Supplementary Material (ESI) for Soft Matter

Biocompatible fluorescent nanoparticles for pH-sensoring

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Materials and Methods

Dextran with the weight-average molecular weight of 54,800 g mol^{-1} and a polydispersity index (PDI) of 1.56 is produced by *Leuconostoc mesenteroides* strain no. NRRL B-512(F). The particle size of the nanospheres was determined by dynamic light scattering (DLS) studies using a laser beam at 633 nm and a scattering angle of 173° (Zetasizer Nano ZS, Malvern Instruments). For SEM studies, one droplet nanoparticle suspension on a mica surface was lyophilized and covered with gold. The images were obtained with the SEM equipment LEO-1530 VP Gemini (LEO, Oberkochen, Germany) operating at 10 kV. All fluorescence spectra were recorded in a 10 mm cuvette on a Fluorolog 3 from Jobin Yvon-Spex at a temperature of (25 ± 1)°C. The wavelength for excitation of the nanoparticle suspensions was 488 nm, if not denoted otherwise. For pH calibration, the sodium phosphate buffer used had a concentration of 0.067 mol L^{-1}. Confocal images were acquired with a Zeiss LSM 510 (Carl Zeiss GmbH, Oberkochen, Germany) inverted laser scanning microscope (LSM) using a C-Apochromat × 63 water immersion objective lens (Zeiss). Fluorescein and sulforhodamine B were excited with the Ar 488 nm and the HeNe 543 nm laser lines, respectively. The fluorescence intensities were recorded with a 505–530 nm band-pass filter and a 560 nm long-pass filter. For quantitative analysis of the fluorescence intensities, the microscopy images were flattened by using a low-pass filter (Kernel size 5x5).

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Synthesis of dextran derivatives

Preparation of sulforhodamine B labeled dextran propionate (SRB-dextran propionate)

For a complete dissolution of dextran in DMAc, 1 g (6.2 mmol) dextran and 0.6 g (14.2 mmol) water-free LiCl were suspended in 25 mL DMAc and stirred at 80°C until a clear solution appears. After cooling down at 8°C, 0.4 mL triethylamine and 0.2 g (0.3 mmol) sulforhodamine B acid chloride dissolved in 2 mL DMAc were added dropwise under stirring. The mixture was allowed to react at 8°C under stirring for 24 h. The product was isolated by precipitation in 300 mL 2-propanol, washed several times with 2-propanol, and dried at 60°C under vacuum. Yield: 1.02 g (51.0%).

The sulforhodamine B labeled dextran (0.5 g; 3.1 mmol) was dissolved in 10 mL DMAc and allowed to react with 3.2 mL (24.7 mmol) propionic acid anhydride and 1.7 g (24.7 mmol) imidazole for 20 h at 80°C. The product was isolated by precipitation in 200 mL ethanol, washed several times with 150 mL ethanol, and dried at 60°C under vacuum. Yield: 0.7 g (64.1%). The complete functionalization of all hydroxyl groups was proven by IR spectroscopy.

Preparation of fluorescein labeled dextran propionate (FITC-dextran propionate)

To a homogeneous solution of 2 g (12.3 mmol) dextran in 20 mL DMSO 1.7 g (24.7 mmol) imidazole and 3.2 mL (24.7 mmol) propionic anhydride were added. The mixture was allowed to react for 5 h at 80°C under stirring and precipitated in 400 mL water, washed twice with 150 mL water, and dried at 60°C under vacuum. Yield: 2.23 g (68.6%). DS_{Pro}: 1.81 (determined by means of ^{1}H NMR spectroscopy after peracetylation).

The resulting dextran propionate (1 g; 3.8 mmol) and 10.4 mg fluorescein isothiocanate (0.0267 mmol) were dissolved in 10 mL DMSO and allowed to react for 5 h at 100°C. The

mixture was precipitated in 300 mL water, washed twice with 150 mL water, and dried at 60°C under vacuum. Yield: 0.82 g (81.2%).

Preparation of the nanoparticle suspensions

The preparation of the nanoparticles was carried out by a dialysis process. A total quantity of 20 mg of dextran ester (SRB-dextran propionate, FITC-dextran propionate, or mixtures at different weight ratios) was dissolved in 5 mL purified DMAc and dialyzed against distilled water (Spectra/Por® membrane, molecular weight cut-off 3500 g mol^{-1}) for 4 d. The deionized water was exchanged 5 times.

Cell experiments

Human foreskin fibroblasts were cultured in RPMI-1640 with 10 % fetal calf serum (FCS) under standard conditions (37°C, 85% humidity). Cells were harvested and sowed out on glass covers for a density of 80% over night. After removing of all non-adherent cells by washing with phosphate buffered saline (PBS), cells were incubated with dextran propionate particles (100 µL particle suspension per 2 mL cell culture medium) for 24 h. Afterwards, all unbound particles were removed by washing 3 times with PBS. Cells were cultured again under standard conditions. The culture medium was exchanged every 3 days. After 4 and 20 days, cells were incubated with PBS at several pH values (4.9 to 8.2) and investigated by confocal laser scanning microscopy.