Evaluation of ultra thin PIPAAm layer modified glasscover slips surfaces by using AFM

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Statement of Purpose: Ultra thin temperature responsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm) grafted layer exhibits cell attachment and detachment properties in response to temperature change. Such properties are dependent on thickness and amount of the grafted PIPAAm, and are observed for extremely ultra thin thickness (for example, 20 nm thickness for tissue culture poly styrene and 5 nm for glass cover slips). Although this phenomenon was not demonstrated clearly, we have proposed a mechanism (Fig. 1). Namely, thinner PIPAAm layer are subjected to dehydration of the PIPAAm chains in the vicinity of basal surfaces. The dehydration progressively promotes dehydration of the grafted PIPAAm chains toward to the outermost regions. In this presentation, we tried to demonstrate the proposed mechanism by investigating the dynamic alternation of thickness of PIPAAm layer in response to temperature. The alternation was measured undere aqueous conditions above and below LCST, using AFM. As a result, thickness of thinner PIPAAm layer was not altered between under atmospheric and aqueous conditions, while thicker layer was done. This difference strongly supports the proposed mechanism. **Methods:**The PIPAAm modified coverglass slips were prepared referring to a previous repot [1]. Cell attachment and detachment assay and polymer density was also carried our according to a previous report. A The depth of ablated domains was obtained by AFM (Tapping mode, spring constant : 0.32 N/m; DNP-S20). All of images were measured in AFM fluid cell. Milli-Q water temperature was varied using a temperature-controlling system (Veeco instruments). **Results:** In the present study, to investigate features of such thickness dependency of cell adhesion/detachment properties in detail, we employed glass coverslips modified with 3-methacryloxypropyl -trimethoxysilane (MPTMS) as the surfaces for PIPAAm-grafting, because thickness of the PIPAAm grafted on the glass are easy to be determined in comparison with that of PIPAAm-TCPS system. Temperature-dependent surface property changes of PIPAAm-grafted coverslips (PIPAAm-CSs) were examined in comparison to those of PIPAAm-TCPS. In addition, we observed thickness of the grafted PIPAAm above and below LCST by AFM. Fig. 1 shows a square domain after removal of PIPAAm composition from 0.84PIPAAm-CS surfaces (polymer density = 0.84ug/cm^2) by excimer layer ablation under atmosphere conditions. The PIPAAm layer at 25°C is thicker than that at 37°C due to hydration of the grafted PIPAAm chains. However, interestingly, thickness of the PIPAAm layer under aqueous conditions (37°C) is as same as that under atmosphere conditions, even though 0.84PIPAAm-CS is immersed into aqueous solution. These images suggest that ultra thin PIPAAm layer suffer from the progressive dehydration and do not swell even under aqueous condition above LCTS. This phenomenon may reflect a feature of the ultra thin polymer layer. Fig. 1 Effect of thermal change on polymer thickness of 0.84PIPAAm-CS (graft density of PIPAAm = 0.84μg/cm2). **Conclusions**: We evaluated PIPAAm –CSs with different polymer thickness. Furthermore, we investigated dynamic alternation of the thickness of the PIPAAm layer above and below LCST under aqueous conditions, using AFM equipment. Consequently, swelling and hydration of PIPAAm chains in thinner PIPAAm layer are restricted, while, for thicker layer, the PIPAAm chains are hydrated and dehydrated in response to temperature change. These results are in agreement with our proposed scheme. That is due to progressive dehydration of the PIPAAm chains generated at the interface of the basal glass coverslips. This manner is also applicable to PIPAAm –TCPS system.

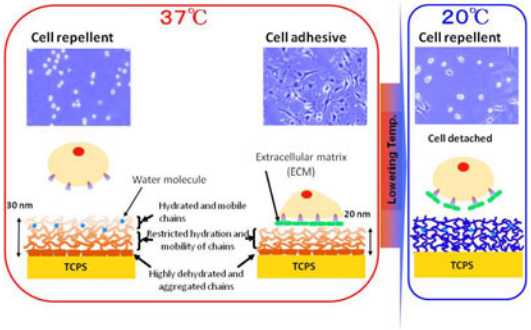

Fig. 1. For ultra thin PIPAAm layer, dehydration of PIPAAm chains in the vicinity of the hydrophobic TCPS surfaces are promoted due to extremely less molecular motion of the polymer chains and the hydrophobic property of the TCPS. This dehydration progressively dehydrates PIPAAm chains towards to outermost regions. Thus, such progressive dehydration induces more dehydrated of PIPAAm chains for thinner polymer surfaces (20 nm), producing cell adhesive property at 37 °C. In contrast, thicker polymer surfaces (30 nm) are not subjected to the progressive dehydration in the outermost regions, showing cell repellent property even at 37 °C.

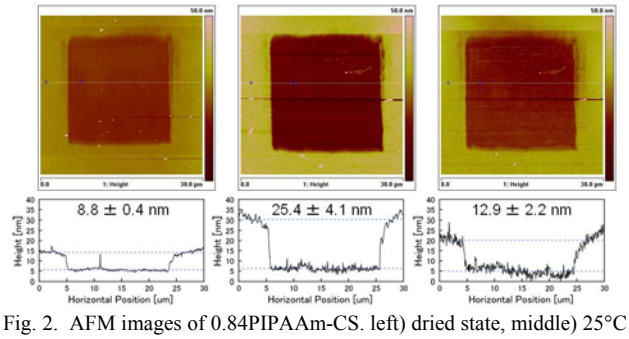

Fig. 2. AFM images of 0.84PIPAAm-CS. left) dried state, middle) 25°C under aqueous conditions, right) 37°C under aqueous conditions. Section profile of the domain was represented below each image. The inserted values in the profile indicate depth of the ablated domain.

References: [1] N. Yamada et al., Makromol Chem Rapid Commun 1990;11:571-576. [2] Y. Akiyama et al, Langmuir 2004;20:5506-5511.