

## Supporting Information

### Dynamic alterations in hepatocellular function by on-demand elasticity and roughness modulation

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#### 1. Fabrication and Characterization of crosslinked PCL elastomers

The crosslinked poly( $\epsilon$ -caprolactone) (PCL) elastomer was prepared by reacting four-arm branched (4b) PCL end-functionalized with acrylates in the presence of two-branched (2b) PCL telechelic linear diacrylates. Two-branched and four-branched PCL were synthesized by  $\epsilon$ -caprolactone (CL) ring-opening polymerization that was initiated with tetramethylene glycol and pentaerythritol as initiators, respectively. Following polymerization, hydroxyl end groups were modified with acryloyl chloride to introduce acrylate functionality onto the branch ends. The structures and the molecular weights were determined by  $^1\text{H}$  NMR spectroscopy (JEOL, Tokyo, Japan) and gel permeation chromatography (JASCO International, Tokyo, Japan), respectively (Table S1 and Figure S1). The degrees of polymerization of each branch for two- and four-branch PCL were 24 and 10, respectively. The 70 wt% 2b-PCL/30 wt% 4b-PCL macromonomers were then dissolved at 45 wt% in xylene containing 2-fold molar excess benzoyl peroxide (BPO) to the end-group of macromonomers. The solution was injected between glass slides with a 0.2 mm thick Teflon spacer. Subsequent thermal polymerization was carried out at 80°C for 180 min to obtain the crosslinked PCL elastomers. The crosslinked PCL elastomers were immersed in acetone to remove the unreacted compounds by swelling and then methanol to shrink it. The crosslinked PCL elastomers was kept under reduced pressure overnight to obtain completely dry samples. The thermal properties of the branched PCLs were measured by differential scanning calorimetry (DSC, Exster X-DSC7000Hitachi HT-SEIKO Instruments, Chiba, Japan) at a 5 °C/min programming rate. The

mechanical properties and elastic moduli of the crosslinked PCL elastomers were characterized by tensile testing (EZ-S500N, SHIMADZU, Kyoto, Japan), taking advantage of a thermo-chamber that allowed the characterization of temperature-dependent mechanical property of samples. The surfaces of crosslinked PCL elastomers were observed by atomic force microscopy (AFM) (SPM-9500J3, Shimadzu Co., Kyoto, Japan) with non-contact mode using a  $\text{Si}_3\text{N}_4$  cantilever (spring constant; 42 N/m), and the sample temperature was controlled using a thermo controller. The surface roughness ( $R_a$ ) before and after shape-memory activation were estimated from AFM scans on  $20 \times 20 \mu\text{m}^2$  area.

The surface wettability of crosslinked PCL elastomers was measured by contact angle analyzer (DropMaster 500, Kyowa Interface Science, Niiza, Saitama, Japan). First, the crosslinked PCL elastomers with or without thermal treatment above  $T_m$  were equilibrated at 37 °C for 1h, and the contact angle measurement was conducted at 37 °C. The thermal treatment of samples and measurement temperature was controlled using hotplate (Microwarm plate, KM-3, Kitazato, Shizuoka, Japan). The swelling behaviors of crosslinked PCL elastomers in aqueous environment were characterized according to the method described in the manuscript's main text. Protein adsorption using BSA as a model on the surface of crosslinked PCL elastomers was investigated with a micro bicinchonic acid (BCA) assay according to the manufacture's protocol (Pierce BCA assay).

For the cell culture experiments, crosslinked PCL elastomers with different mechanical and surface states were achieved by simple thermal treatment with or without heating above  $T_m$ . Tissue culture polystyrene dishes were also employed as a static control substrate system. Prior to cell cultivation, the stiff and soft crosslinked PCL elastomers were coated with  $10 \mu\text{g mL}^{-1}$  fibronectin and equilibrated in a 37°C incubator for 1 h. Human hepatocellular carcinoma cells (HepG2) were

seeded on the crosslinked PCL elastomers at a density of  $3.0 \times 10^4$  cells  $\text{cm}^{-2}$  and cultured in Dulbecco's modified Eagle's medium (DMEM) in the presence of 10% fetal bovine serum (FBS) and 1 % antibiotics at 37°C. For dynamic cell culture experiments that can induce softening and smoothening of stiff crosslinked PCL elastomer, the cells were transferred to a 41°C incubator following 1, 2, or 3 days of incubation at 37°C. The cells were subjected to a 41°C heat treatment for 1 h prior to returning the samples to the incubator. The cell morphology was monitored and imaged using a phase contrast microscope (Olympus IX71, Tokyo, Japan). The total number of cell on each sample (indicative of proliferation) was determined through immunostaining and fluorescent imaging. Cells were fixed with 4% paraformaldehyde and treated with Rhodamine phalloidin for F-actin staining and DAPI for nucleus staining. The cell culture medium was changed with 1 mL of fresh medium for collecting the supernatant every 24 h after HepG2 were seeded on the substrates set in 24-well plate. The albumin concentration secreted in the medium was quantitatively determined by Human Albumin ELISA Quantification kit (Bethyl Laboratory, Inc., Montgomery, TX, US) according to manufacture instruction.

**Table S1.** Summary of GPC results for synthesized tetra- and double-branched PCL polymers and macromonomers

<b>Sample</b>		<b>Mn</b>	<b>Mw</b>	<b>PDI</b>
4b10PCL	Polymer	4770	5110	1.07
	Macromonomer	5100	5410	1.06
2b20PCL	Polymer	5860	6600	1.12
	Macromonomer	5960	6690	1.12

Solvent: THF, Molecular weight was determined by GPC relative to PEO standards.

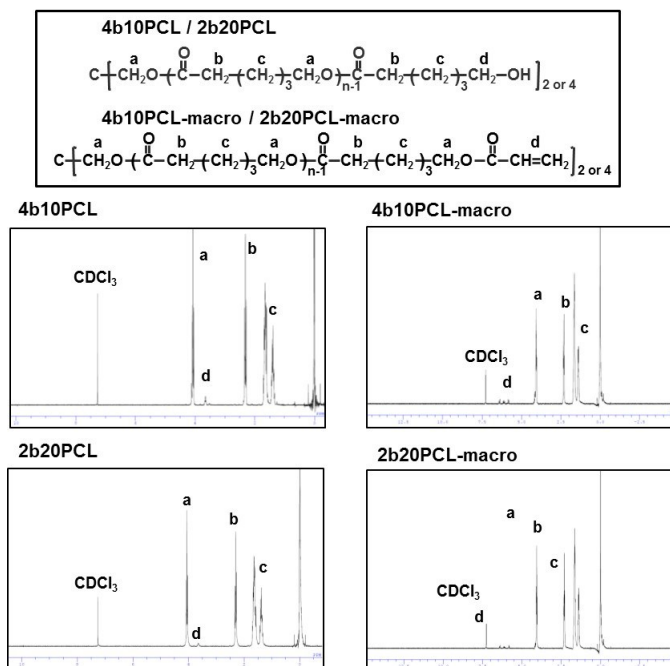


Fig. S1 Chemical structures and  $^1\text{H}$  NMR spectra for tetra- and double-branched PCL polymers and macromonomers in  $\text{CDCl}_3$ .

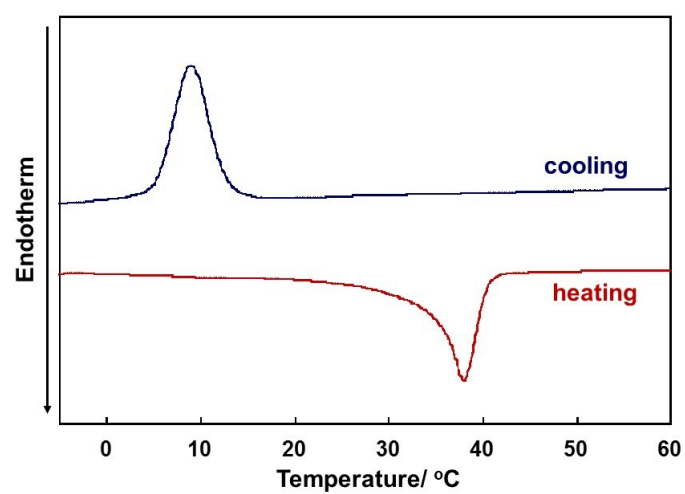


Fig. S2 DSC curves of crosslinked PCL elastomers with 70/30 wt% of 2b/4b-PCL macromonomers during heating (red) and cooling (blue) processes. DSC scans were conducted with a heating/cooling rate of 5 °C/min.

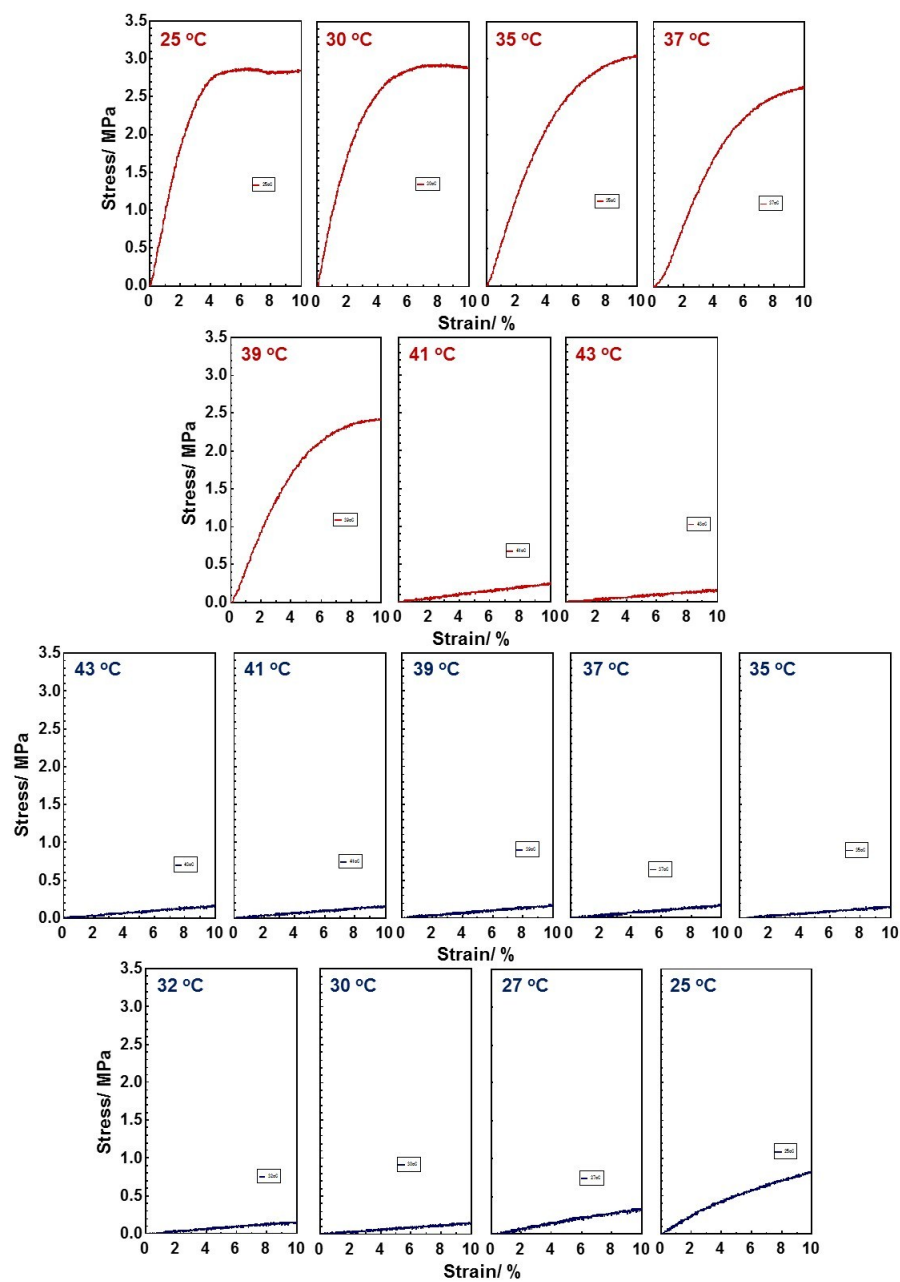


Fig. S3 Temperature-dependent stress-strain curves of crosslinked PCL elastomers with 70/30 wt% of 2b/4b-PCL macromonomers during heating (red) and cooling (blue) processes.

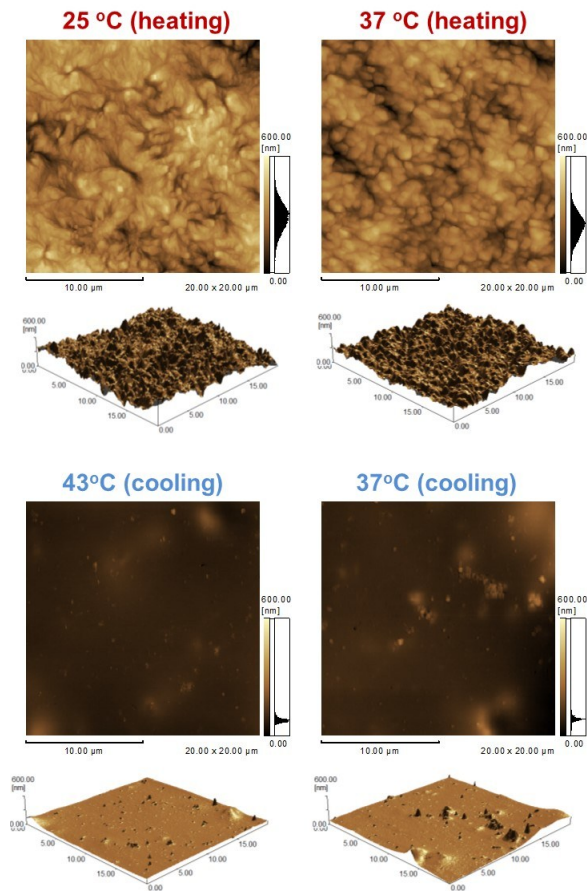


Fig. S4 2D and 3D topographical surface images of crosslinked PCL elastomers with 70/30 wt% of 2b/4b-PCL macromonomers observed by AFM in (upper, red) heating and (bottom, blue) cooling processes. The sample was equilibrated at 43 °C for 1 h prior to performing measurements during cooling. The scan size is 20 x 20  $\mu\text{m}$  and shown with a fixed height 600 nm for each image.



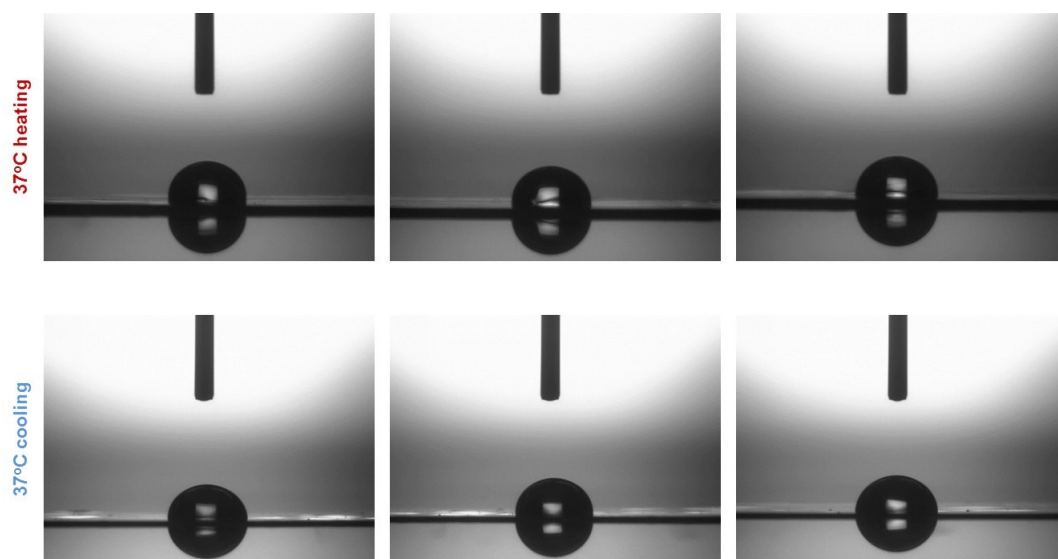


Fig. S5 Photographs of water contact angle on stiff and rough (top), and soft and smooth (bottom) crosslinked PCL elastomers at 37 °C. Representative images are shown that correspond to water droplets on different positions of each sample.

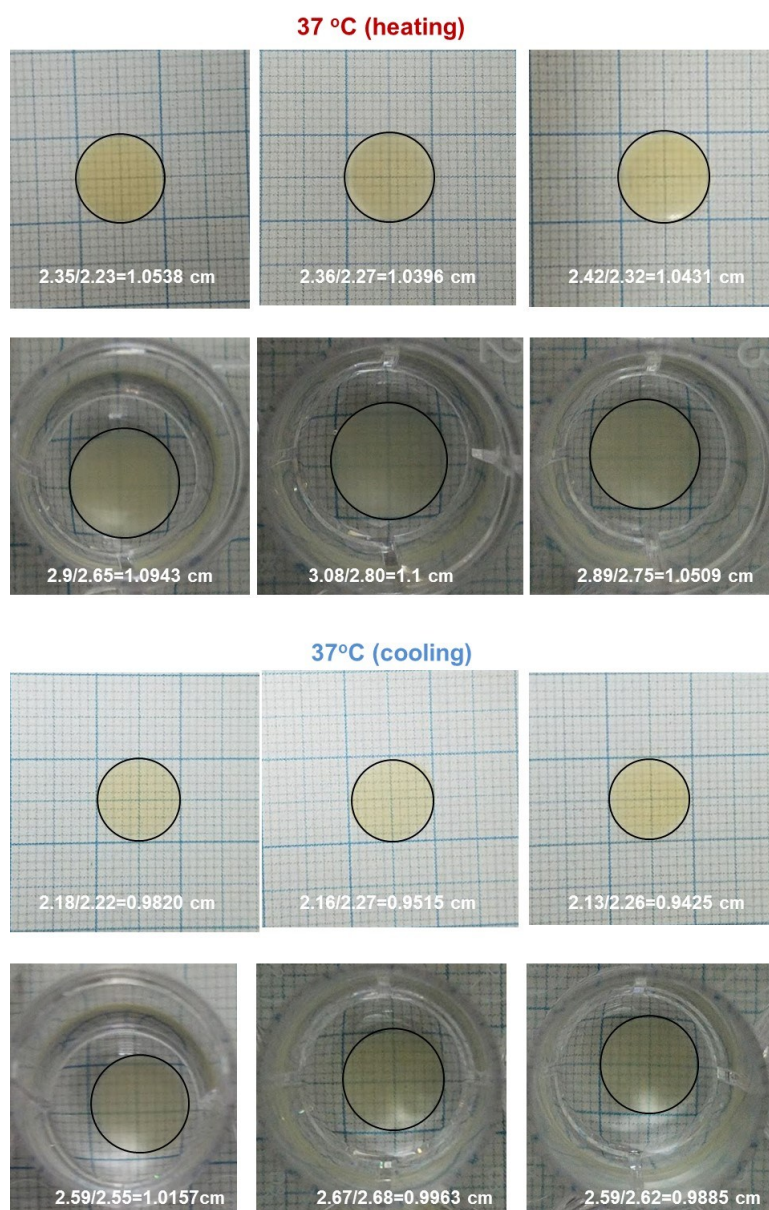


Fig. S6 Photographs of stiff and rough (top), and soft and smooth (bottom) crosslinked PCL elastomers in either air or PBS. The crosslinked elastomers were immersed in PBS at 37 °C for 2 days. The photographs of (top line of each groups) dried PCL elastomers with different mechanical and surface state were taken to characterize the swelling properties.

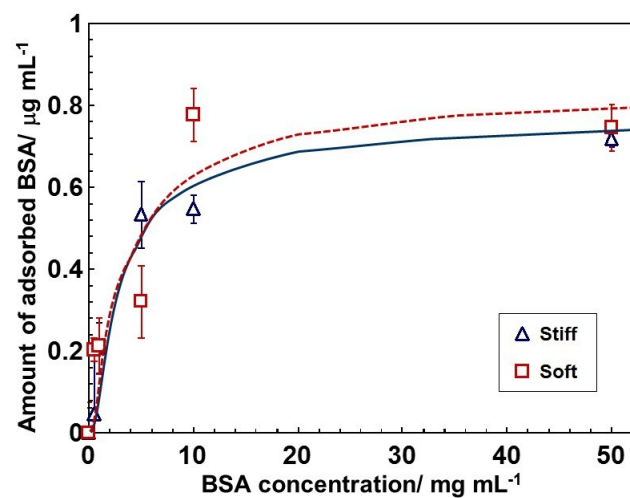


Fig. S7 Amount of albumin adsorption on stiff and soft crosslinked PCL with 70/30 wt% of 2b/4b-PCL macromonomers at 37 °C as a function of BSA concentration. Amount of adsorbed BSA in 1 mL was quantitatively characterized by micro bicinchoninic acid (BCA) protein assay. The surface area of substrates was  $\sim 4 \text{ cm}^2$ .

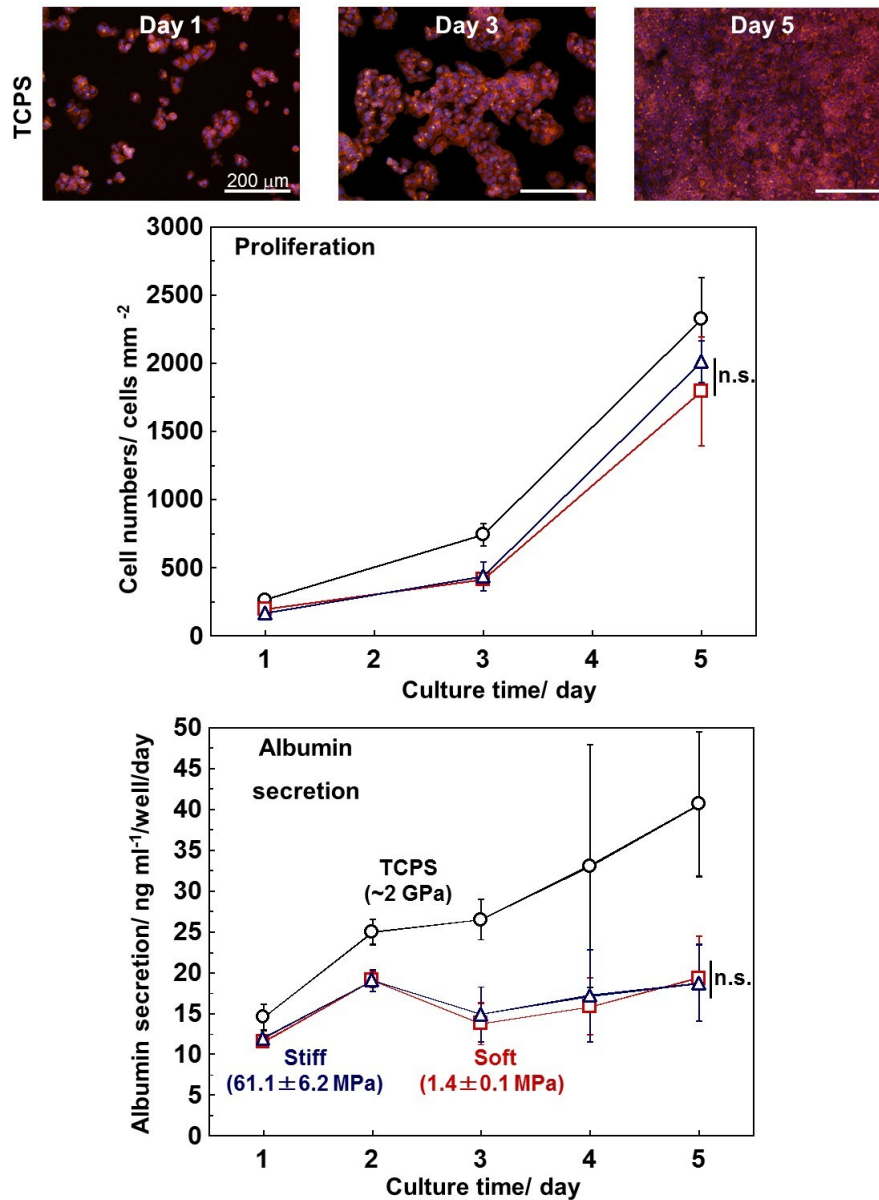


Fig. S8 Immunofluorescent staining images of HepG2 cells on TCPS as a control substrate. Immunofluorescent staining was performed against actin (red) and nucleus (blue) after cultivation for 1, 3 and 5 days. The middle graph shows the adhesion and proliferation of HepG2 estimated from the immunostaining images and bottom graph shows change of albumin secretion from HepG2 cells cultured on TCPS as well as stiff (blue) and soft (red) crosslinked PCL elastomers at 37°C. The amounts of secreted albumin were quantified by ELISA assay after cultivation for 1, 3, and 5 days ( $n > 3$ , data represent means  $\pm$  SD, n.s. not significant,  $t$ -test).

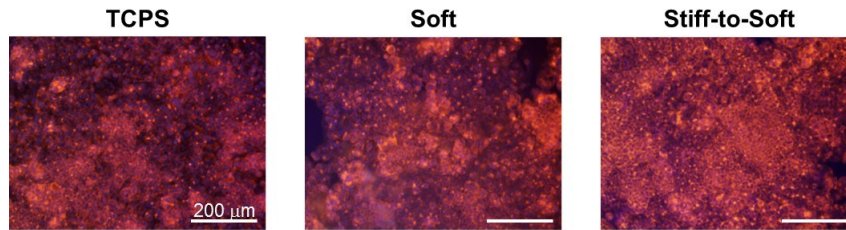


Fig. S9 Immunofluorescent staining images of HepG2 cells cultured on TCPS as a control substrate, soft and stiff-to-soft crosslinked PCL elastomers with 70/30 wt% of 2b/4b-PCL macromonomers at 37°C. Cells were cultured on the substrates at 37°C for 2 days, and the HepG2-seeded materials were then subjected to heating at 41°C for 1 h. Immunofluorescent staining was performed against actin (red) and nucleus (blue) after cultivation for 5 days (after 3 days of heating treatment).