Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Protein-Coated Nanofibers to Promotion of T Cell Activity

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Materials. Amphiphile **1** and **2** were prepared according to the procedures described previously.[18] Con A-coated beads were prepared by a traditional procedure for ligand coupling that is the formation of amide bond a primary amino group of legends and the carboxylic acid group of the beads (polystyrene latex, ~400nm DIA. 2mg/mL), mediated by carbodiimide activation. PBS solution (pH 7.4, 1×), RPMI medium 1640, fetal bovine serum and Penicillin-streptomycin were purchased from Gibco (USA). Tween 20 and albumin from bovine serum were purchased from Aldrich Korea. TMB seingle solution was perchased from Invitrogen. Fluorescein labeled Con A was perchased from Vector Labs (USA). Antibodies were perchased from abcam (UK). Cryo-preserved Jurkat cell line (human T lymphocyte, KCLB No. 40152) and media were purchased from Korean Cell Line Bank (Seoul, Korea).

Supporting Figure

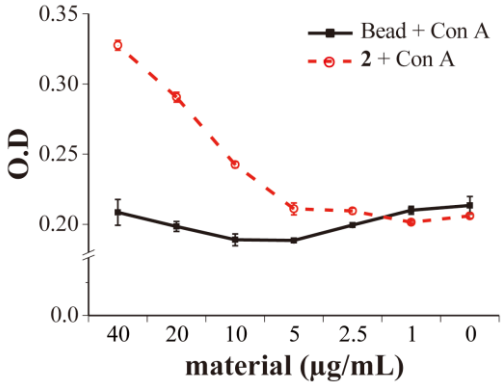

Fig. S1 Comparison of IL-2 production by Con A-coated bead and Con A-coated nanofiber. The error bars mean the standard deviations, and some are smaller than the symbols.

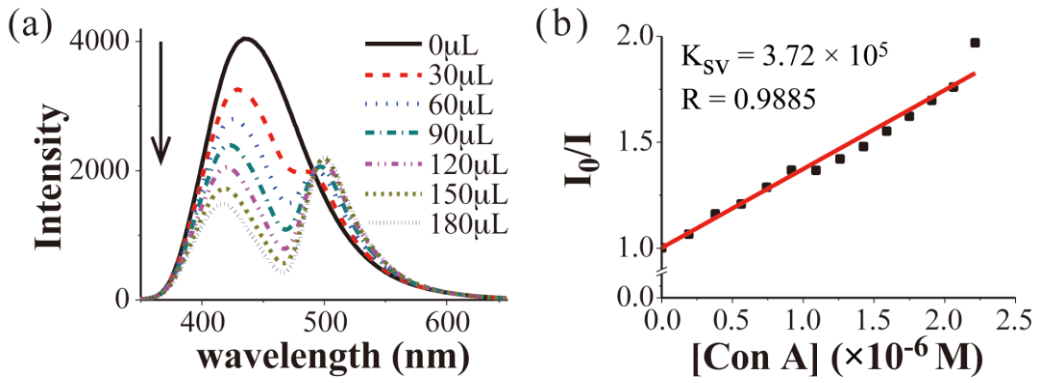

Fig. S2 The results of FRET experiments upon the addition of fluorescein-labeled Con A (2 mg per mL), respectively, to a solution containing amphiphile **2**, (a) the whole fluorescence spectra and (b) a Stern-Volmer plot of the fluorescence signal. λ_{ex} = 409 nm.

Techniques. The fluorescence spectra were obtained from a Hithachi F-7000 fluorescence spectrophotometer. The transmission electron microscope (TEM) was performed at 120 kV using JEOL-JEM 2010. The optical and fluorescent image was observed with a Nikon Eclipse TE2000-U inverted fluorescence microscope equipped with a DXM1200C digital camera. The absorbance of ELISA assay was observed from a BioTek ELx-800 plate reader. Cells were cultured at a Sanyo MCO-18AC CO_2 incubator.

Fiber Generation and TEM sample preparation. 60 μM of amphiphiles was dissolved in 1 mL pure water in 3 mL vial and placed in a water bath at room temperature with mild sonication. After 2 hours, the aqueous solutions were slowly dropped on a carbon-coated copper grid and the solution was allowed to evaporate under 30 °C. The samples were stained by depositing a drop of uranyl acetate onto the surface of the sample-loaded grid then dried at 30 °C.

The cryogenic transmission electron microscopy (cryo–TEM) experiments were performed with a thin film of premixed solution of amphiphiles with Con A (1 mL) transferred to a lacey supported grid. The thin aqueous films were prepared under controlled temperature and humidity conditions (97 ~ 99%) within a custom–built environmental chamber in order to prevent evaporation of water from sample solution. The excess liquid was blotted with filter paper for 2 ~ 3 seconds, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. The grid was transferred, on a Gatan 626 cryoholder, using a cryo–transfer device and transferred to a JEM–2010 TEM. Direct imaging was carried out at a temperature of approximately –175 °C and with a 120 kV accelerating voltage, using the images acquired with a Dual vision 300 W and SC 1000 CCD camera (Gatan, Inc.; Warrendale, PA)

Fluorescence Resonance Energy Transfer Experiments (FRET). Stock solutions of Fluorescein labeled Con A were resuspended in PBS to 2 mg per mL. Each solution of 600 μM of amphiphile **1** and **2** were diluted in PBS, pH 7.4 buffer to 120 μM. Aliquots of Fluorescein labeled Con A were added to the solution, the concentration of Con A is given in amount of monomer (MW = 25,000). After each addition, the sample was allowed to equilibrate for 2 min prior to recording a spectrum. Additions of Con A were continued until no change in the fluorescence signal was observed. Fluorescein emission was measured on a Hithachi F-7000 Fluoresence Spectrophotometer using 5 nm slit widths, a PMT voltage of 400 V, excitation wavelength of 316 nm for **1** and 354 nm for **2**, and an emission scan was from 326 ~ 700 nm and 364 ~ 700 nm, respectively. The signal was corrected for the dilution factor, according to the equation: $F_{i,corr} = F_{i,obs} \times V_i/V_0$, where $F_{i,corr}$ is the corrected intensity for point I, V_i is the volume after the i^{th} addition, and V_0 is the initial volume (typically 300 μL). The fluorescence maximum was then plotted.

Cell Culture. Jurkat cells were grown in RPMI Medium 1640 (Gibco, USA, 22400089) with 10% fetal bovine serum (Gibco, USA, 16000044) and 100 units/mL penicillinstreptomycin (Gibco, USA, 15070063) at 37 °C, 5% CO_2.

Cell viability assay. The viability of Jurkat cells in the nanofibers was investigated using the Trypan blue assay and the EZ-Cytox Cell viability assay kit (water-soluble tetrazolium salt method).

EZ-Cytox assay (dehydrogenase assay): After 2days of seeding the cells (1×10^4 cells per mL), the WST reagent solution (10 μL) was added to each well of a 96-well microplate that contained 100 μL of cells in the nanofibers at various solution concentrations (0, 1, 2.5, 5, 10, 20, 40 μg per mL) under control conditions (96 wells for cell culture). The plate was then incubated for 3 hours at 37 °C. The absorbance was measured at 450 nm using a plate reader. At the same time, nanofibers without cells were incubated for 1, 3 and 5 days to obtain the background signal. As a result, we were able to calculate the final value: (total signal − background signal = original signal → (original signal / control signal) × 100 = viability %).

Sandwich ELISA. The amount of interleukin 2 that is released from cell was evaluated with the sandwich ELISA method. Jurkat cells were grown in RPMI with 10% fetal bovine serum and 100 units per mL penicillin-streptomycin for 2 days. Jurkat cells (1.0×10^5 per mL) were incubated with preincubated solution at various concentrations (0, 1, 2.5, 5, 10, 20, 40 μg per mL) of mannose-functionalized nanofibers with Con A at 37 ºC and 5% CO_2 condition. After 24 hours of cell culture, cell media were harvested and clarified at 2,000 rpm in an eppendorf tube for 4 min at RT. The supernatant was transferred to a new tube, and the pellet was discarded. For the sandwich assay, samples were loaded on 96 wells which were coated with rabbit anti-IL-2 polyclonal antibody (abcam, United Kingdom, ab9618). After incubation and blocking with BSA solution, we used mouse anti-IL-2 monoclonal antibody (abcam, United Kingdom, ab38151) to form sandwich complexes. At the final step, horseradish peroxidase-conjugated anti-mouse IgG was used and reacted with TMB solution. And the absorbance of solution was measured at 450 nm.

Fluorescence Optical Microscopy Experiment. Jurkat cells (1.0×10^5 per mL) were incubated with preincubated solution of mannose-functionalized nanofibers with Con A (40 μg per mL) at 37 ºC and 5% CO_2 condition. After 2 hours, the portion of cell culture was dropped on a coverglass-bottom dish (200 μL) for checking fluorescence co-localization studies of the Jurkat cells with Con A-coated nanofibers.