Mechanical-stress assisted modulation of the properties of temperature-responsive cell culture surface

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Introduction: Cell sheets from various tissues and organs were fabricated with temperature-responsive cell culture surface (TRCS).[1] The nano-scaled poly(*N*-isopropylacryl-amide) (PIPAAm) hydrogel modified tissue-culture polystyrene (TCPS) was prepared as conventional TRCS by using EB irradiation (PIPAAm-TCPS). Surface properties of PIPAAm-TCPS were strongly influenced by its deposited PIPAAm hydrogel thickness and graft polymer density. The polymer thickness as well as the polymer graft density of resulting PIPAAm-TCPS was necessary to be precisely optimized to express temperature-induced cell attachment and detachment character, depending on cell species. In this research, we hypothesized that TRCS properties could be controlled by applying the mechanical-stress to TRCS, and could modulate cell adhesion and detachment character by the applied mechanical-stress. In this presentation, in order to explain this hypothesis, we prepared PIPAAm modified PDMS (PIPAAm-PDMS) surface as TRCS and investigated the properties of mechanical-stress applied PIPAAm-PDMS surfaces.

Methods: PIPAAm-PDMS was prepared according to a previous report.[2] In brief, IPAAm monomer dissolved in 2-propanol 10 wt% - 50 wt% monomer in 2-propanol) was spread onto PDMS surfaces (4-well PDMS chamber purchased from STREX, Inc.) followed by O_2 plasma and (3-aminoproyl)trimethoxysilane (APTMS) treatments. And then, the surface was subjected to EB irradiation treatment to graft PIPAAm on the PDMS surface. PIPAAm-PDMS was characterized by FT-IR / ATR, XPS analysis, contact angle measurement and cell detachment / attachment assay. For the cell attachment and detachment assay, PIPAA-PDMS with 14.1 μg/cm^2 of PIPAAm graft density was used. Mechanical-stress was applied to the PIPAAm-PDMS surfaces with a special device (purchased from STEX, Inc.) in uniaxial direction. In this experiment, 20% of stretch ratio was used. Unstrethced PIPAAm-PDMS was equal to PIPAAm-PDMS at original state.

Results: PIPAAm-PDMS surfaces with stretched was characterized by various methods as mentioned above. Through the characterization, we confirmed that PIPAAm was successfully grafted onto PDMS surfaces. The characterization also showed that PIPAAm component was not chemically deposited on PDMS surfaces without the APTMS treatment. Contact angle values of unstretched and stretched PIPAAm-PDMS surfaces revealed that upstretched PIPAAm-PDMS surface was more hydrophilic. This increase of hydrophobicity is probably because the graft polymer density and the graft PIPAAm layer thickness of the PIPAAm-PDMS decrease by applying the uniaxial-stretch stress to the PIPAAm-PDMS. Cell adhesion assay also supported that the stretched PIPAAm-PDMS was more hydrophobic and more cell adhesive than unstrethed PIPAAm-PDMS (Fig. 1). At 3 hours after seeding cells, more cells were adhered and spread on the stretched PIPAAm-PDMS surface. This result indicate that the stretched PIPAAm-PDMS surface is more cell adhesive, being in agreement with the contact angle value. The area of adherent cell on each PIPAAm-PDMS surfaces also supported the cell adhesive character of stretched PIPAAm-PDMS surface.

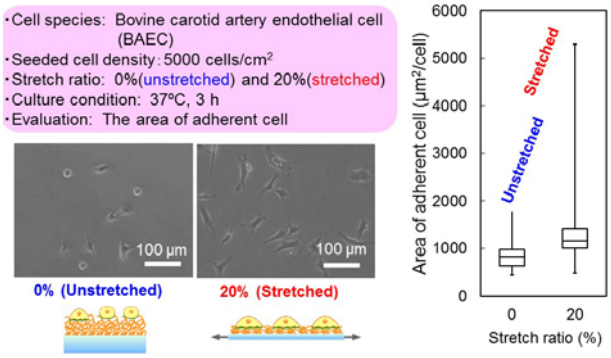

Figure. 1. Cell adhesion assay of the unstrethced and stretched PIPAAm-PDMS surfaces. Experimental conditions for this assay was summarized in pink-highlighted square. Photographs showed cell adhesion character on the unstrethced (left) and stretched (right) PIPAAm-PDMS surfaces. From photographs, the area of adherent cell was determined. These results suggested that, by applying mechanical-stress to PIPAAm-PDMS surface, the surface property as well as cell attachment and detachment behavior on the surface were modulated.

After 24 hours cell-culture, we restored the stretched PIPAAm-PDMS to the unstrethed state (original state) by applying the shrinking-stress to the stretched PIPAAm-PDMS, accompanying with lowering temperature from 37 to 20 degree. Cells were detached more rapidly from the shrinking-stress applied PIPAAm-PDMS.

Conclusions: The PIPAAm-PDMS was expected to be a new type of TRCS, which possessed a stretchable property. This new TRCS would be applicable to cell sheets stretching, which is anticipated to enhance their physiological function.

References: (1) Akiyama Y. et al., Langmuir. 20, 5506-11, 2004. (2) Akiyama Y. et al., Journal of Robotics and Mechatronics. 25, 631-6, 2013.

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