

Supporting Information

Chemicals

Poly-L-arginine hydrochloride (PLA; MW > 70,000) and poly-L-glutamic acid (PLG; MW > 50,000-100,000) were purchased from Sigma-Aldrich (USA). Fluorescein-4-isothiocyanate (FITC) was purchased from Dojindo (USA). All buffers were prepared using double-distilled water (ddH₂O) from a Millipore system (> 18 MΩcm). 550 nM propidium iodide (Sigma) was prepared using PBS.

Instrumentation

SEM measurements were performed with JSM6330F scanning electron microscope (JEOL, Japan) at 3, 5, 10 kV. TEM analysis was performed with a JEM-1230 transmission electron microscope (JEOL). Pressure filtration on nanoporous membranes was performed using an Advantech syringe filter holder unit and a syringe pump (KDS210, KD Scientific, USA).

Preparation of protein nanotubes (PNTs) by layer-by-layer assembly

PNTs were prepared according to a previously reported method¹. PLA dissolved in 10mM sodium phosphate buffer (1 mg/ml, pH 7) containing 0.1 M NaCl was filtered through a track-etched polycarbonate membrane (pore diameter: 400 nm). Five mL of the PLA solution was filtered through the membrane at a rate of 0.25 mL/min and excess PLA was washed by filtering with 10 mL of water (1 mL/min). The membrane was then removed from the filter holder and dried under a low vacuum. Next, PLG in 10 mM sodium phosphate buffer (1 mg/mL, 5 mL) was filtered through the dried polycarbonate membrane for the second layering. Excess PLG was washed away and dried in a vacuum. This process was repeated for 4 cycles and the polycarbonate membrane was gently cleaned on both sides and dried under a vacuum for long time. Finally, polycarbonate template was dissolved in dimethyl formamide (DMF) and gently removed from the polycarbonate by repeated washing with DMF. The white powder obtained after washing with DMF was freeze-dried and dispersed in PBS for cell interaction studies.

SEM and TEM observations

For SEM observations, protein nanotubes were put on carbon tape and sputter-coated with palladium-platinum using a SC-701 Quick coater (Sanyu, Japan). Later, cells treated with PNTs were fixed with gluteraldehyde and post-fixed with osmium tetroxide. Next, the cell samples were dehydrated in ethyl alcohol mixtures and finally dried at critical point using CO₂ in a HCP-2 critical point dryer (Hitachi, Japan). Before observations, cell samples were coated with osmium using an OPC80N osmium plasma coater (Filgen, Japan)

For the TEM observation, carbon-coated copper grids were hydrophilized using JEOL Datum for 30 s and dried powder of PNTs were fixed on the copper grids. Samples were observed at 80 kV and images were recorded.

Cell interaction studies

HeLa cells (RIKEN Cell Bank, Japan) were grown in Dulbecco's modified Eagle's medium (DMEM; Wako, Japan) supplemented with 10% fetal bovine serum (FBS; Life Technologies) at 37°C under 5% CO₂. Cells were passaged regularly to maintain exponential growth. One day before use, cells were plated in a 35 mm plastic dish (Asahi Techno Glass, Japan) with a plastic coverslip (Thermanox Nunc, Rochester, USA) and 35 mm glass bottom dish (zellkontakt GmbH) (2 mL, 5×10^4 cells). The cells were rinsed with DMEM and incubated with PNT for 5 h at 37°C under 5% CO₂. After this, the cells were incubated with propidium iodide (PI) for 5 min, and washed with Hanks' balanced salt solution (Wako, Japan). The cell membrane was stained using CellMask™ Orange plasma membrane stain (Life Technologies) according to the manufacturer's instructions. The cells were visualized with a Nikon C2 confocal fluorescence microscopy system (Nikon, Japan). A Z-stack comprising around 40 optical sections covering 200 nm thicknesses was captured using a confocal laser scanning microscope. The 3D reconstruction and image analysis was conducted to study the location and length of the nanotubes penetrating the inside of the cell.

Cell viability studies

Cell viability studies were conducted based on the interaction of protein nanotubes with HeLa cells. Cells were incubated with PNTs for 24 h and cells were washed two times with PBS to remove dead or non-adherent cells. Before treating with PNTs cell number was counted and maintained a same number of cells for control and test experiments. After incubating with PNTs, cell were examined carefully and counted in both control and test. We didn't use any biochemical analysis for this experiment and we anticipate that may be these chemicals can go through the PNTs to give a negative result.

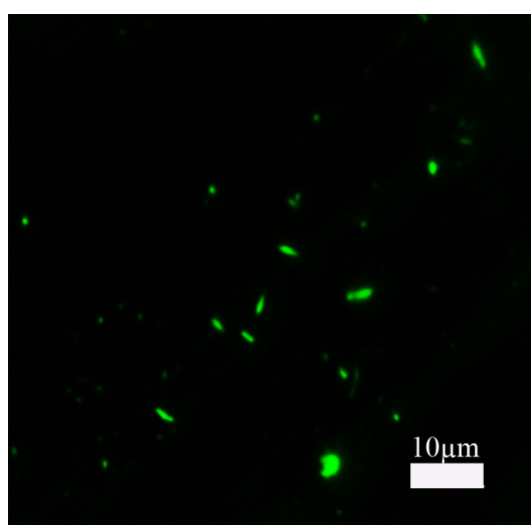


Fig. S1. Fluorescent microscopy image of FPNT in PBS for cell interaction studies. FPNT solution were placed on a glass slide and covered with a cover slip before microscopy analysis.

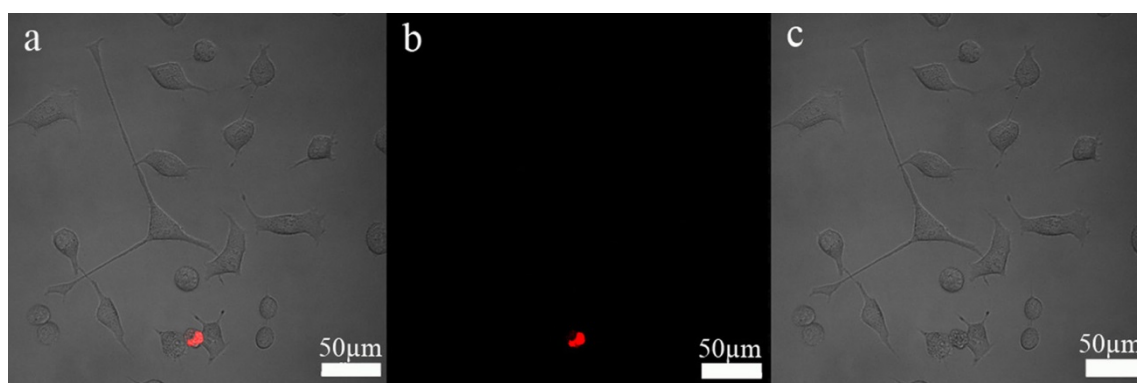


Fig. S2. Confocal microscopy image of HeLa cells treated with propidium iodide (PI) without protein nanotubes. It is clearly visible in the images that only dead cells are stained with PI. (a) Merge (b) PI and (c) DIC

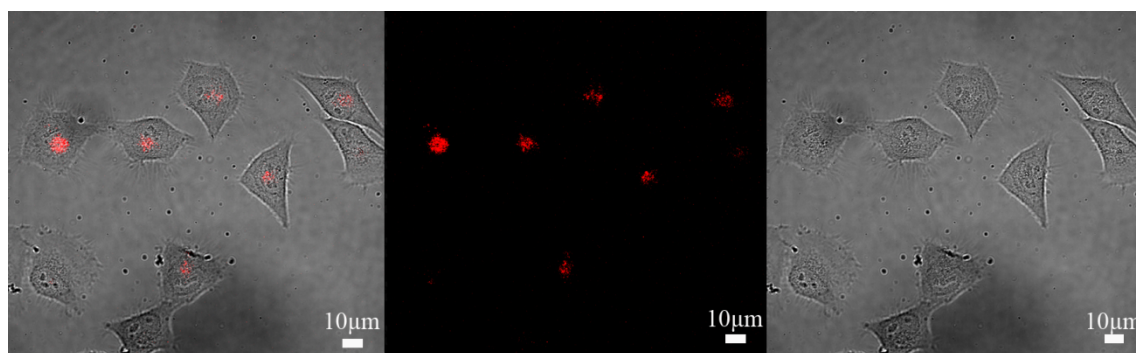


Fig. S3. Confocal microscopy images of HeLa cells treated with protein nanotubes and PI. PI is localized only in the PNT penetrated cells. Scale bar represents 10 μm . (a) merge (b) PI and (c) DIC

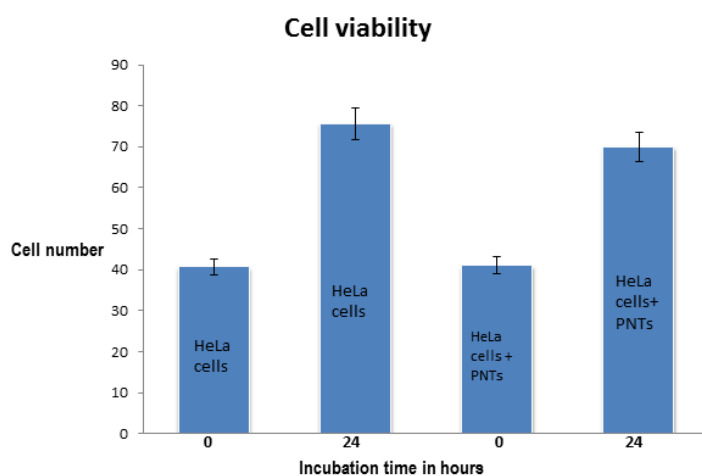


Fig. S4. Cell viability assay of HeLa cells. Number of cells were counted before and after treating with PNTs (100 $\mu\text{g}/500 \mu\text{L}$) in a 24 well plate for a period of 24h. Healthy cells were only considered for counting. Control and test samples were given similar experimental conditions. Cells were counted in 3 different areas and calculated the average.

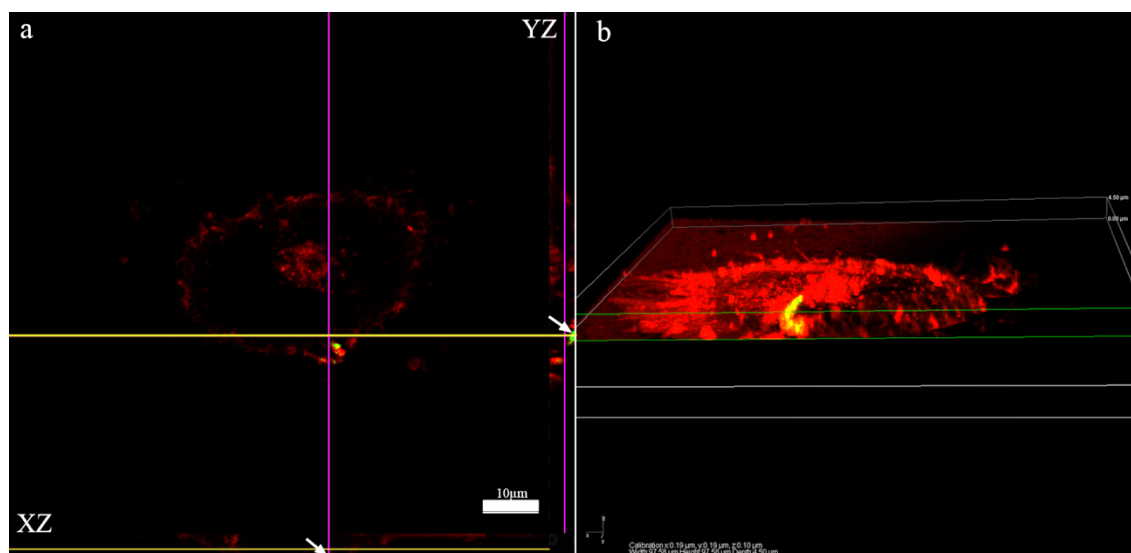


Fig. S5. 3D reconstruction of Z stack images (obtained by confocal laser scanning microscope) of HeLa cells treated with FPNTs (green) and cell membrane stain, CellMask™ (red, pseudo coloured). (a) An orthogonal section through XZ (yellow line) and YZ (pink line) planes shows that nanotube is penetrated into the cell. Arrows represent the location of nanotubes in the cell. (b) A cross-sectional display of the 3D surface rendered image. We have calculated the approximate length of the penetrated nanotube in cells from these 3D reconstructions. (Length: 1.17 μm; XY: 0.68 μm Height 0.95 μm)

References

1. X. Qu, N. Kobayashi and T. Komatsu, *ACS Nano*, 2010, **4**, 1732-1738.