Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

Effective Segregation of Cytocompatible Chitosan Molecules by Silica-Surfactant Nanostructure Formation Process

Motohiro Tagaya [a, b, *]

[a] *Department of Materials Science and Technology, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan.*

[b] *Top Runner Incubation Center for Academica-Industry Fusion, Nagaoka University of Technology, 1603-1 Kamitomioka, Nagaoka, Niigata 940-2188, Japan.*

*** Author to whom correspondence should be addressed:**

Tel: +81-258-47-9345; Fax: +81-258-47-9300, E-mail: tagaya@mst.nagaokaut.ac.jp

Experimental Procedure S1

The force curves were measured at the scan rate of 30 nm/s and displacement sensitivity of 40 mV/nm. A silicon probe mounted on a cantilever (SII Micro Cantilever SI-AF01, SII Investments, Inc.) was employed. The decline in the saturated region in the approaching curve was calculated to be the viscoelasticity. The tip−sample stiffness, *S*, can be represented by Eq (S1).

$$S = (\Delta F/\Delta x) - k \quad \text{(S1)}$$

where k is the spring constant of the cantilever and is considered to be 0.2 nN/nm in this study. ΔF is the modulated force and Δx is the amplitude of the tip on the sample. These regions in the curves are drawn in Scheme S1. On the other hand, S can be also represented by Eq (S2).[18b, 18c]

$$S = 2aE^* \quad \text{(S2)}$$

where a is the contact radius on the monolithic film, which is estimated to be ca. 20 nm according to the tip radius used in this study. Based on the Eqs (S1) and (S2), E^*, which is the Young's modulus of the monolithic film, was also calculated.

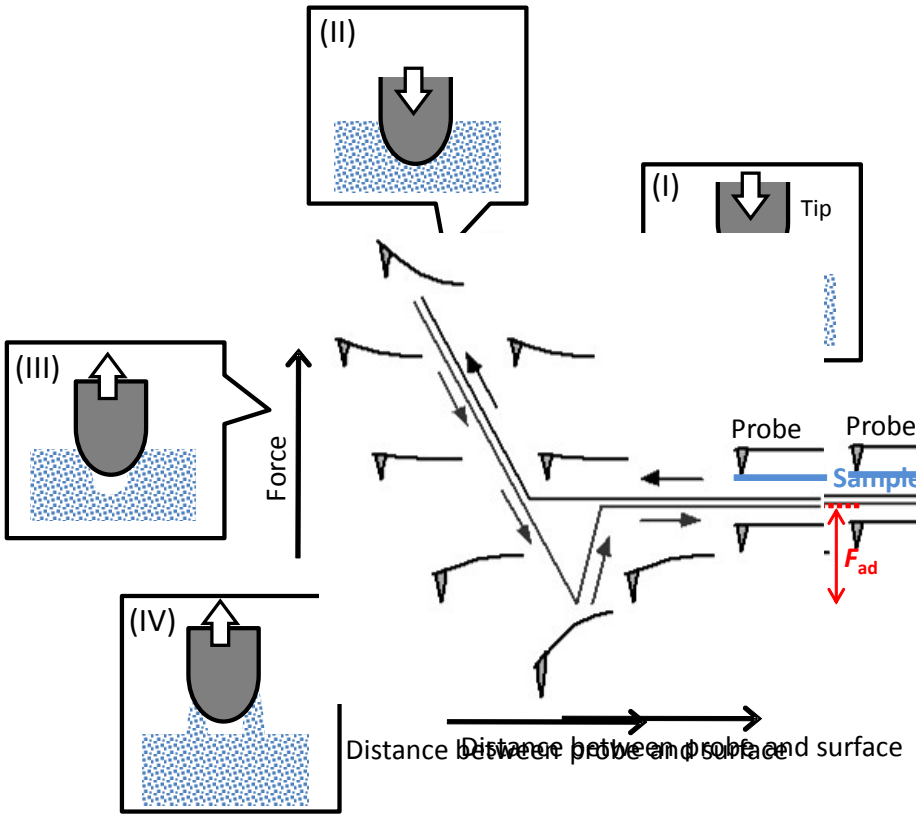
All the measurements were repeatedly conducted five times to obtain the average values. The statistical analysis of all the measurements was evaluated using the Student's *t* test.

Experimental Procedure S2

For the cell viability measurement, the cell suspensions were added and cultured on a 96-well poly(styrene) culture plate for evaluating the cell viability (BD Falcon™ Co., Ltd.). The cell viability was evaluated using the MTT assay method by 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide at the culture times of 6 h, 24 h and 36 h.

Before the measurements, all the cell surfaces were washed with PBS twice. 10 μL of the Cell Counting Kit-8 solution (Cayman Chemical Co., Ltd., MTT reagent, Cat. No. 10009591) was added and stored at 37 °C for 3 h in a humidified atmosphere of CO_2 (5 %), and the solusion was removed and then 1 mL of crystal dissolving solution (Cayman Chemical Co., Ltd., Cat. No. 10009593) was added and shook for 1 min. The absorbance at 570 nm of the resulting solution was then measured by a microplate spectrophotometer (Power Scan ®HT, DS Pharma Biomedical Co., Ltd.) and the absorbance of 1 mL of 10% FBS/DMEM was subtracted as the blank value. The average value between five samples was used, and the maximum absorbance in all the samples was set at 100 %.

Scheme S1



Scheme S1. Scheme of the force curve measurement process as a function of the distance between the probe and film surface, which indicates the different interfacial interactions at the points (I)−(IV). At a large separation in (I), the interactions between the tip and sample surface are negligible as such that the spring cantilever remains in an undeflected state. As the sample approaches the tip, the interactions between the tip and sample surfaces at a certain separation cause a deflection of the spring cantilever upward or downward, depending on the nature of the force. With a further approach to each other in (II), the interfacial forces increase in magnitude until the cantilever irreversibly jumps and contacts the sample surface. From this point onward, the cantilever and sample move together until reaching its set upward limit. The sample subsequently retracts back in (III). If there is an adhesion force (F_{ad}) between the tip and the sample as shown in (IV), the sample will pull the tip downward as it retracts until the restoring force of the cantilever spring exceeds the adhesion force at which time the cantilever jumps back to its normal position. The F_{ad} in the curve was defined on the force axis.

Fig. S1

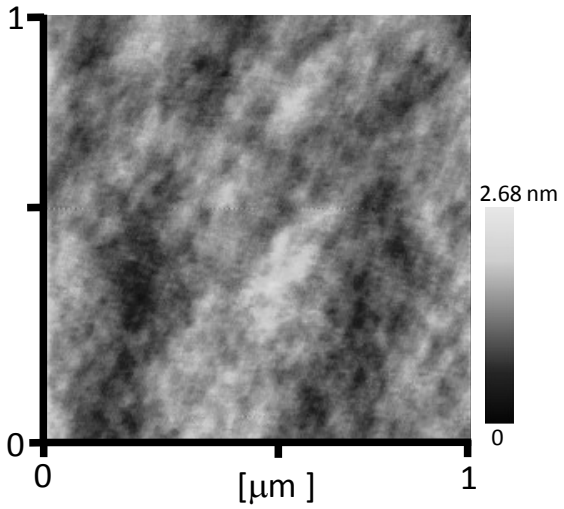


Fig. S1. AFM topographic image of the Chi alone prepared by casting Chi solution on **0w**-film in an observation area of 1 x 1 μm^2, which exhibits the R_{rms} value of 0.86 $\pm$ 0.11 nm.

Fig. S2

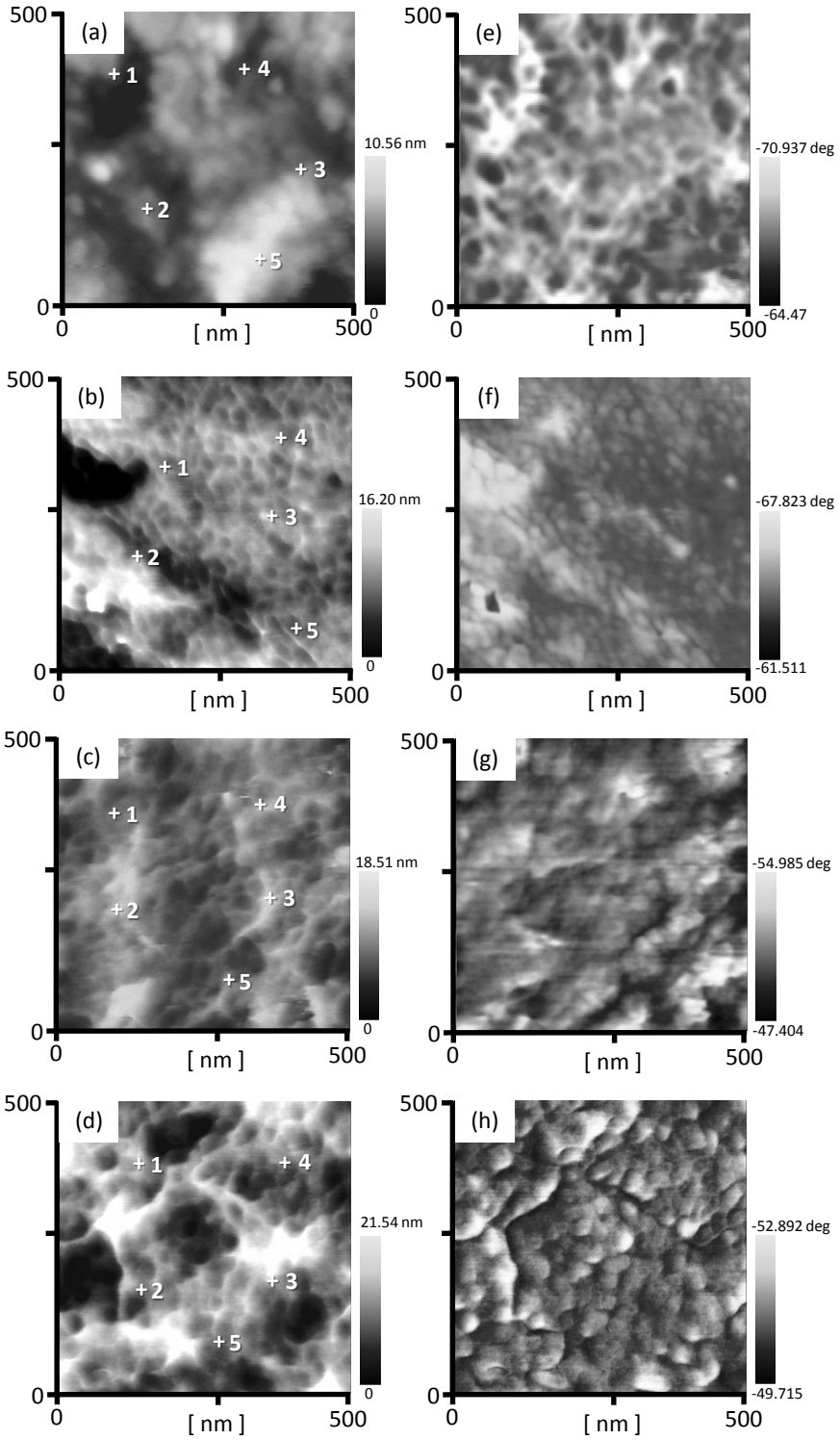


Fig. S2. AFM (a-d) topographic and (e-h) phase-shift images of the (a, e) **0w**- (b, f) **1w**- (c, g) **5w**- and (d, h) **10w**-films in an observation area of 500 x 500 nm^2, and the marks numbered with1−5 were the measured pints for the force curves in Fig. 3 in the manuscript.