Convenient preparation of patterned temperature-responsive cell culture surface by using thioxanthone based photo-initiator modified polystyrene surfaces

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Purpose: Micro-patterned temperature-responsive cell culture surfaces were expected to be useful for fabrication of micro-sized cell sheets or cells sheet consisting of co-cultured cells, which mimicking complex structure of tissue and organ. Several methods for the preparation of patterned surface were developed.[1, 2] However, those methods had troublesome problems. For example, in the case of preparing patterned surfaces by using photoregist material, the fabrication process for the preparation was complicated and had lost of steps, which include coating and removing the photoregist material and so on. By contrast, when electron beam irradiation (EB) and stainless mask were used for the preparation, patterned surface with less than 100 μm was difficult to be prepared. Recently,[3] a facile method for preparation of temperature-responsive cell culture surface (TRCS), which was poly(*N*-isopropylacrylamide) (PIPAAm) chains grafted polystyrene (PSt) surface, was developed by using thioxanthone (TX) based photo-initiator modified PSt surfaces. The graft polymer chains might be a polymer brush structure.[4] An advantage of the method was that TRCS was conveniently prepared without expensive equipment such as EB and plasma. As another advantage, it was expected that radical molecules remaining on the PIPAAm grafted surface were used for second polymer grafting even after the first photo-induced polymerization and grafting.[4] Namely, the block-copolymer containing PIPAAm segment was created on the PSt surface. In this presentation, the facile method was applied to the preparation of patterned TRCS.

Methods: Procedure for immobilizing photo-initiator and grafting PIPAAm on PSt surfaces was carried out according to a previous method.[3] In brief, thiosalicylic acid dissolved in conc. sulfuric acid (20mM, 2mL) was added to commercially available PSt dish surface (Corning, 35 mm size). The dish was left at 60 ºC for 3 h. During the reaction, the PSt surface was modified with sulfate and TX based photo-initiator groups. After the modification, the reaction solution was removed and the surface was washed with water and was dried at 45 ºC. IPAAm monomer solution containing a catalyst (*N*-methylethanolamine (Nmet) (100 mM)) was added to the dish. Polymerization and polymer chain grafting reaction were carried by visible light (405 nm, 70 mW/cm^2) or LED light irradiation. Resultant PIPAAm grafted PSt surface (PIPAAm-PSt) was immersed in cold water for 24 h and was washed with water and then dried at 45 ºC. To prepare TRCS with micro-patterned surface, PIPAAm-PSt surfaces were modified with polyacrylamide (PAAm) chains, being irradiated with visible light or LED light through the photomask in the presence of acrylamide monomer and Nmet (Upper scheme in Fig. 1), again. The PAAm grafted region was cell adherent surface.

Results: PIPAAm chains were grafted onto TX modified PSt surface by visible light irradiation. The PIPAAm-PSt showed characteristic of TRCS. In order to TRCS with micro-patterned surface, PAAm was further grafted on PIPAAm-PSt surface with visible light irradiation thorough the photomask. In this experiment, photomask having strip pattern was used (Upper scheme in Fig. 1). Striped pattern of PAAm was successfully grafted on the PIPAAm-PSt. As shown in bottom photographs of Fig. 1, at 37 ºC, BSA molecules labeled with Alxa 488 fluorescent dye were preferentially adsorbed on the PIPAAm chains grafted region. Bovine aortic endothelial cells (BAECs) were favorably adhered to and proliferated on the patterned PIPAAm graft region. By decreasing temperature to 20 ºC, BAECs were detached as ribbon-shaped cell sheets (the width of cell sheet was about 100 μm) within 7 minutes. We also tried to fabricate micrometer size cell sheet, which was useful for cell sheet based chip, with the patterned surfaces.

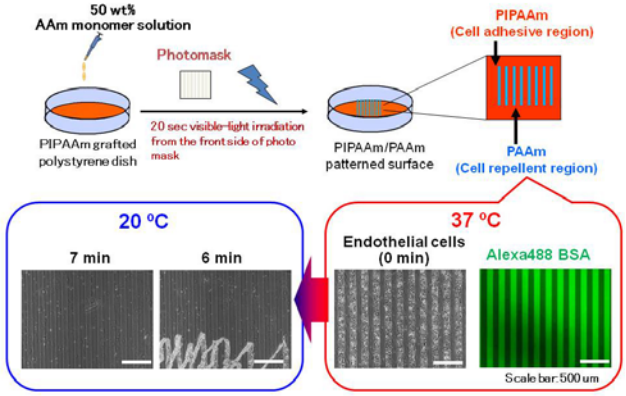

Figure 1. Preparation of stripe pattern of temperature-responsive cell culture surface by using the visible light irradiation and the photomask (upper scheme) and the characterization of patterned surface (bottom photographs).

Conclusions: PAAm chains were successfully grafted onto PIPAAm-PSt surface with visible light induced polymerization and grafting method through the photomask. The successful graft of PAAm suggested that remaining radical molecules on PIPAAm-PSt were activated to initiate polymerization by photo irradiation. Namely, block-copolymer containing PIPAAm and PAAm segments and PIPAAm chains were grafted on PSt surface. The PAAm component exhibited protein and cell repellent character. This method is expected to be a convenient method for preparation of TRCS with micro-patterned surface.

References: 1) Tsuda Y. Biomaterials. 2005;26:1885-93. 2) Tsuda Y. Biomaterials. 2007;28: 4939–46. 3) Akiyama Y. SFB 2013 Annual Meeting and Exhibition, abstract 787, Boston MA. 4) Jia X. Macromol. Chem. Phys. 2009;210:1876-82.