Facile method of preparing of temperature-responsive cell culture surface by using photoinitiator immobilized polystyrene surfaces

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Statement of Purpose: Temperature-responsive cell culture surfaces (TRCS), poly(*N*-isopropylacrylamide) (PIPAAm) grafted on tissue culture polystyrene (TCPS) (PIPAAm-TCPS), have been utilized to fabricate various kinds of cell sheets.[1] Currently, PIPAAm-TCPS prepared by electron beam irradiation (EB) method is commercially available products.[2] Besides the EB method, plasma irradiation method is also available for preparation of PIPAAm based TRCS.[3] However, as some researchers pointed out, equipments for the EB and plasma irradiation were so expensive that average laboratory could not prepare the TRCS.[4, 5] In addition, the commercially available TRCS was not as cheap as TCPS. Thus, development of alternative facile method of preparing TRCS has been desired. It was considered that polymer grafting without requiring special chemical reagents and equipment might overcome these problems. Based on the consideration, we have developed facile method of preparing of PIPAAm based TRCS by using photoinitiator immobilized PSt surfaces and light visible light. In this presentation, PIPAAm grafted polystyrene by the approach was characterized as TRCS.

Methods: Thiosalicylic acid dissolved in conc. sulfuric acid (20mM, 2mL) was added to polystyrene cell culture dish (35 mm size) and then, the dish was left at 60 ºC for 3 h, to modify the polystyrene surface with sulfate and thioxanthone photoinitiator groups by referring a previous report.[6] After the reaction, the reaction solution was carefully removed and the surfaces was vigorously washed with water. The polystyrene dish was dried at 45 ºC. IPAAm monomer solution containing *N*-methylethanolamine (100 mM) as catalyst was added to the dish. Polymerization and polymer grafting reaction were carried by visible light irradiation (405 nm, 70 mW/cm^2) for 30 min. Following polystyrene surfaces were immersed in cold water for 24 h, and the surfaces was washed and dried at 45 ºC. These obtained sample surfaces were characterized by FT-IR / ATR, XPS and cell detachment and attachment assay.

Results: Fig. 1. shows FT-IR / ATR spectra of PIPAAm grafted polystyrene surfaces, which was prepared at various monomer concentration (1.0 (1.0IPAAm-PSt), 3.0 and 5.0 wt%) by using photoinitiator immobilized This-PSt and visible light (Fig. 1 (C)-(E)). For comparison, polystyrene (PSt) (Fig. 1 (A)) and polystyrene surfaces modified with sulfate and thioxanthone groups (Thio-PSt) (Fig. 1 (B)) were also characterized. Broad peaks were newly observed in Tho-PSt around 1680 cm^{-1} and 1200 cm^{-1}, which were characteristic of sulfonic groups. XPS analysis suggested additional formation of thioxantone groups on the Thio-PSt surfaces. By irradiation of UV light to the Thio-PSt containing IPAAm monomer and catalytic agent solution, peaks at 1650 and 1600 cm-1 corresponding to amide I and II of the grafted PIPAAm were clearly observed, indicating successfully grafting of PIPAAm on the PSt surfaces.

Cells attachment and attachment character of the obtained samples was investigated. As shown in Fig. 2. (A), cells were well adhered to and proliferated on 1.0IPAAm-PSt at 37 ºC. However, cells failed to detach from the surfaces as well as TCPS. By contrast, 5.0IPAAm-PSt surfaces exhibited temperature dependent cell attachment and detachment character (Fig. 2 (B)). After cells were proliferated and become confluency on 5.0IPAAm-PSt, cells were recovered as a cell sheet from the surfaces as well as conventional PIPAAm grafted TCPS by decreasing temperature.

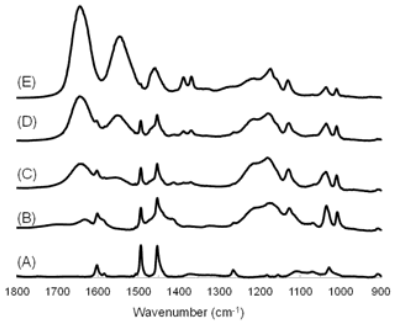

Figure 1. FT-IR / ATR spectra of (A) PSt, (B) Thio-PSt, (C) 1.0, (D) 3.0 and (D) 5.0IPAAm-PSt.

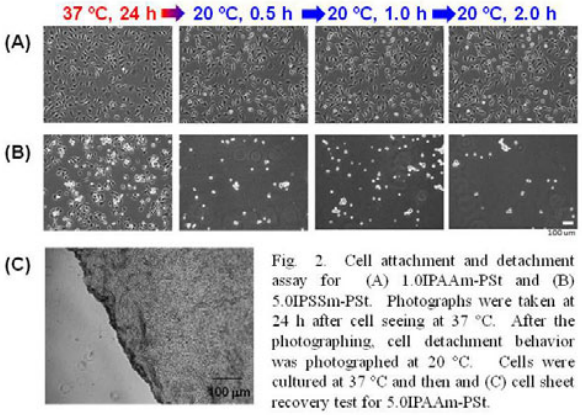

Fig. 2. Cell attachment and detachment assay for (A) 1.0IPAAm-PSt and (B) 5.0IPSSm-PSt. Photographs were taken at 24 h after cell seeing at 37 ºC. After the photographing, cell detachment behavior was photographed at 20 ºC. Cells were cultured at 37 ºC and then and (C) cell sheet recovery test for 5.0IPAAm-PSt.

Conclusions: Thio-PSt, photointiaotor immobilized polystyrene surfaces, was readily prepared, and PIPAAm was readily grafted on the PSt surface by the visible light. The PIPAAm-PSt showed characteristic of TRCS, demonstrating TRCS was readily prepared without special chemical reagent and equipment.

References: [1]Yang J. et al, Biomaterilas, 2005, 26(33), 6415, [2] http://www.nuncbrand.com/en/page.aspx?ID=11850 , [3]Canavan HE., et al, Langmuir, 2007, 23, 50, [4] Nash ME., et al, ACS Appl. Mater. Interface. 2011, 3, 1980, [5] von Recum HA., J. Biomed. Mater. Res. 1998, 40, 631, [6]Temel G. and Arsu N., J. Photochem. Photobiol. A: Chem. 2009, 202, 63.