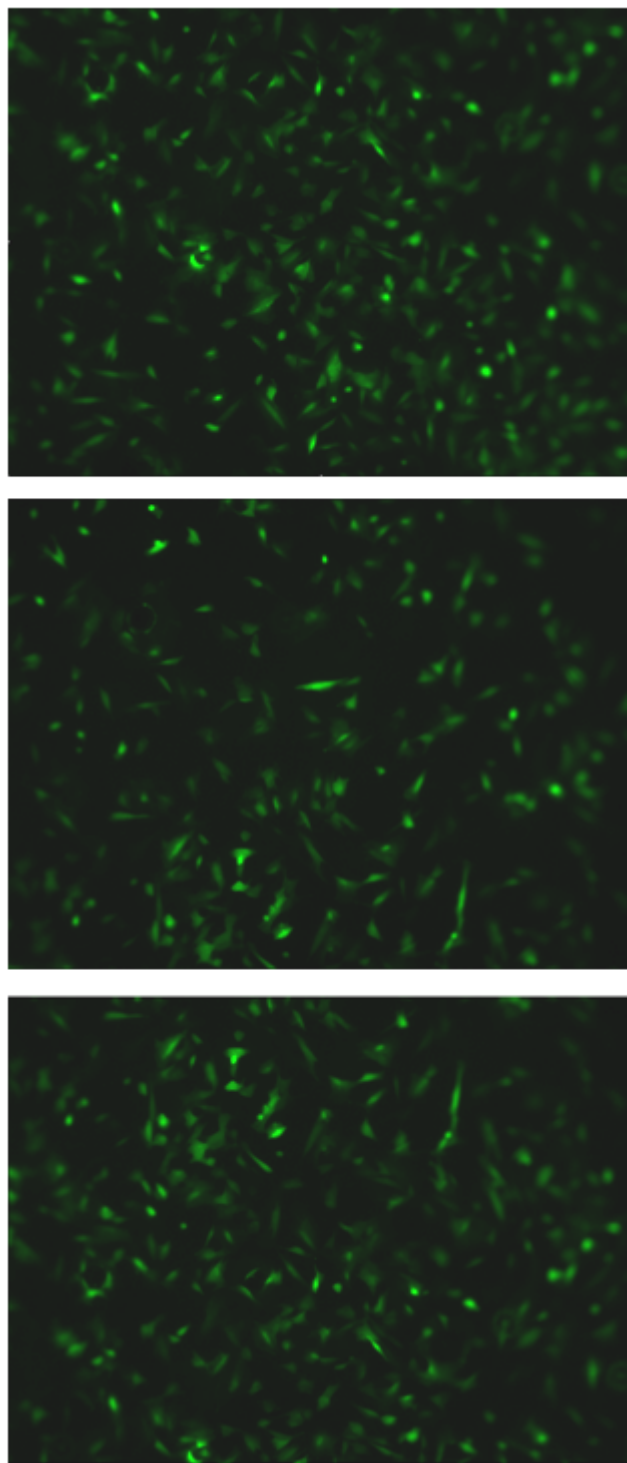


Fig. D2. Fluorescent images of GFP-hMSCs cultured on a static planar PCL surface at 28 °C. Static planar PCL surface 1 of 3.

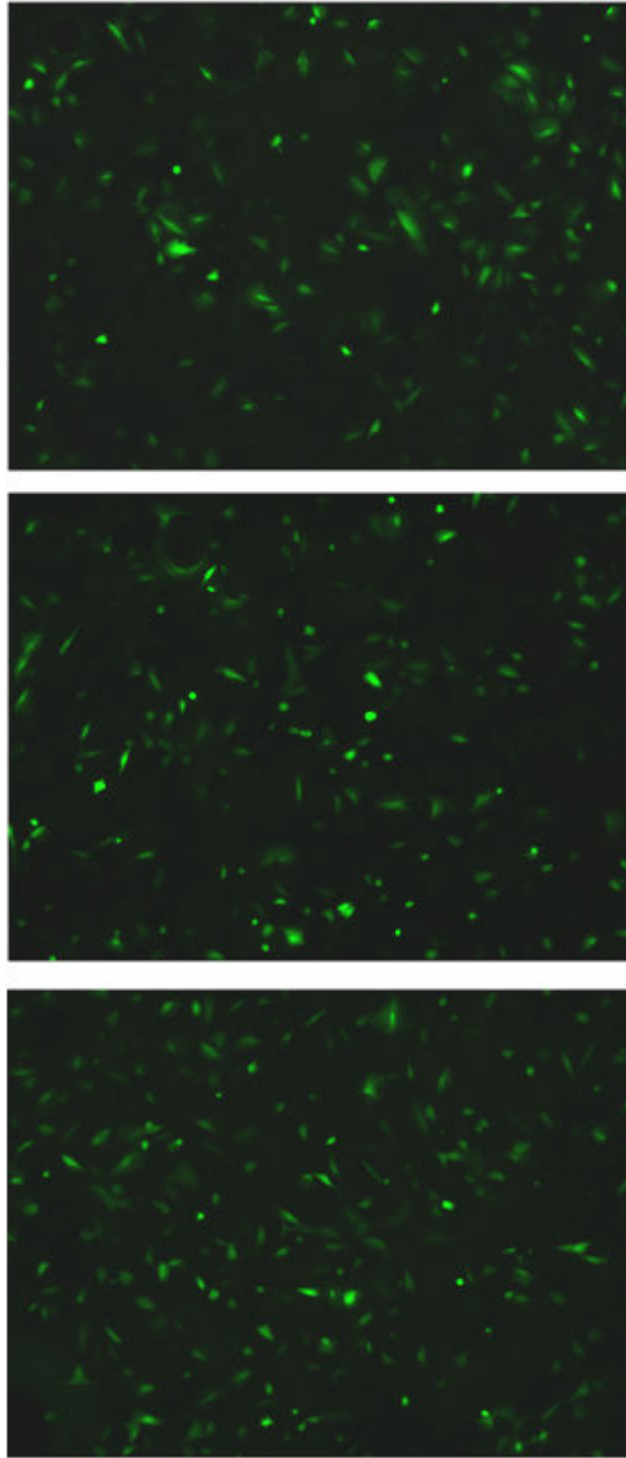


Fig. D3. Fluorescent images of GFP-hMSCs cultured on a static planar PCL surface at 28 °C. Static planar PCL surface 2 of 3.

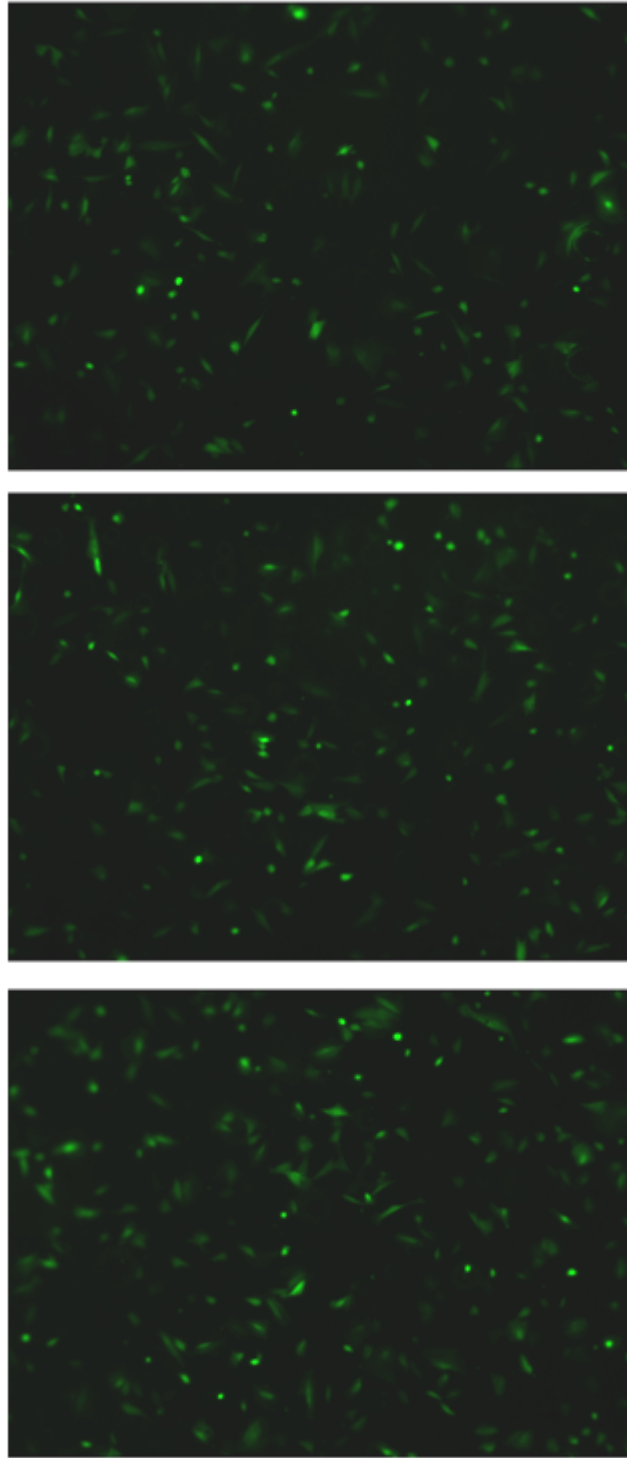


Fig D4. Fluorescent images of GFP-hMSCs cultured on a static planar PCL surface at 28 °C. Static planar PCL surface 3 of 3.

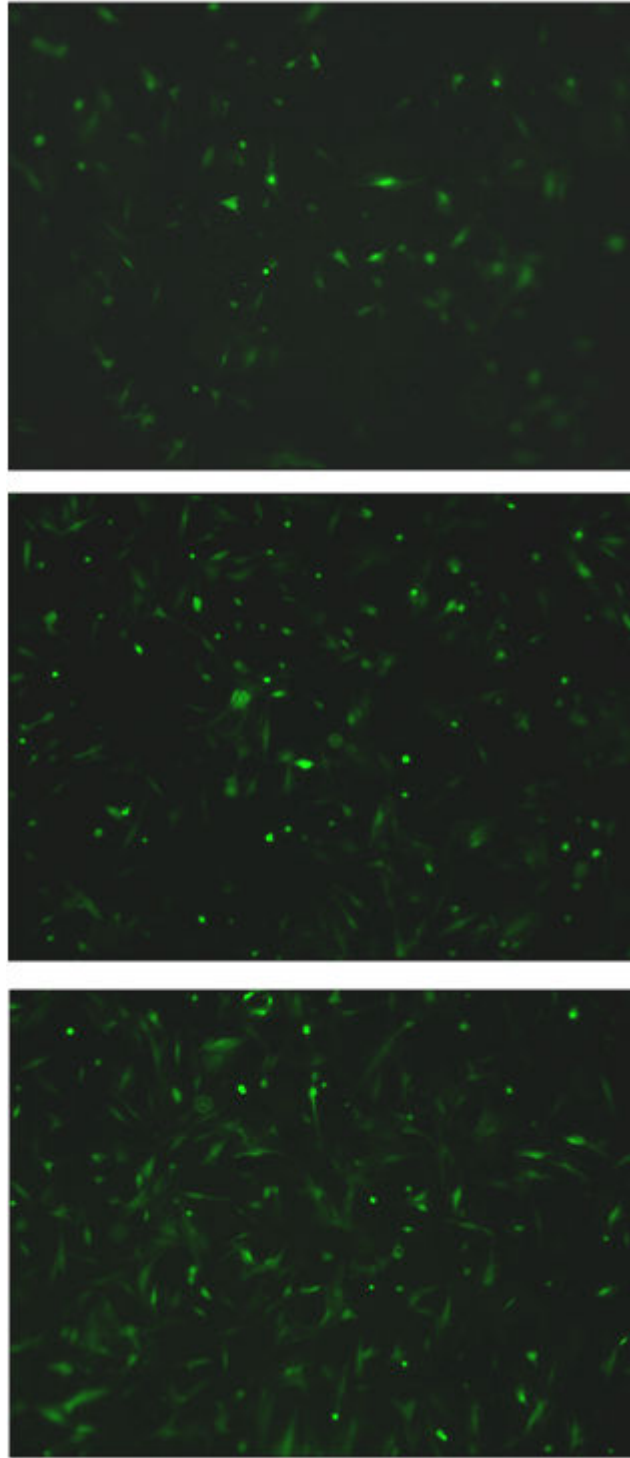


Fig. D5. Fluorescent images of GFP-hMSCs cultured on a static planar PCL surface at 37 °C. Static planar PCL surface 1 of 3.

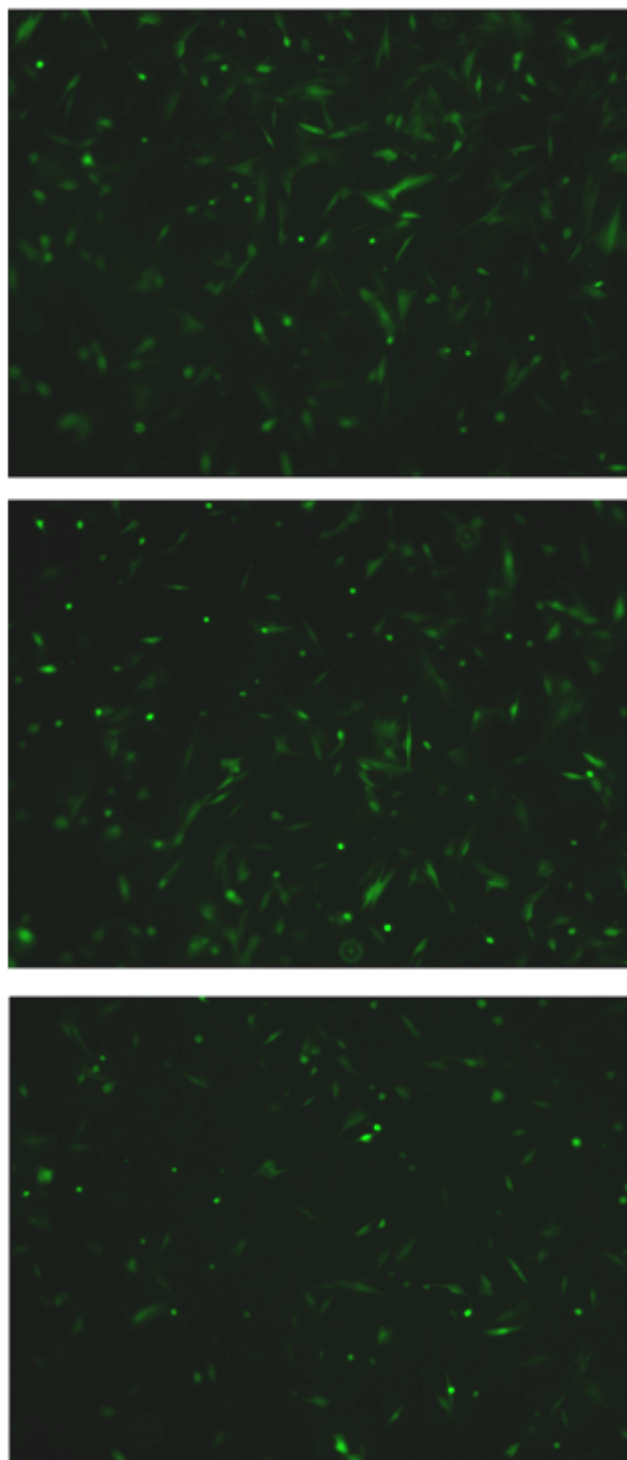


Fig. D6. Fluorescent images of GFP-hMSCs cultured on a static planar PCL surface at 37 °C. Static planar PCL surface 2 of 3.

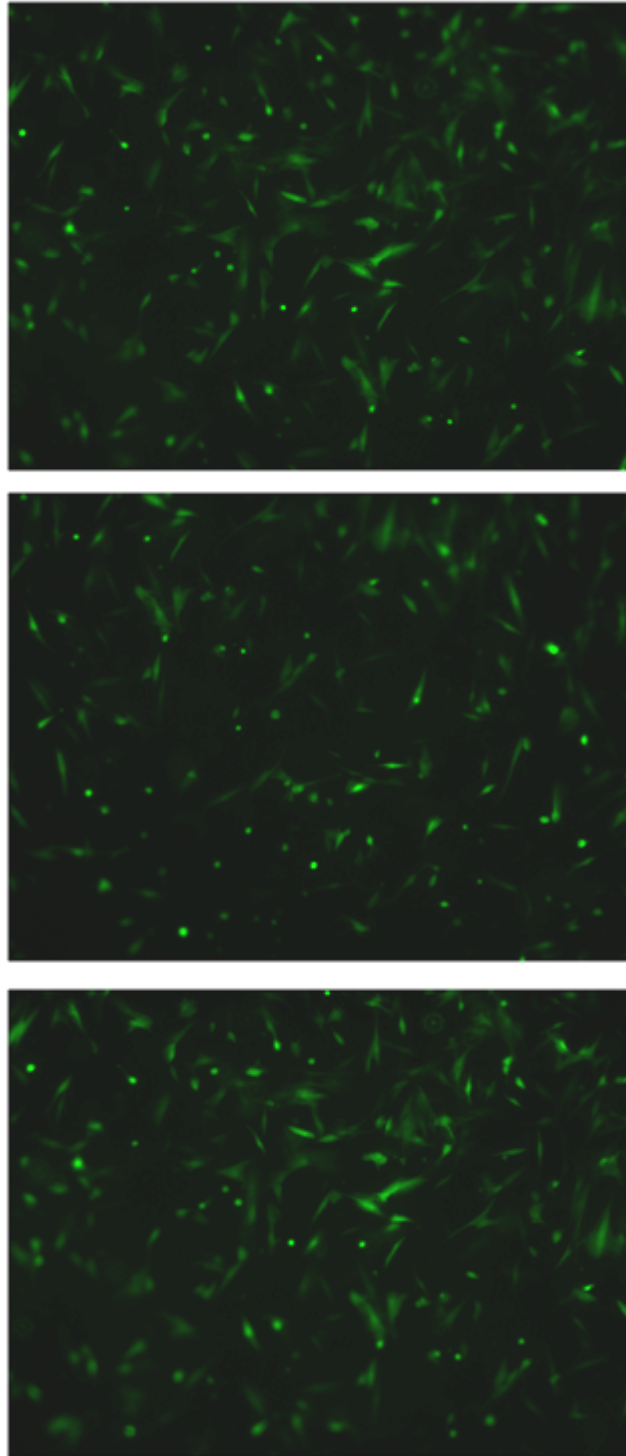


Fig. D7. Fluorescent images of GFP-hMSCs cultured on a static planar PCL surface at 37 °C. Static planar PCL surface 3 of 3.

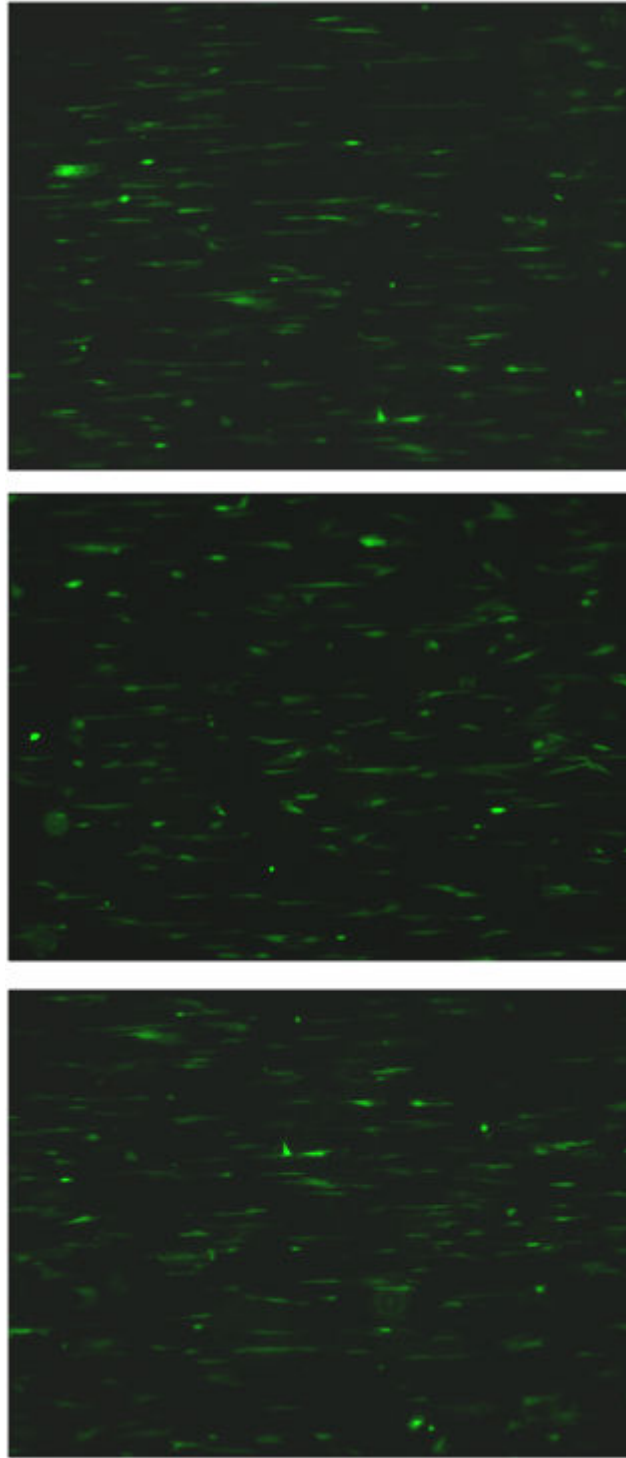


Fig. D8. Fluorescent images of GFP-hMSCs cultured on a static 3 x 5 μm PCL channel microarray at 28 °C. Static 3 x 5 μm PCL channel microarray 1 of 3.

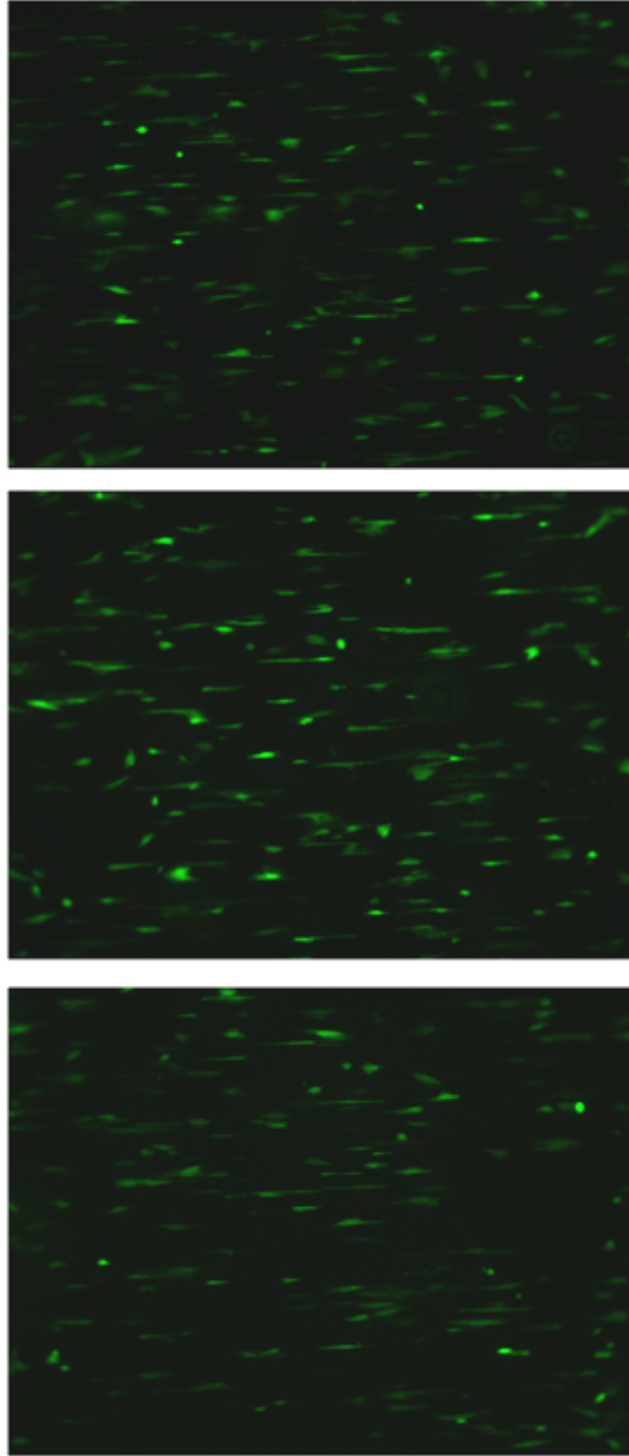


Fig. D9. Fluorescent images of GFP-hMSCs cultured on a static 3 x 5 μm PCL channel microarray at 28 $^{\circ}\text{C}$. Static 3 x 5 μm PCL channel microarray 2 of 3.

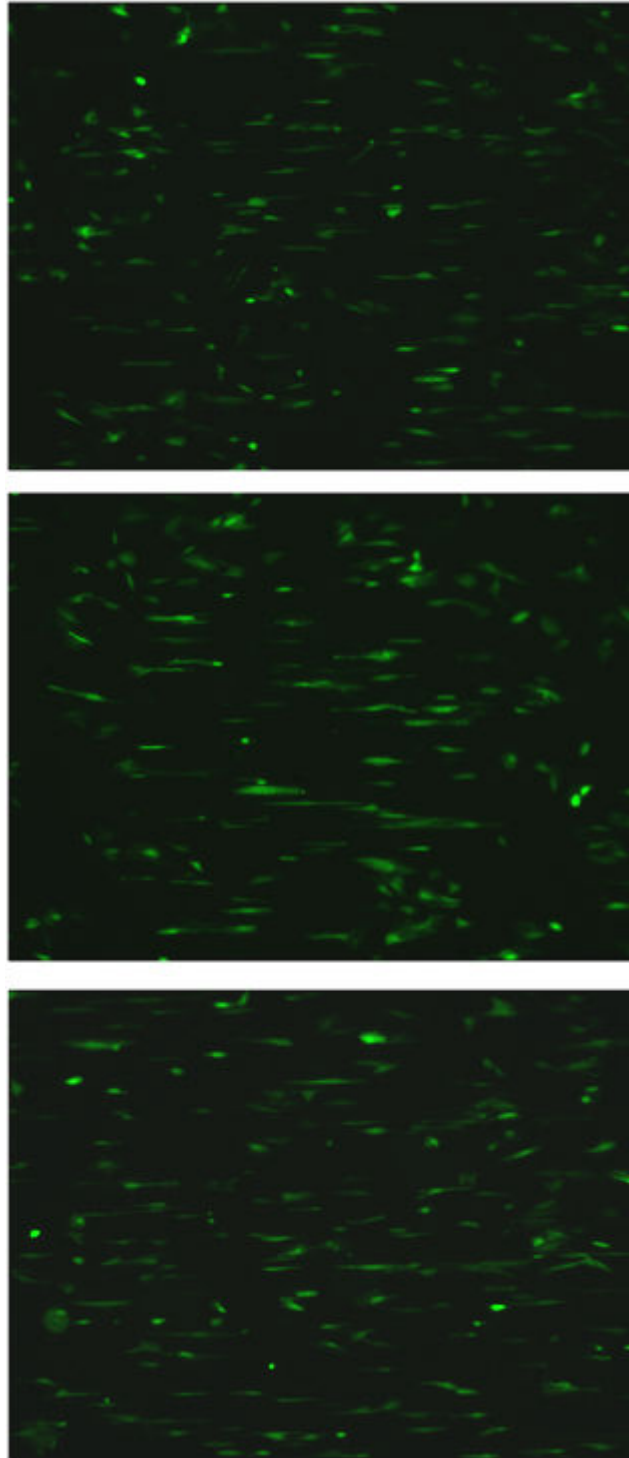


Fig. D10. Fluorescent images of GFP-hMSCs cultured on a static 3 x 5 μm PCL channel microarray at 28 °C. Static 3 x 5 μm PCL channel microarray 3 of 3.

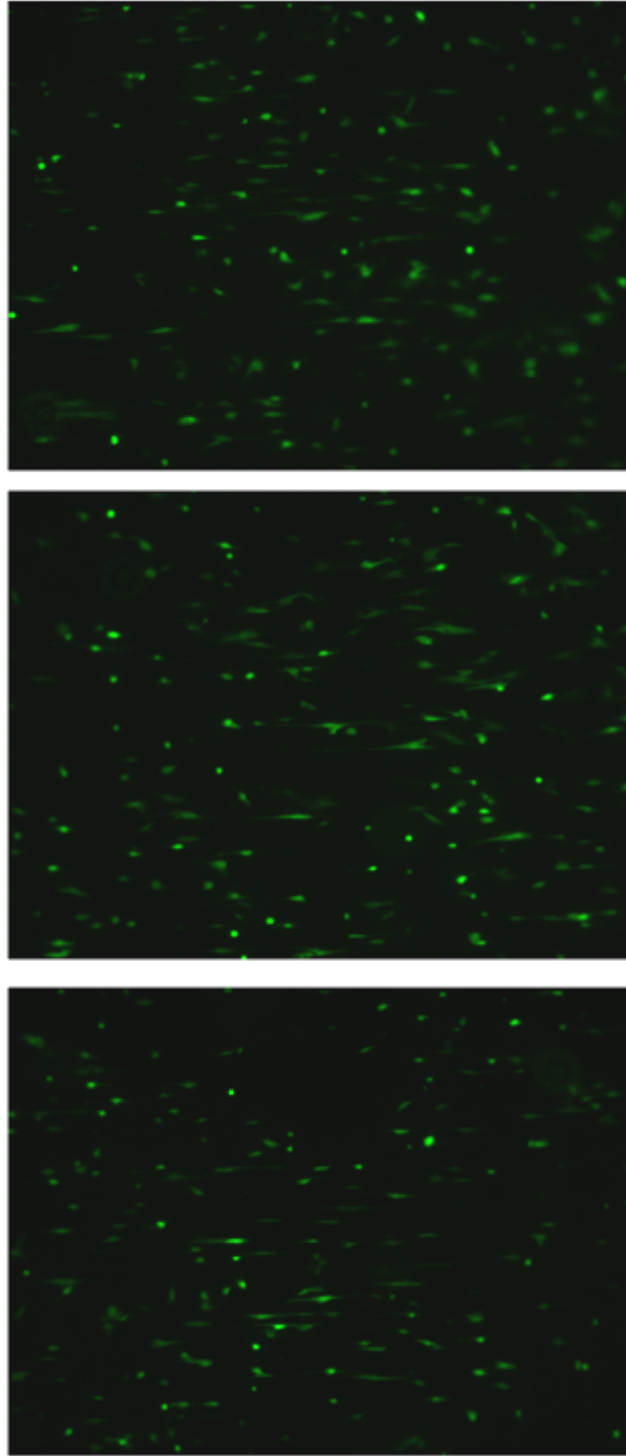


Fig. D11. Fluorescent images of GFP-hMSCs cultured on a static 3 x 5 μm PCL channel microarray at 37 °C. Static 3 x 5 μm PCL channel microarray 1 of 3.

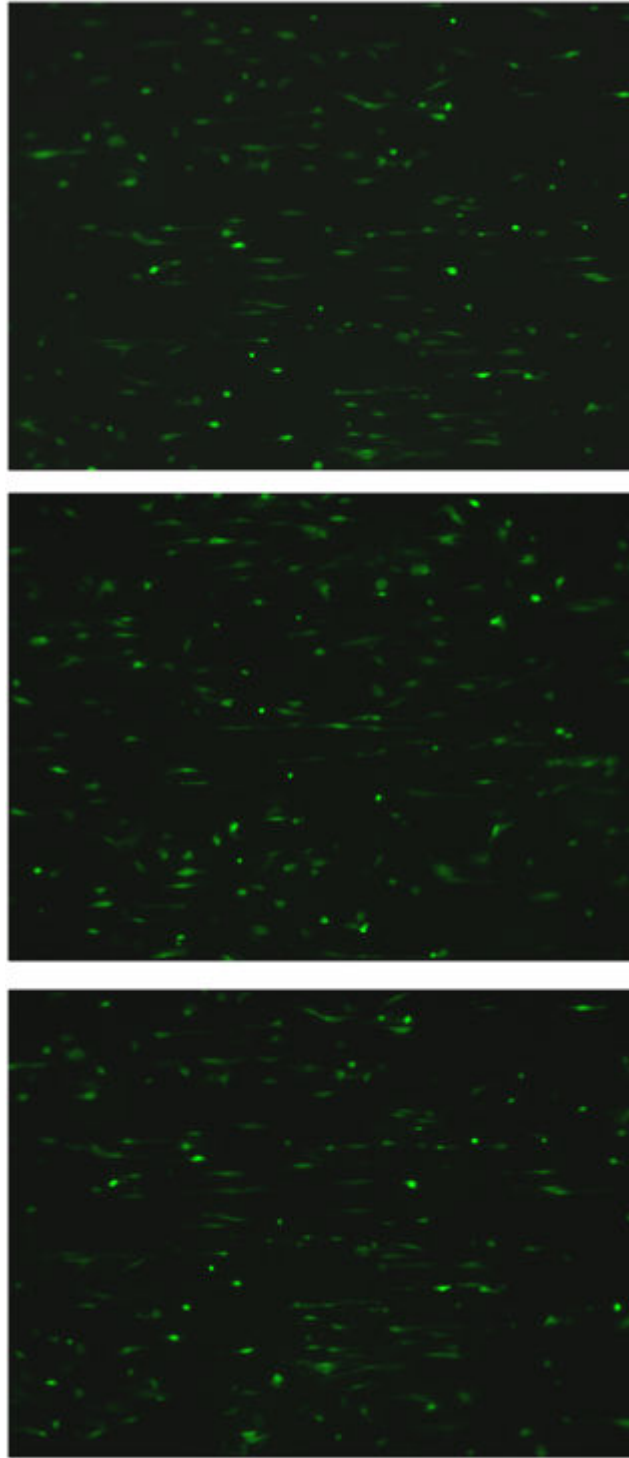


Fig. D12. Fluorescent images of GFP-hMSCs cultured on a static 3 x 5 μm PCL channel microarray at 37 °C. Static 3 x 5 μm PCL channel microarray 2 of 3.

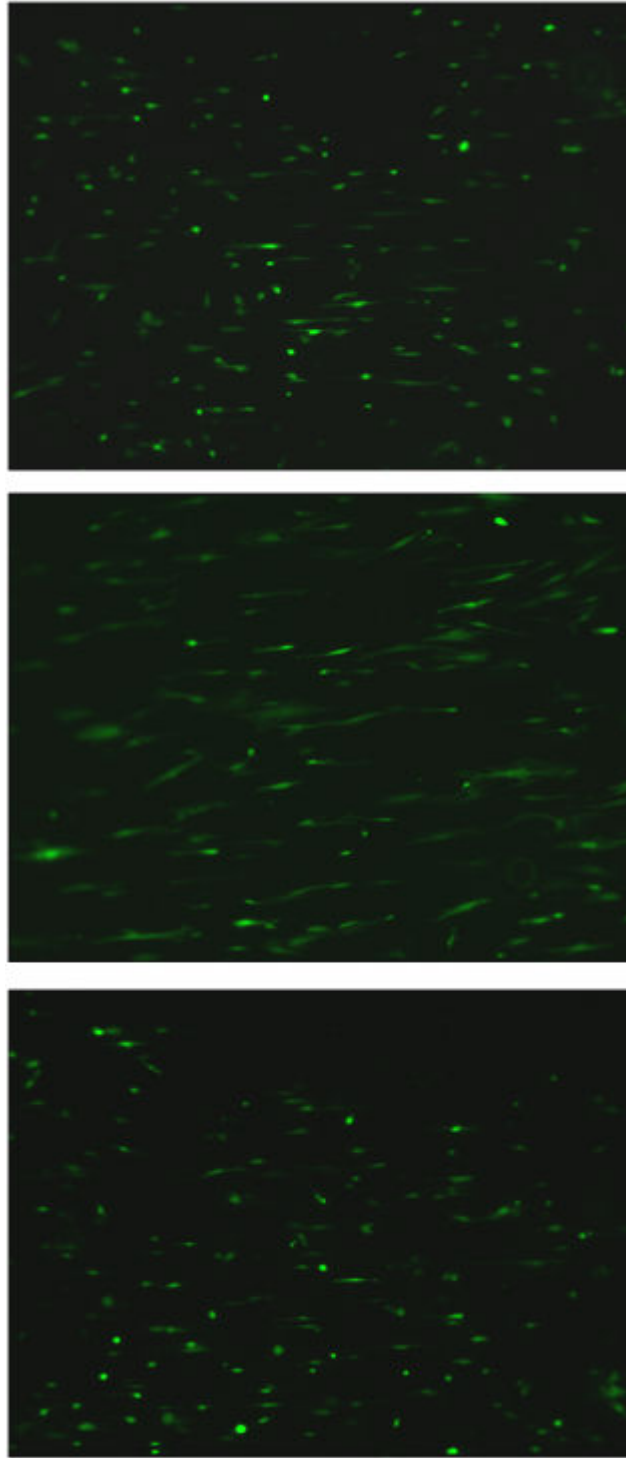


Fig. D13. Fluorescent images of GFP-hMSCs cultured on a static 3 x 5 μm PCL channel microarray at 37 °C. Static 3 x 5 μm PCL channel microarray 3 of 3.

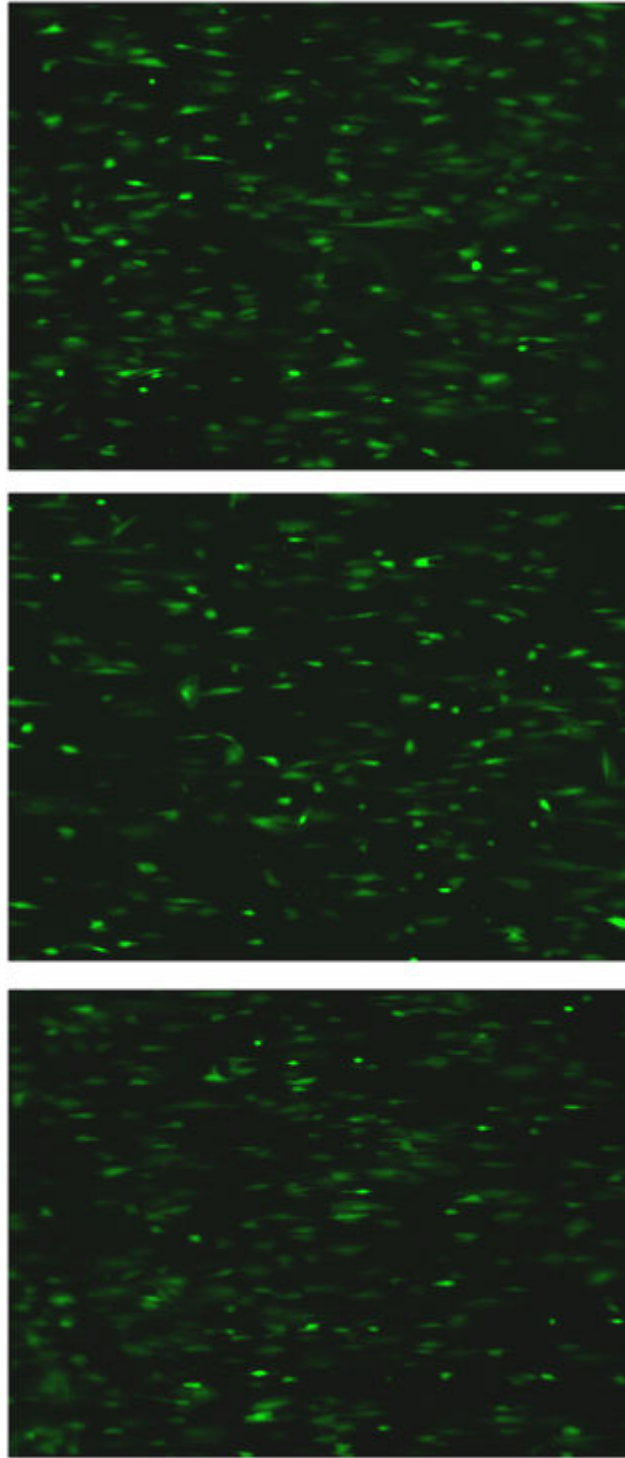


Fig. D14. Fluorescent images of GFP-hMSCs cultured on a temporary 3 x 5 μm PCL channel microarray at 28 °C. Temporary 3 x 5 μm PCL channel microarray 1 of 3.

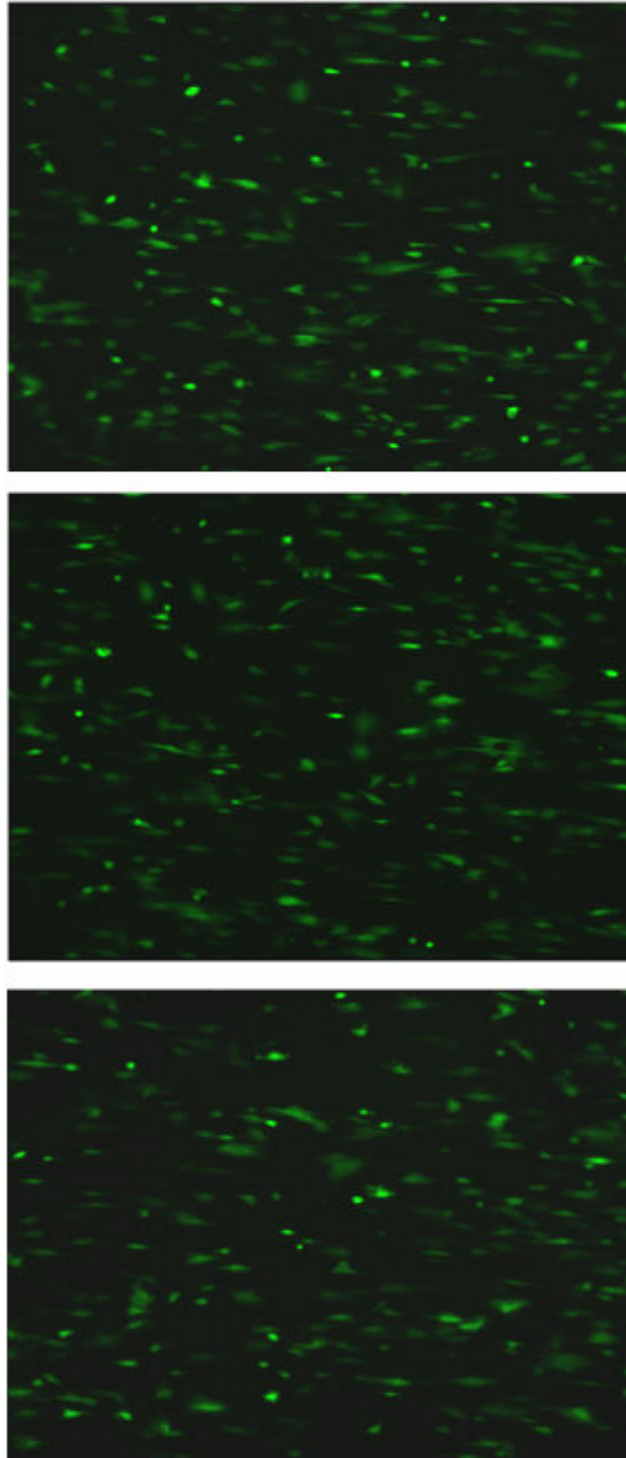


Fig. D15. Fluorescent images of GFP-hMSCs cultured on a temporary 3 x 5 μm PCL channel microarray at 28 °C. Temporary 3 x 5 μm PCL channel microarray 2 of 3.

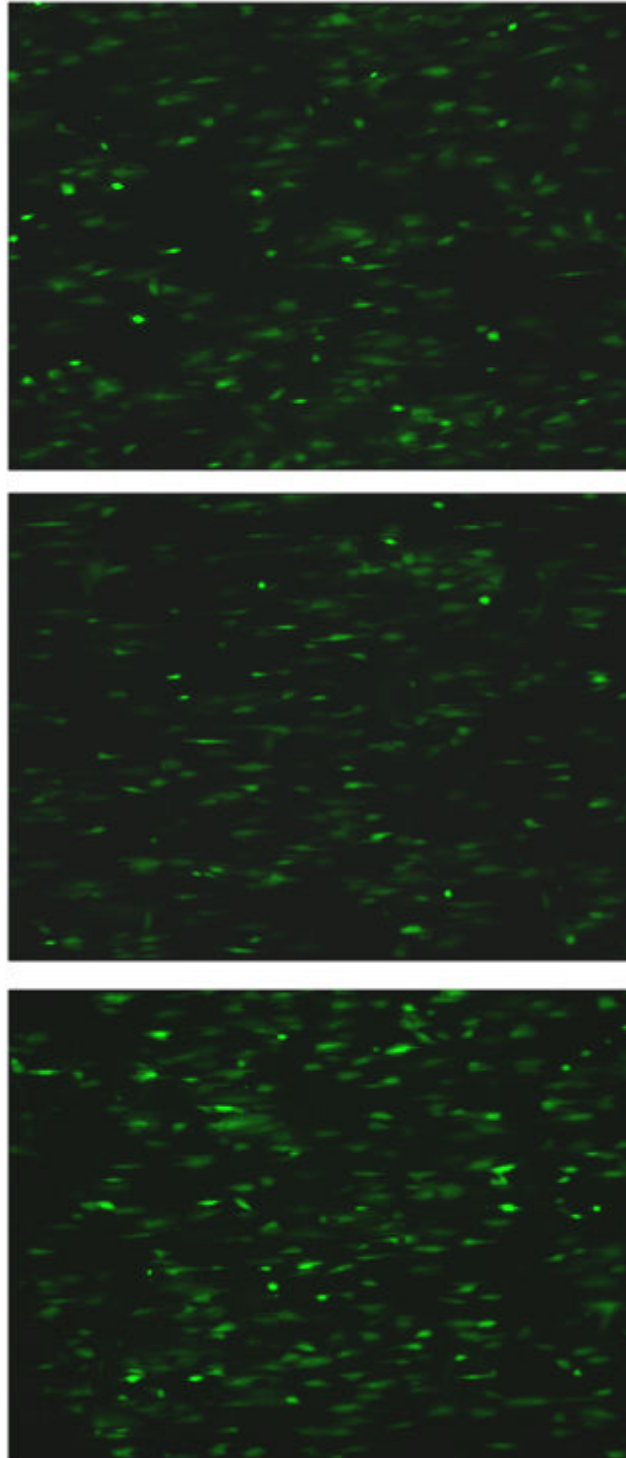


Fig. D16. Fluorescent images of GFP-hMSCs cultured on a temporary 3 x 5 μm PCL channel microarray at 28 °C. Temporary 3 x 5 μm PCL channel microarray 3 of 3.

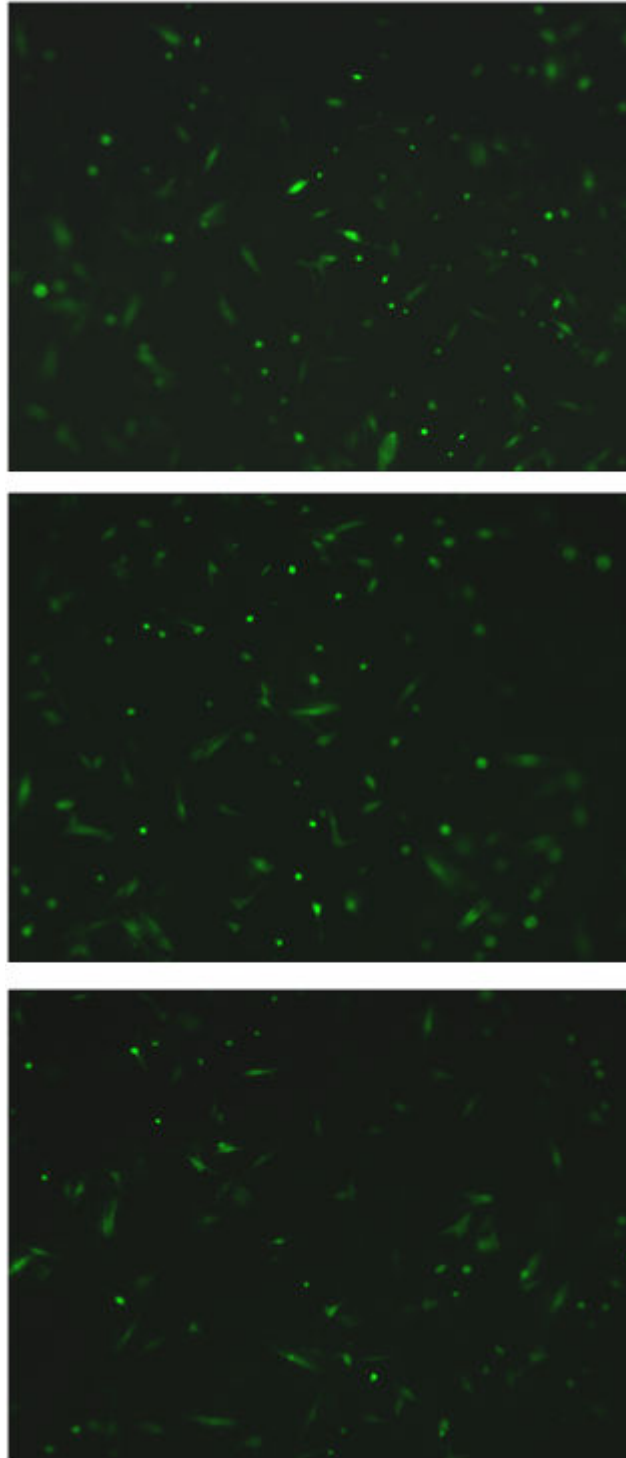


Fig. D17. Fluorescent images of GFP-hMSCs cultured on a recovered planar PCL surface at 37 °C. Recovered planar PCL surface 1 of 3.

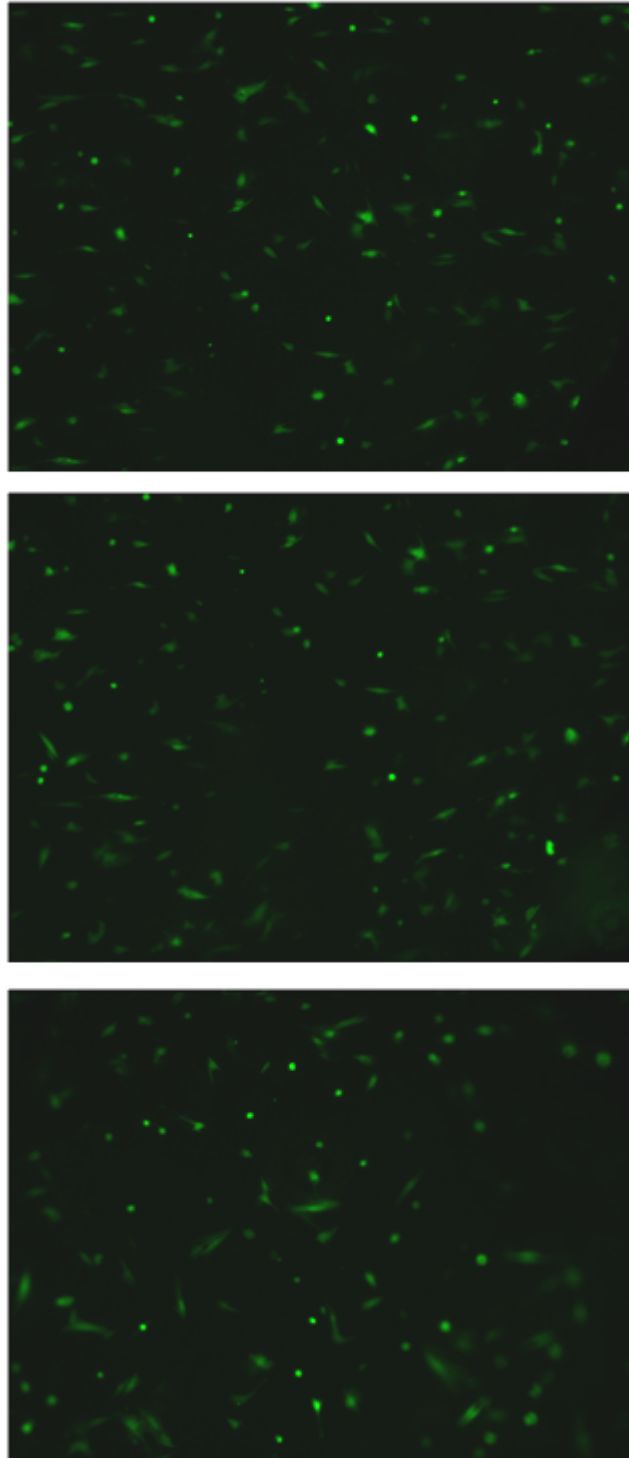


Fig. D18. Fluorescent images of GFP-hMSCs cultured on a recovered planar PCL surface at 37 °C. Recovered planar PCL surface 2 of 3.

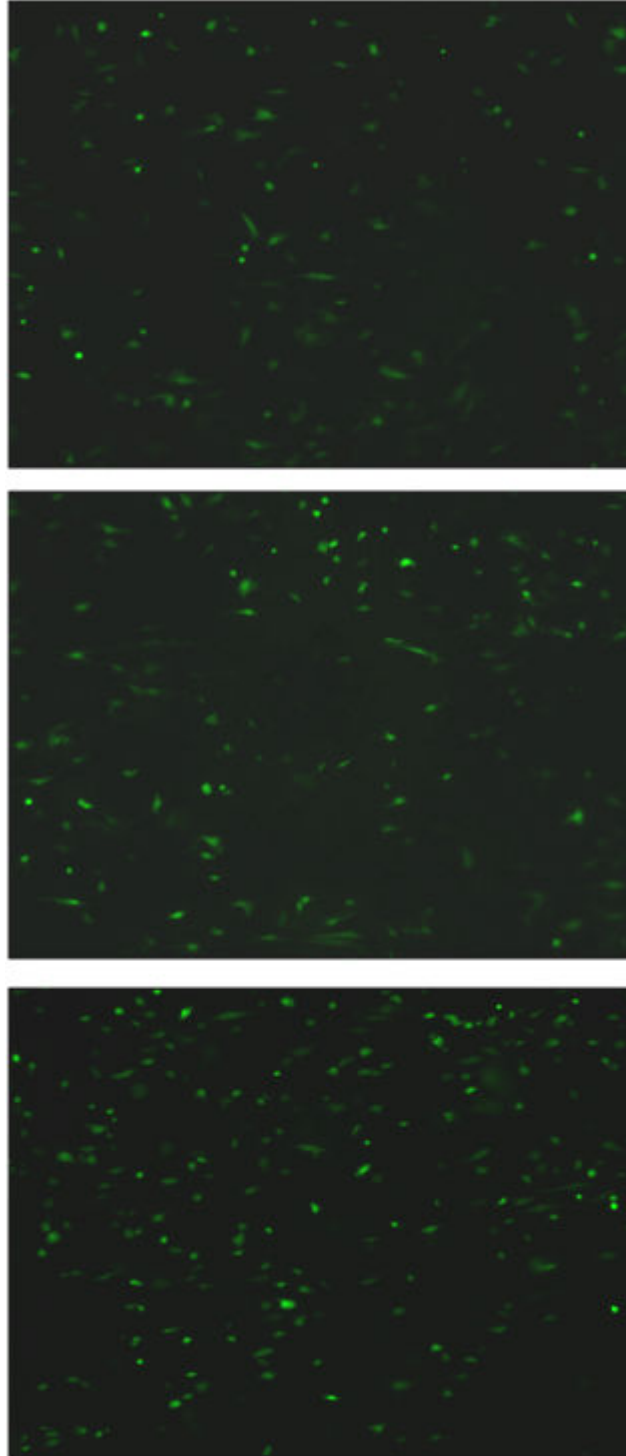


Fig. D19. Fluorescent images of GFP-hMSCs cultured on a recovered planar PCL surface at 37 °C. Recovered planar PCL surface 3 of 3.

Appendix E

Supporting Information: Chapter 4

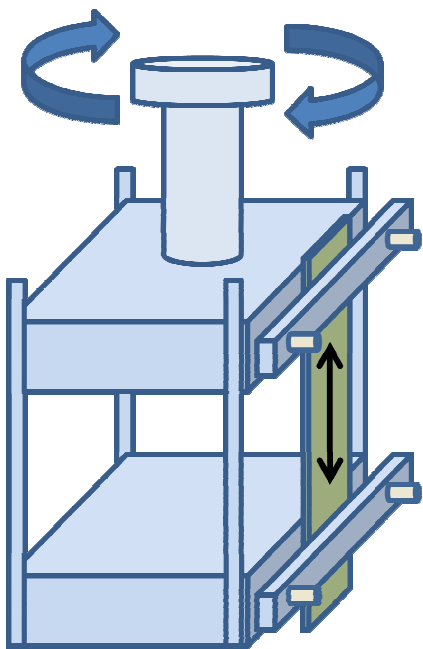


Fig. E1. Schematic of vice used to stretch AuNP films.

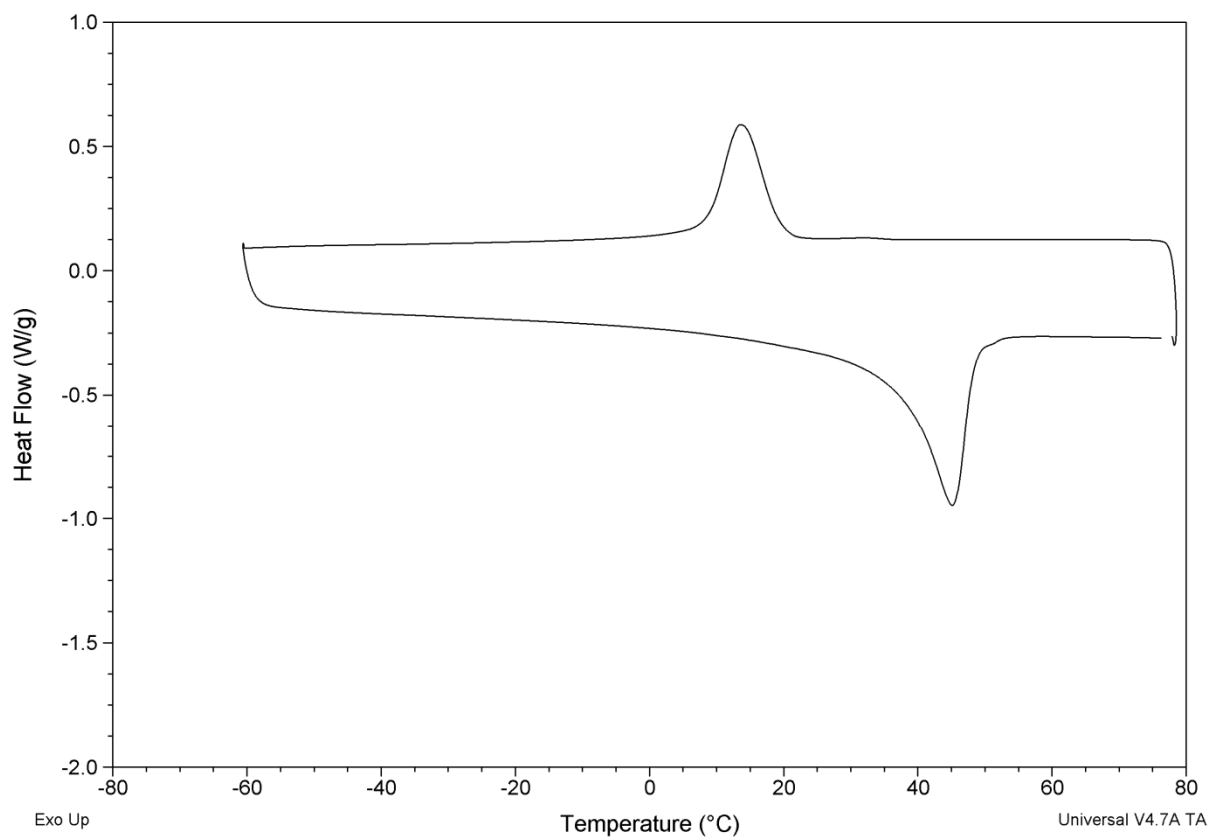


Fig. E2. DSC of a neat PCL network synthesized from 13,200 gmol^{-1} branched precursors.

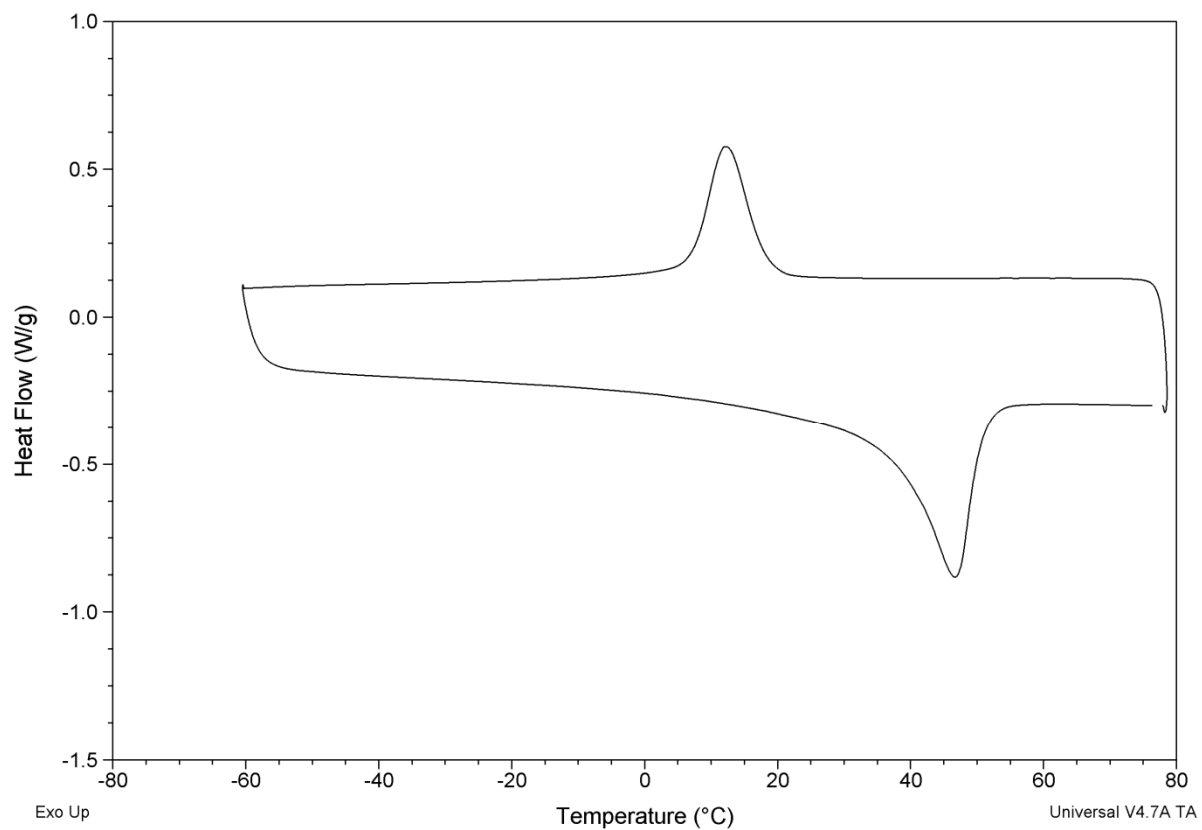


Fig. E3. DSC of a Au nanoshell/PCL nanocomposite film comprised of 0.1 wt% gold nanoshells and $13,200 \text{ gmol}^{-1}$ branched PCL precursors.

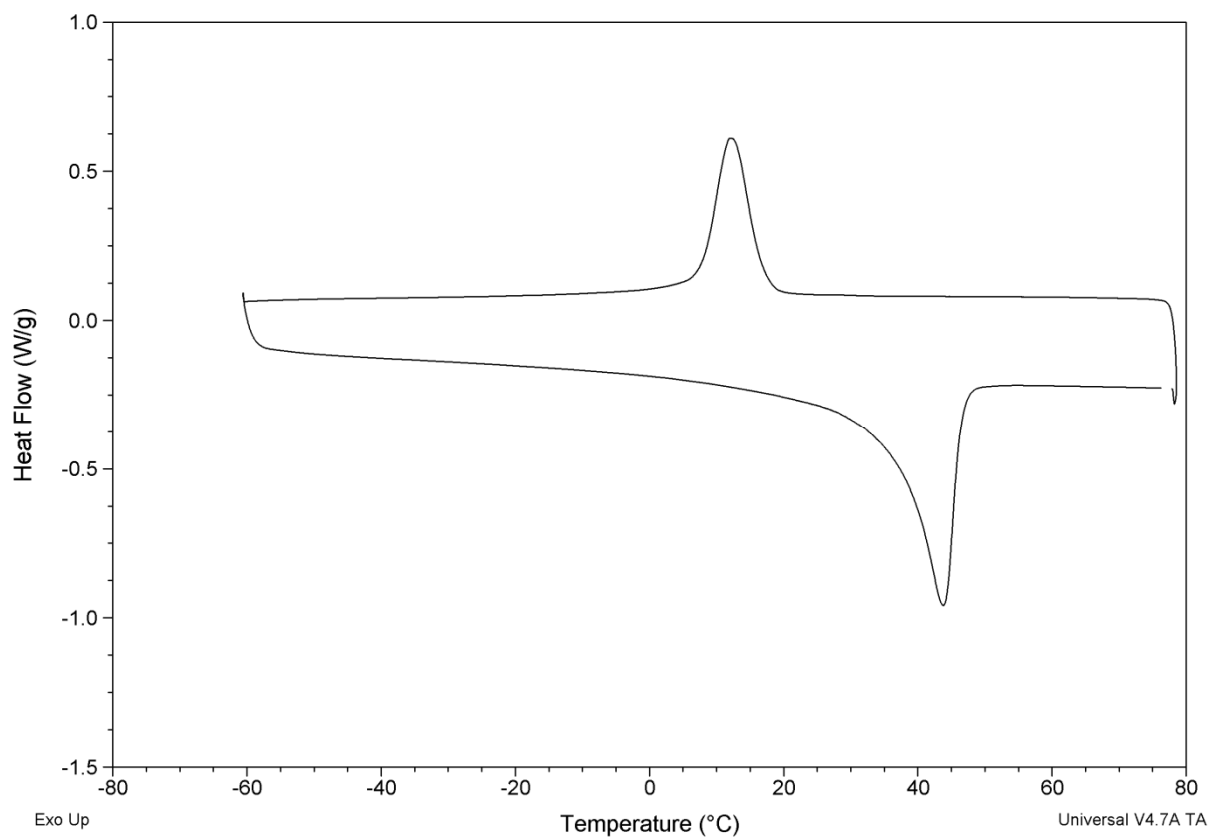


Fig. E4. DSC of a Au nanoshell/PCL nanocomposite film comprised of 0.5 wt% gold nanoshells and 13,200 gmol^{-1} branched PCL precursors.

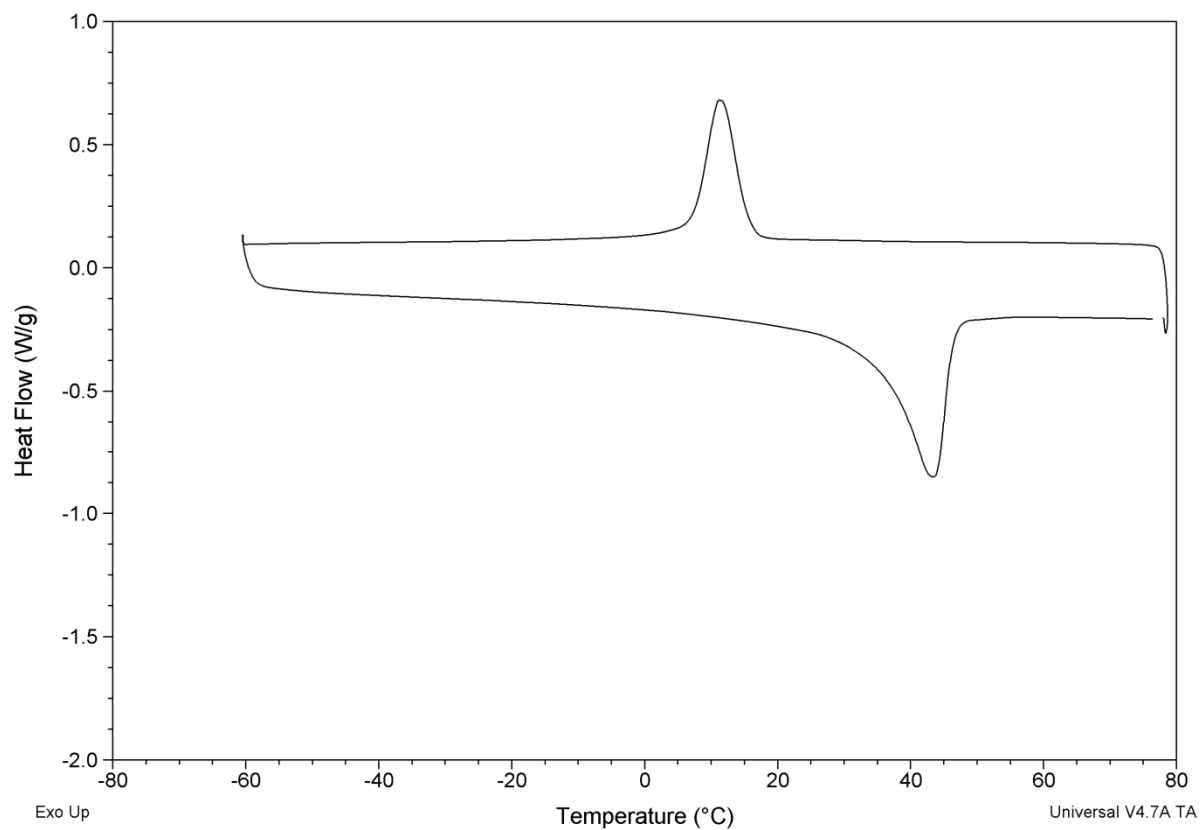


Fig. E5. DSC of a Au nanoshell/PCL nanocomposite film comprised of 1.0 wt% gold nanoshells and $13,200 \text{ gmol}^{-1}$ branched PCL precursors.

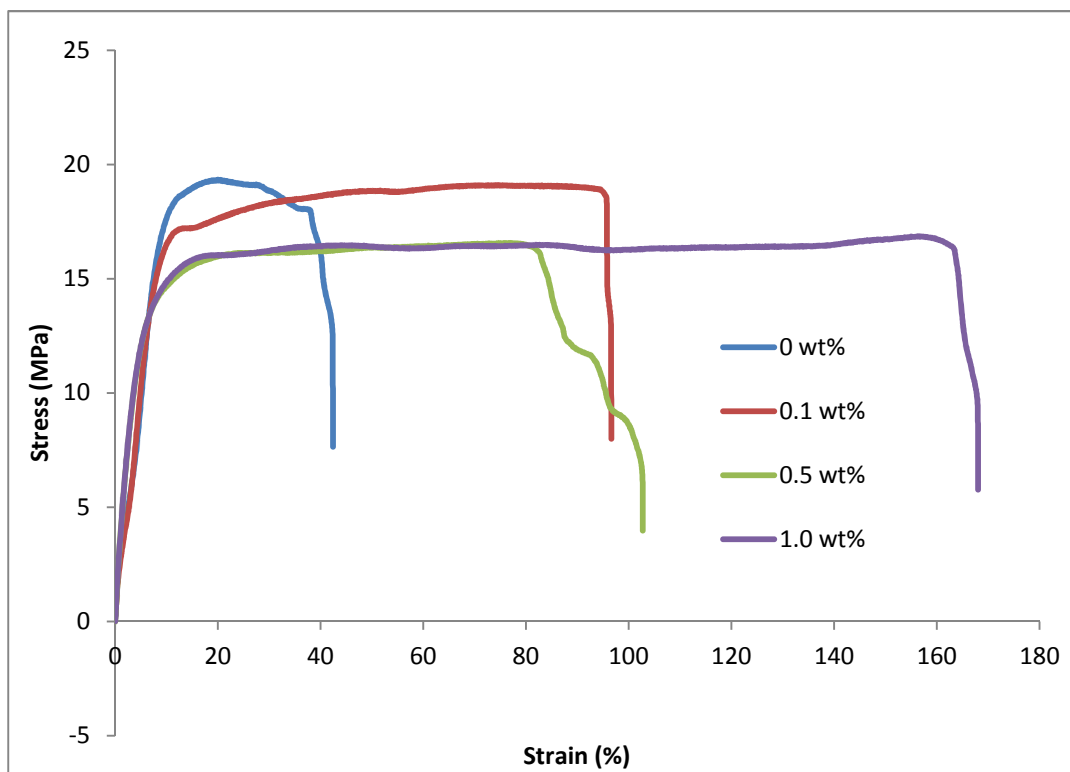


Fig. E6. Stress-strain behavior of polymer nanocomposites synthesized from branched PCL precursors ($\langle M_n \rangle = 13,200 \text{ gmol}^{-1}$) containing vary weight fractions of 150 nm Au nanoshells at 22 °C.

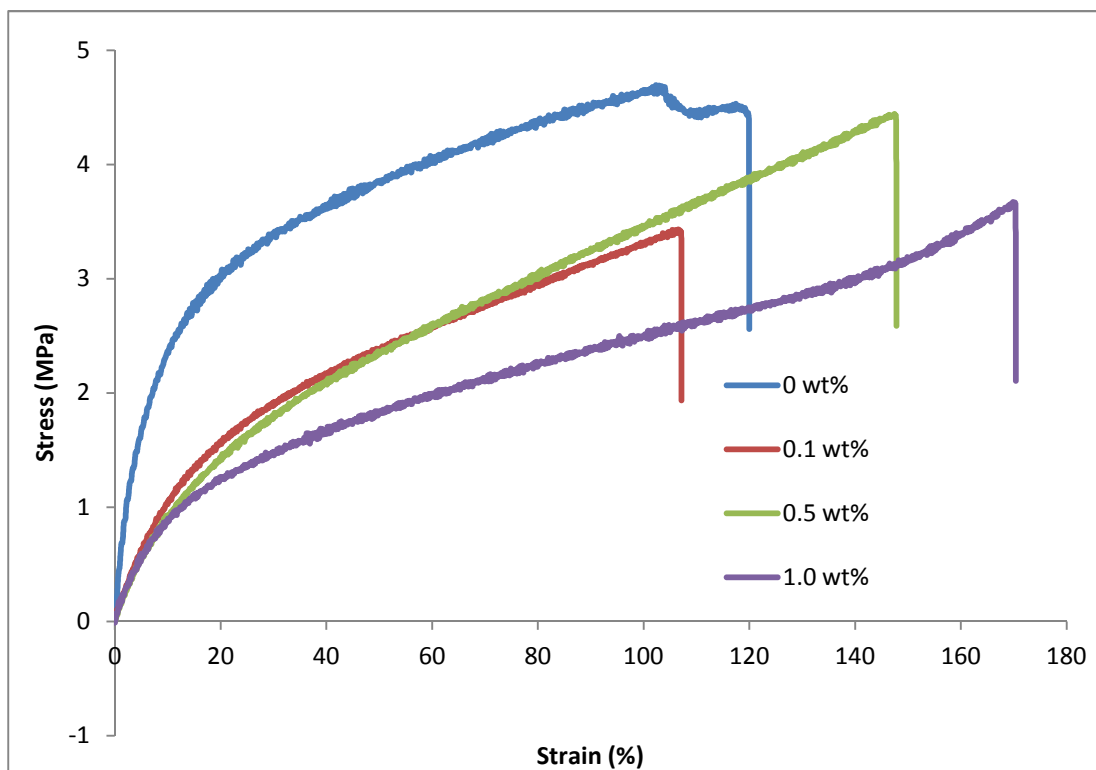


Fig. E7. Stress-strain behavior of polymer nanocomposites synthesized from branched PCL precursors ($\langle M_n \rangle = 13,200 \text{ g mol}^{-1}$) containing vary weight fractions of 150 nm Au nanoshells at 60 °C.

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