Supporting Information for "Fabrication of autofluorescent protein coated mesoporous silica nanoparticles for biological application"

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Experimental Section

Materials

Tetraethylorthosilicate (TEOS) and 3-Aminopropyl-triethoxysilane (APTEOS) were from Acros. Glucose oxidase was from Aspergilus niger (GOD, Mw ~ 186000). Bovine hemoglobin (Hb, Mw ~ 64500) were from Sigma-Aldrich and used as received. Amplex red reagent (Molecular Probes) was used for enzymatic activity experiments. Cetyltrimethylammonium bromide (CTAB), β-D-glucose and glutaraldehyde (GA) were obtained from Beijing Chemical Reagent Co., Beijing, China. MTT were purchased from Amresco. The water used in all experiments was prepared in a three-stage Millipore Milli-Q Plus 185 purification system and had a resistivity higher than 18.2 MΩ.cm.

Preparation of surface-functionalized MSNs

The MSN samples with a hexagonal well-ordered pore structure were synthesized using a base-catalyzed sol-gel method.[1] 0.25 g CTAB and 0.87 ml 2M NaOH were stirred in 120 ml water. Then the mixture was heated to 80 ^{o}C. 1.25 ml TEOS was introduced dropwise to the surfactant solution. The reaction mixture was stirred for 2 h at 80 ^{o}C. The dispersion was washed thoroughly with methanol and dried under vacuum at room temperature. To modify the particle surface with -NH_2 groups, 0.28 ml APTEOS was used to react with 0.1 g dry MSNs. To avoid APTEOS hydrolysis before reacting with MSNs, the reaction was allowed to proceed in 30 ml toluene. The mixture was ultrasonicated for 10 min then refluxed for 20 h. The final products were filtered, washed with methanol and dried under vacuum. To remove the surfactant template (CTAB), the as-synthesized NH_2-MSN nanoparticles were refluxed in acidic ethanol solution with 0.37% HCl for about 24 h.

Preparation of protein coated MSNs

All experiments of preparing protein coated MSNs were performed at room temperature. First of all, GA adsorbs to the MSNs and then the aldehyde groups of GA is reacted with -NH_2

groups on the surfaces of MSNs by mixing the particles with 0.025% GA in pH 7.2 phosphate buffer solution (PBS) for 12 h. Then the coated particles were purified by three cycles of centrifugation and PBS washing. Next, the GA coated MSNs were dispersed into 4 mg/ml Hb solutions in PBS and reacted for 12 h, followed by washing with PBS. The GA and Hb were alternately adsorbed until three layers of GA/Hb were coated on the surface of MSNs. By the same method, another three layers of GA/GOD were assembled on the GA/Hb coated MSNs. Finally, the protein coated MSNs, MSN@$(Hb/GOD)_3$, were fabricated by GA as cross-linker. We also fabricated different protein layer numbers samples, MSN@$(Hb/GOD)_2$ and MSN@(Hb/GOD) with the same method.

Preparation of protein film on the glass substrate

The mixture solution consisting of Hb (4 mg/ml) and GOD (4 mg/ml) was dropped onto the glass substrate. A protein film was formed under vacuum conditions and observed by CLSM.

Quantitative analysis of the glucose sensitivity using fluorescence spectrofluorometer.

80 ul MSN@protein dispersed solution (~4mg/ml in PBS) was dropped into 900 ul different concentration glucose solution (0, 0.03, 0.12, 2, 4, 16 and 32 mg/ml in PBS). After being mixed well, the mixture was added 20 ul Amplex Red (0.5mM in PBS) and measured using fluorescence spectrofluorometer instantly. Fluorescence intensity was recorded at 585 nm with excitation wavelength 550 nm.

Cell culture

The cancer cell line, HeLa cells and normal cell line, Human embryo skin fibroblast (ESF) cells were used as cell experiments. Standard cell culture techniques were used for all cell experiments. Briefly, cells were cultured at 37 ^{o}C in a DMEM medium (Gibco BRL, USA) supplied with fetal bovine serum (FBS) (10%), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (25 mg/ml) in a humidified atmosphere with 5% CO_2. For the following experiments, cells were detached from culture flasks using PBS containing EDTA (0.02%) and trypsin (0.05%) and seeded to a 35 mm glass-bottom Petri dish. After the cells were incubated in the logarithmic growth phase, 50 ml MSN-based dispersed sample solutions (10 mg/ml) were added for 12 h of co-culturing. Then, the cells were washed twice with PBS to remove the dispersed nanoparticles and dead cells in the growth media. FM 4-64 solution (0.025 mg/ml), used for membrane staining, was added to the incubation mixture. Then, the stained cells were observed by CLSM.

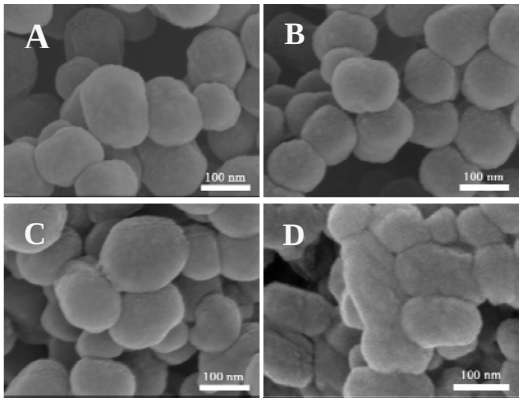
In vitro cytotoxicty assay

Hela cells were cultured into 24-well plates for 24 h, and then were incubated with the MSN@protein samples for 12 h or 24 h. After incubation, 100 ml sterile filtered MTT in PBS was added to each well to reach a final MTT concentration of 1 mg/ml and incubated with the cells for 4 h at 37 ^{o}C. Then dimethyl sulfoxide (DMSO) was added to dissolve the purple formazan crystals, followed by measuring the absorbance at 570 nm with a UV-Vis spectrophotometer.

Characterization and instrumentation

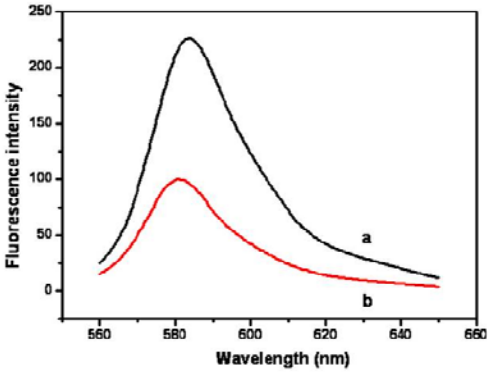
SEM and TEM images were obtained with Hitachi S-4300 and Philips Tecnai G2 F20UT respectively. For TEM observation, the samples were stained with 1% phosphotungstic acid. The amount of proteins coated on the surface of MSNs was determined comparing the absorbance at 595 nm before and after assembly using the Bradford protein assay with a U-3010 spectrophotometer (HITACHI, Japan). Production of resorufin from the coupled reaction of GOD and Hb was investigated to follow the color change of mixture via the spectrophotometer. The time scan of the coupled enzymatic reaction was recorded at 570 nm. Hb solution and GOD solution were equally mixed in a cuvette. The reaction triggered after adding glucose immediately followed by time scan. The same experiment was carried out for MSN@protein suspension with exception that the immobilized Hb was used in the coupled reaction rather than Hb solution. A Hitachi Model F-4500 fluorescence spectrofluorometer was used to measure the fluorescence of the samples. CLSM images were taken with an Olympus FV 1000 confocal system, equipped with 488 nm and 559 nm laser excitation.

Figure S1



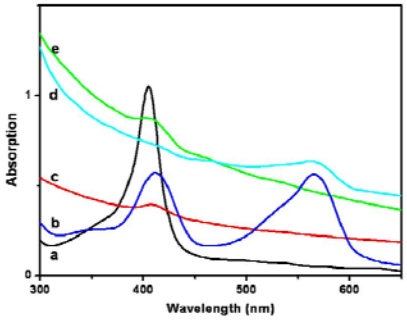
SEM images of protein coated MSNs with different assembly layer numbers. (A) MSN; (B) MSN@(Hb/GOD); (C) MSN@$(Hb/GOD)_2$; (D) MSN@$(Hb/GOD)_3$.

Figure S2



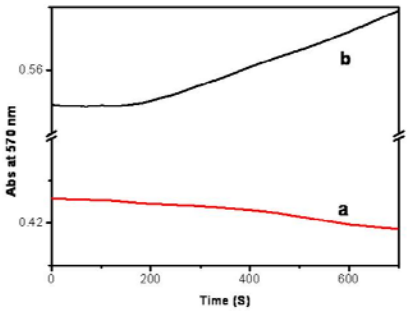
Fluorescence emission spectra in two systems by excitation at 550 nm: (a) protein solution upon the addition of glucose and Amplex Red (black line), (b) MSN@(Hb/GOD)$_3$ dispersed solution upon the addition of glucose and Amplex Red solution (red line).

Figure S3



UV-vis spectra of Hb and GOD mixed solution (a); the protein mixed solution upon the addition of glucose and Amplex Red (b); MSN@(Hb/GOD)$_2$ dispersed solution (c); MSN@(Hb/GOD)$_3$ dispersed solution upon the addition of glucose and Amplex Red (d); MSN@(Hb/GOD)$_3$ dispersed solution (e).

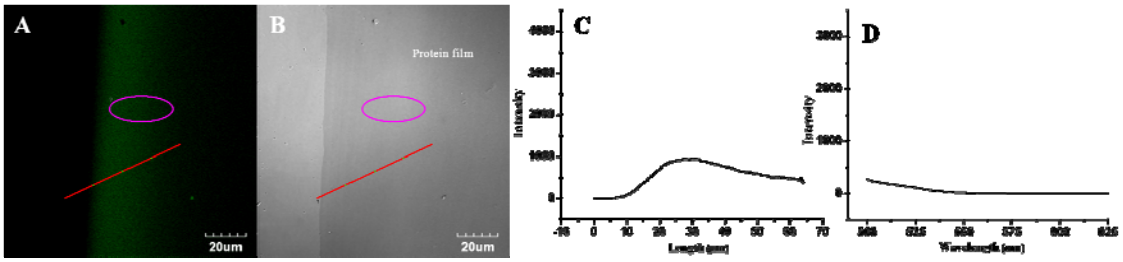
Figure S4



Absorption of resorufin at 570 nm change as a function of time in two systems: (a) MSN@protein dispersed solution (red line), (b) the dispersed solution upon the addition of glucose and Amplex Red solution (black line).

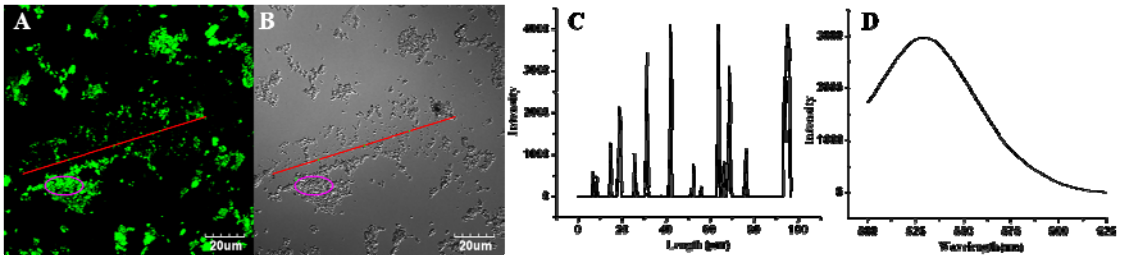
For proving the enzymatic activity of coupled protein on the surface of MSN, production of resorufin from the coupled reaction of GOD and Hb was also investigated using the spectrophotometer. Because resorufin has excitation/emission maxima of ~570/585 nm, the time scan of enzymatic reaction systems (MSN@protein) were recorded at 570 nm. As shown in Figure S4, the absorption at 570 nm increased after adding Amplex Red and β-D-glucose into MSN@protein solution (black curve). It is proved that the coupled reaction was in progress with time. Under the same experimental condition, the reaction did not happen because the absorption at 570 nm did not increase when no Amplex Red (red curve). It might because MSN@protein particles precipitation happened and the signal was slightly decreased with time (red curve). The results proved the coupled protein still retain their enzymatic activity when immobilized on the surface of MSN.

Figure S5



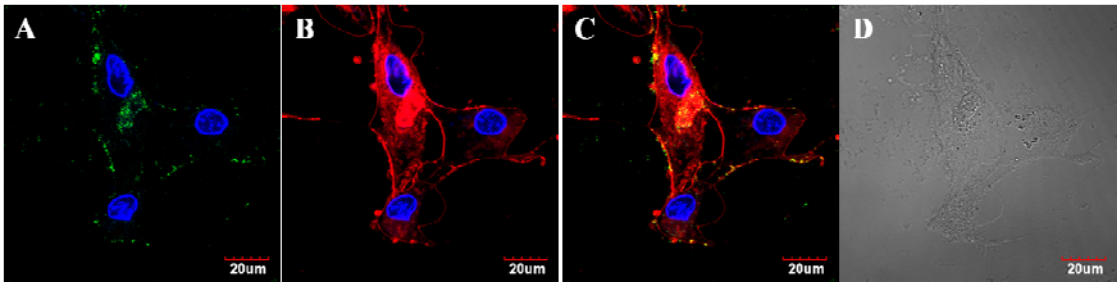
CLSM image of protein film obtained by excitation at 488 nm (A) and the corresponding pseudo-bright field image (B); fluorescent intensity profile of red line region (C); fluorescence spectra of selected purple line region (D). From figure A and C, the fluorescence of protein film is very weak (compared to Figure S6 C). It might come from trace heme of Hb molecule. There is no obvious emission peak by excitation at 488 nm.

Figure S6



CLSM image of MSN@protein particles obtained by excitation at 488 nm (A) and the corresponding pseudo-bright field image (B); fluorescent intensity profile of red line region (C); fluorescence spectra of selected purple line region (D). All the results were obtained by the same test parameters with ones of the protein film sample. Compared with Figure S5, the fluorescent intensity of MSN@Protein sample has been notably enhanced. The peak at 525 nm in fluorescence spectra was come from schiff's base.[2]

Figure S7



CLSM images of ESF cells stained with FM 4-64 and Hoechst 33342 incubated with MSN@protein by excitation at 405 nm, 488 nm and 559 nm. The corresponding images of the MSN@protein nanoparticles (green) (A); FM 4-64 labeled cell membranes (red) (B); the overlapped image (C) and the pseudo-bright field image (D). (in Figure A-C, blue fluorescence come from cell nucleus marker, Hoechst 33342)

Caption of the movie in supporting information

It is a 3-dimensional stacked x-y cross-sections of a cell observed from bottom to top by CLSM. The yellow dots represent MSN@protein nanoparticles which come from overlapped effect between autofluorescence of MSN@protein (green) and plasma membrane marker (red). In the movie, the cell membrane is clearly visible in outline. Furthermore, some MSN@protein particles located at inner layer of cell membrane outline. Therefore, we believe certain amount MSN@protein particles are embedded into cell membranes.

References:

1. S. Huh, J. W. Wiench, J.-C. Yoo, M. Pruski and V. S. Y. Lin, *Chemistry of Materials*, 2003, **15**, 4247-4256.
2. W. Wei, L. Y. Wang, L. Yuan, Q. Wei, X. D. Yang, Z. G. Su and G. H. Ma, *Advanced Functional Materials*, 2007, **17**, 3153-3158.