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Focus on the interlude between topographic transition and cell response on shape-memory surfaces

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ABSTRACT

Shape-memory surfaces with on demand, tunable nano-patterns have been developed to observe time dependent changes in fibroblast cell alignment using temperature-responsive poly(ε-caprolactone) (PCL) films. The PCL films were prepared by crosslinking tetra-branched and linear PCLs, each with acrylate end-groups. Permanent surface patterns were generated by crosslinking the PCLs in a mold. Temporary surface patterns were later embossed into the crosslinked PCL. NIH 3T3 cells cultured on the temporal nanopatterns showed marked alignment along the pattern direction, regardless of their ridge and groove widths. Then, the direction of grooves was suddenly transitioned 90° to the temporary ridges. Holographic microscope revealed that the application of heat quickly and completely transitioned temporal to permanent patterns within 30 s. However, it took more than 2 h for cells on substrate with 500 nm grooves to change their orientation, while it took more than 8 h on substrate with 2000 nm grooves. This different alignment behavior can be explained by the different adhesion strength and reorganization of cytoskeletal proteins on nano-*v.s.* micro-patterns. Dynamically tunable nano-structured surfaces, therefore, can be used to study the effects of surface nano-geometries on time-dependent cytoskeleton remodeling under biological relevant conditions.

1. Introduction

There has been widespread interest in understanding cell–substrate interactions for the development of medical implants and the production of pharmaceuticals. More recently, the cell–substrate interactions is considered to be important extrinsic factors that regulate cell fate because determination of cellular phenotypes is considered to be governed by a complex set of extrinsic cues in collaboration with intrinsic gene regulatory machinery [1,2]. Among those extrinsic cues, an increasing number of studies have shown that the mechano-structural stimuli such as elasticity, topography and mechanical force alter cell migration, overall morphology, the structure of the cytoskeleton, expression of specific genes, as well as the lineage of stem cell differentiation [3]. Engler et al., for example, first reported that differentiation of mesenchymal stem cells (MSCs) is highly sensitive to substrate stiffness [4]. Kim et al. developed an engineered myocardial tissue that mimics the structural and functional properties of native myocardial tissue and specifically the underlying extracellular matrix (ECM) architecture [5]. They found that the engineered tissue structure and function were highly sensitive to variation of the nanoscale topographic features of the substratum. They also reported that cells can recognize the variation of topographic pattern density and anisotropy, resulting in migration toward the denser area from their initial positions [6,7]. Recent researches have also suggested that changes in tissue stiffness are related to specific disease characteristics. Healthy lung tissue, for example, has been shown to have an elastic modulus in the range of 5–30 kPa when deformed at physiologically relevant rates, whereas tissues treated with proteases to mimic progression of alveolar disease showed a loss in mechanical rigidity [8]. Furuya et al. reported that mechanical stimulations to the cellular network of subepithelial fibroblasts in intestinal villi evoke cell-shape dependent ATP release and Ca^{2+} wave propagation, which may play crucial roles in intestinal functions [9]. Georges et al. reported that increases in liver stiffness precede myofibroblast

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activation and matrix deposition, resulting in fibrosis in chronic liver disease [10]. Lam et al. use atomic force microscopy to measure the mechanics and dynamics of single platelets. When exposed to stiffer microenvironments, platelets generated higher stall forces, which indicates that platelets may be able to contract heterogeneous clots more uniformly [11]. These studies would pave the way to develop technologies to reverse or prevent cell dysfunction and disease.

Despite recognition of the importance of the mechano-structural properties of extracellular environments, relatively little is known about the effects of dynamic changes in those properties on cell behavior. Many cell types have been known to react to dynamic changes in the extracellular environments at the nanometer and micrometer scales, by altering their adhesion, motility and orientation [12,13]. Therefore, there has recently been significant effort aimed at moving away from traditional "static" biomaterials towards new "dynamic" biomaterials that provide specialized cell behavioral cues in temporally defined manners. In this context, "smart" or "stimuli-responsive" materials have emerged as powerful tools for basic cell studies as well as promising for biomedical applications. Recent examples of smart materials include temperature-responsive polymer-grafted surfaces where the surface energy can be controlled with temperature. Okano and co-workers have successfully developed dynamically switchable surfaces that exhibit temperature-responsive hydrophilic/hydrophobic alterations with external temperature changes, which, in turn, result in thermally modulated interactions with biomolecules and cells [14,15]. Photo-responsive polymer-based substrates can also regulate cellular functions spatially since the light irradiation can be applied locally with subcellular resolution [16,17]. Concerning dynamically switchable surfaces with mechano-structural properties, we have proposed dynamic cell culture platforms to direct cell fate using temperature-responsive poly(ε-caprolactone) (PCL) [18–20]. Since PCL is a semi-crystalline polymer that shows crystal–amorphous transition over the melting temperature (T_m), it has been also considered as a class of temperature-responsive polymers. One of the great advantages of PCL over other temperature-responsive polymers is that surface properties such as wettability and charge are independent of temperature. We have previously shown how mechanical properties of PCL influence cell behavior using MSCs [21], skeletal myoblasts [22], fibroblasts [20], and neonatal cardiomyocytes [23,24]. We have also fabricated shape-memory substrates from PCL because the crosslinked PCL offers reversible crystallizable regions that can fix a temporary shape and have dual-shape capability, showing a shape memory effect [19]. We have successfully tuned the direction of aligned cells on shape-memory nanopatterns to a perpendicular direction without use of any biochemical reagents. Interestingly, 90% of cells did not change their direction 1 h after the topographic transition. By 36 h, 70% of cells finally realigned parallel to the permanent grooves that emerged.

This report focuses on the interlude between the topographic transition of shape-memory nanopatterns and cell response on them. More specifically, we have monitored time-dependent changes in surface nanotopographic features associated with shape-memory transition as well as the cell morphology or alignment. First, permanent nanopatterns were generated by crosslinking the PCLs in a mold that has parallel ridges with width of 100, 400, or 1000 nm. Temporary surface patterns were then overwritten onto the permanent pattern. The time-dependent surface shape-memory transition of nanopatterns was observed by holographic microscopy. Finally, we analyzed cell alignment on the PCL films before and after the shape-memory activation, the surface of which was programmed to transition from temporary grooved nanopatterns to permanent grooved nanopatterns which are perpendicular to the original shape.

2. Experimental section

2.1. Materials

ε-Caprolactone (CL) was purchased from Tokyo Kasei (Tokyo, Japan), and purified by distillation over calcium hydride under reduced pressure. Pentaerythritol and acryloyl chloride were also purchased from Tokyo Kasei and used as received. Triethylamine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and dehydrated by distillation over potassium hydroxide. Tin octanoate and other chemicals were also purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Benzoyl peroxide (BPO) was purchased from Sigma (St. Louis, MO, USA) and used as received. A mixture of *m*-, *o*-, and *p*-isomers of xylene was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as received.

2.2. Fabrication of crosslinked PCL films

Shape-memory PCL films were prepared by crosslinking tetra-branched PCL with acrylate end-groups in the presence of linear PCL telechelic diacrylates according to our previous reports [18,25,26]. Briefly, two-branched and four-branched PCL were synthesized by a CL ring-opening polymerization that was initiated with tetramethylene glycol and pentaerythritol as initiators, respectively. Acryloyl chloride was then reacted with the end of the branched chains (Fig. 1(a)). The structures and the molecular weights were estimated by nuclear magnetic resonance (^{1}H NMR) spectroscopy (JEOL, Tokyo, Japan). The obtained PCL macromonomers were dissolved in xylene containing BPO and the solution was injected between a nanopatterned silicon wafer mold and a flat glass slide with a 0.2 mm thick Teflon spacer. Nanopatterned molding of silicon wafer substrates was performed by using an electron beam (EB) lithography system (ELS-7500EX, Elionix, Hachioji, Japan). The PCL macromonomers were cured for 180 min at 80 °C. The thermal properties of the PCL films were measured by differential scanning calorimetry (DSC) (6100; SEIKO Instruments Inc, Chiba, Japan).

2.3. Surface shape-memory assays

To program temporary surface patterns, the films were compressed in a thermo chamber. A compressive stress of 0.1 MPa was applied to the samples at 38 °C, and maintained for 5 min. The embossing stress was then released at 4 °C after 10 min of cooling. Samples had a temporary surface pattern that could be triggered to transition to the permanent surface pattern by heating (Fig. 1(b)). Nanopatterned PCL surfaces were observed by atomic force microscopy (AFM) (SPM-9500J3, Shimadzu Corporation) in non-contact mode using a Si_3N_4 cantilever (spring constant; 42 N/m), and the sample temperature was controlled using a thermo controller. The time-dependent surface shape-memory transition was also observed by holographic microscopy (Lyncée tec R2100, Switzerland).

2.4. Cell alignment assay

NIH 3T3 fibroblasts were seeded at a density of 1.0×10^4 cells cm^{-2} on the temporal grooved surface and cultured in Dulbecco's Modified Eagle's Medium (DMEM) in the presence of 10% fetal bovine serum (FBS) at 32 °C for 48 h. For the surface shape-memory experiment, the cells were transferred to a 38 °C