

## Design of Smart Biodegradable Materials with Dynamically Tunable Stiffness for Control of Cell Functions

Koichiro Uto<sup>1</sup>, Mitsuhiro Ebara<sup>1</sup>, Takao Aoyagi<sup>1,2</sup>

<sup>1</sup>Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki, 305-0044 JAPAN, <sup>2</sup>Graduate School of Pure Science and Applied Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8577 JAPAN

**Introduction:** In the field of biomaterials, controlling the mechanical property of cell culture substrates is of crucial importance as it can provide an important role in regulating cell functions, including their adhesion, morphology, proliferation and migration (Wells, H.G., *Hepatology* 2008; 47: 1394-1400.). Recently, a new focus has emerged since the stiffness of materials was also found to have an effect on higher order cellular processes such as cell differentiation (Disher D.E. et. al., *Science* 2005; 310: 1139-1140. and Engler, A.J. et. al., *Cell* 2006; 126: 677-689.). Most of these studies deal with polyacrylamide (PA) gel as cell culture substrate, of which elasticity can be tuned by varying the cross-linker concentration. The detail effects of their surface stiffness on temporal cellular responses, however, have been unraveled because dynamic control of the stiffness of PA gel is a big challenge. Besides, further modification of PA gels with cell adhesive peptides has been required. From these perspectives, we newly designed biodegradable scaffolds with dynamically tunable stiffness using semi-crystalline polymer. Poly( $\epsilon$ -caprolactone) (PCL) is known to show ‘on-off’ crystallinity changes over its melting temperature. We successfully controlled its melting point around biological temperature by crosslinking branched PCL with various branch numbers and molecular weight. In this study, we investigated the thermal properties and feasibility of the cross-linked PCL as a cell culture substrate for controlling cell functions.

**Methods:** Two-branched (2b20) and four-branched (4b10) PCL were synthesized by a CL ring-opening polymerization that was initiated with tetramethylene glycol and pentaerythritol as initiators, respectively (Uto, K. et. al., *J. Control. Release* 2006; 110: 408-413.). The average degrees of polymerization of each branch estimated from <sup>1</sup>H NMR were 18 and 10, respectively. Then, an acryloyl chloride was reacted to the end of the branched chains. The obtained macromonomers were dissolved in xylene containing benzoyl peroxide (BPO), and thermal polymerization at 80 °C was carried out to obtain the cross-linked materials. The thermal properties of the 2-, 4-branched PCLs were measured by differential scanning calorimetry (DSC). The mechanical properties of the cross-linked materials were characterized by a tensile test. The surface properties of cross-linked materials were characterized by contact angle measurement and atomic force microscopy (AFM). For cell culture experiments, NIH3T3 and HepG2 cells were seeded and cultured, and their morphologies were observed by inverted microscopy using a digital camera.

**Results:** Precise control of transition temperature of cross-linked materials was successfully achieved by mixing of 2b20 and 4b10PCL macromonomer. Figure 1A shows the melting temperature ( $T_m$ ) and enthalpy change ( $\Delta H$ ) of cross-linked material comprised of two components

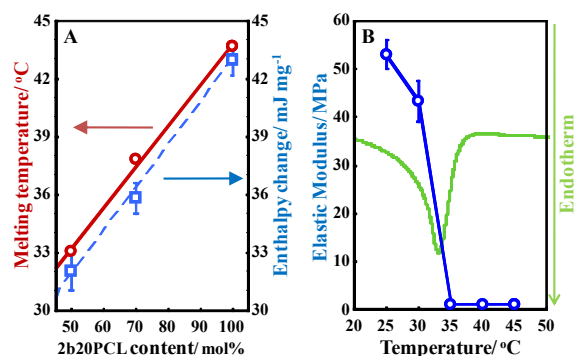


Figure 1. (A) Thermal property of 4b10/2b20PCL compounded cross-linked materials as a function of 2b20PCL content. (B) Elastic modulus and DSC curve of 50/50 (mol%) PCL cross-linked materials as a function of temperature.

characterized by DSC. As seen in the figure, the  $T_m$  and  $\Delta H$  proportionally increased with the 2b20PCL macromonomer content. In other words, the transition temperature of cross-linked materials could be easily adjusted to near biological temperature by changing mixing ratio of 2b20 and 4b10. To examine the mechanical property of the cross-linked materials against the temperature change, we carried out a tensile test of 4b10/2b20PCL (= 50/50 mol%) material as shown in Figure 1B. While the cross-linked membranes are relatively stiff below the melting point, they suddenly became elastic around melting temperature. On the other hand, the 4b10PCL cross-linked material, which had no endothermic peak, was always rubbery and showed a constant elastic modulus over this temperature range. Transition temperature of elastic modulus is consistent with  $T_m$  observed from DSC, and we found that this cross-linked material's elasticity changed dynamically and significantly around biological temperature. The cell adhesion and growth on this material was almost comparable to that on tissue culture polystyrene (TCPS), regardless of without surface modification and coating. Therefore, cross-linked material has potential as a novel cell culture substrate with dynamically tunable mechanical property by temperature change.

**Conclusions:** Thermally-sensitive smart materials with dynamically tunable stiffness were successfully prepared by mixing of 2b20 and 4b10PCL macromonomers. The mixing ratios could precisely modulate the transition temperature of the materials. The dramatically mechanical property control of materials was achieved near biological temperature, although the surface wettability of cross-linked material did not affect temperature change. This material is surely available as a smart cell culture substrate for controlling cell functions such as the control of differentiation induction of stem cell.