SUPPORTING INFORMATION

Anisotropic multicompartment micro- and nano-capsules produced via embedding into biocompatible PLL/HA films

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Experimental Section. Materials. Poly-L-lysine (PLL, 15-30 kDa), PLL labeled with fluorescein isothiocyanate (PLL-FITC, 15-30 kDa), poly(allylamine hydrochloride) (PAH, 70 kDa), poly(styrene sulfonate) (PSS, 70 kDa), poly(diallyldimethylammonium chloride) (PDADMAC, 200- 350 kDa) and PAH labeled with tetramethylrhodamine-isothiocyanate (PAH-TRITC) were purchased from Sigma-Aldrich (Germany). Hyaluronic acid (HA, 360 kDa) was purchased from Lifecore Biomedical (Chaska). Large (4.78 μm) and small (0.5 μm) silica particles (SiO_2) were purchased from Microparticles GmbH, Germany. Tris buffer containing 10 mM Tris and 15mM NaCl at pH 7.4 was used. 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 18.2MΩ cm.

Preparation of the films. The polyelectrolyte multilayer films ($(HA/PLL)_{12}/PLL$) were prepared by the Layer-by-Layer (LbL) deposition technique using a dipping robot (Riegler & Kirstein GmbH, Berlin, Germany). The films were deposited onto a microscopy cover glass (12 mm in diameter, Marienfeld GmbH, Germany). Before deposition, the glass slides were cleaned by consecutive incubation in hot solutions (60ºC) of 2% (v/v) Hellmanex (Hellma GmbH, Germany) and 0.1 M HCl for 15 min for each solution, followed by multiple rinsing steps with pure water. Film buildup was pursued at 25ºC by alternating dipping of the glass slides into polymer solutions (0.5 $mg{\cdot}mL^{-1}$) in Tris buffer with an intermediate washing step with buffer. Before deposition, polyelectrolyte solutions were filtered through a 0.45 μm filter. Each dipping step lasted 10 min. To prepare the film labeled with FITC, PLL-FITC was added to the PLL solution used for film deposition at 30:1 w/w unlabeled/labeled PLL.

Adsorption of big and small particles. Large SiO_2 particles (4.78 μm) were coated with polyelectrolyte multilayer $(PDADMAC/PSS)_4$ using LbL deposition.

Polyelectrolytes were prepared in 0.5 M NaCl at a concentration of 2 $mg \cdot mL^{-1}$. The coated microparticles were adsorbed on the $(HA/PLL)_{12}$/PLL film by the addition of 100 μL of the particle suspension (0.5 $mg \cdot mL^{-1}$) on top of the glass cover slip coated with the freshly prepared film. After 15 min of incubation, the film was washed with Tris buffer. The same procedure was followed for microcapsules. The core of polyelectrolyte coated microparticles was dissolved in HF (0.3 M) solution and washed several times with water. Microcapsules were incubated with dextran-Alexa Fluor 555 (3 kDa, Invitrogen, Germany) at concentration of 0.5 mg/mL and thermally treated at 60°C for 20 min resulting in shrinkage of microcapsules and entrapment of fluorescent dextran molecules. Further, 100 μL of 0.5 $mg \cdot mL^{-1}$ smaller SiO_2 particles (0.5 μm) coated with PAH-TRITC were incubated for 15 min with the $(HA/PLL)_{12}$/PLL film with entrapped large SiO_2 particles/capsules. Finally, the film was washed with Tris buffer.

Destruction of the films. The film with the entrapped particles was removed from the glass substrate by the addition of 200 μL of 0.1 M NaOH followed by 5 min of incubation in this solution. This treatment allows the complete removal of the multilayer films, without destroying the particles, by the deprotonation of PLL molecules. Then, 0.8 mL of Tris buffer was added and the free large SiO_2 particles with adsorbed small SiO_2 particles on half of their surface were collected and imaged by Confocal Laser Scanning Microscopy.

Confocal Laser Scanning Microscopy (CLSM). CLSM micrographs of the films labeled with FITC and after particle adsorption were taken with a Leica confocal scanning system mounted onto a Leica Aristoplan and equipped with a 63x dry objective. CLSM micrographs of the anisotropic constructs were taken with a 100 x oil immersion objective with a numerical aperture of 1.4.

Scanning Electron Microscopy (SEM). SEM images of anisotropic particles were recorded with a Gemini 1550 (Zeiss, Oberkochen, Germany) at an acceleration voltage of 3.0 KeV. A drop of the anisotropic particle suspension was placed on a glass slide and allowed to dry at room temperature.

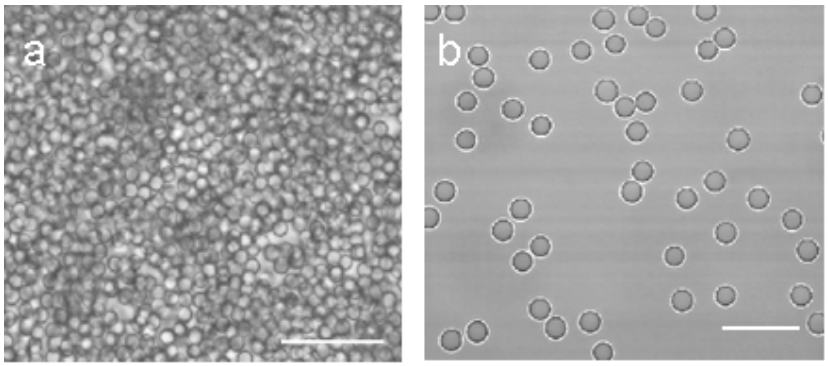

Figure S1. Transmission CLSM images of high (a) and low concentration (b) of $(PDADMAC/PSS)_4$ coated large SiO_2 particles (4.8 μm) embedded into a $(HA/PLL)_{12}/PLL$ film. The concentration of SiO_2 particles was 5 $mg \cdot mL^{-1}$ in (a) and 0.5 $mg \cdot mL^{-1}$ in (b). Deposition of particles at high concentration (a) results in a non-uniform distribution on the surface of the films, particles sitting one on top of the others. Scale bars correspond to 30 μm in (a) and 20 μm in (b).

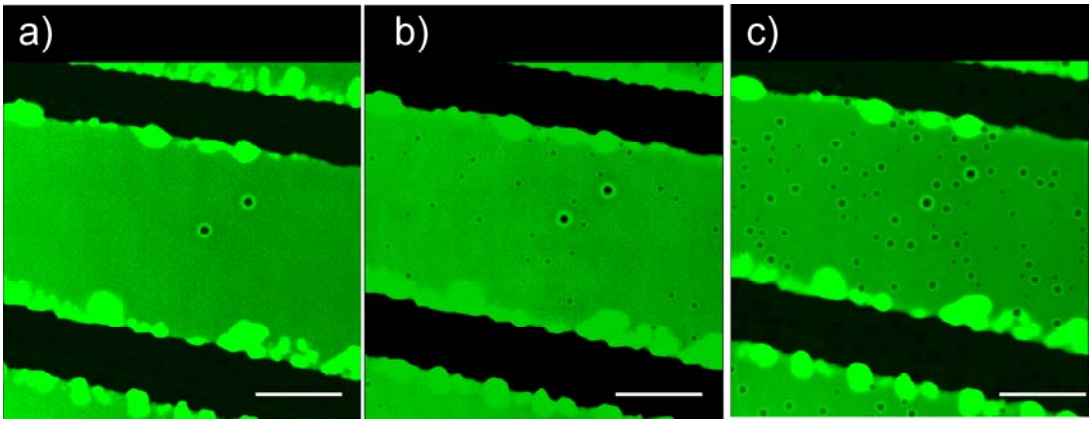

Figure S2. Fluorescence micrographs of polymeric films with embedded particles for different time after addition of containers (dark circular dots): a) 2 containers embedded in the films after 30 min; b) freshly added containers after 30 seconds; c) embedding of the containers from b) after 2 minutes. The black lines are scratches of the films made to enhance the contrast. The scale bars correspond to 35 μm.

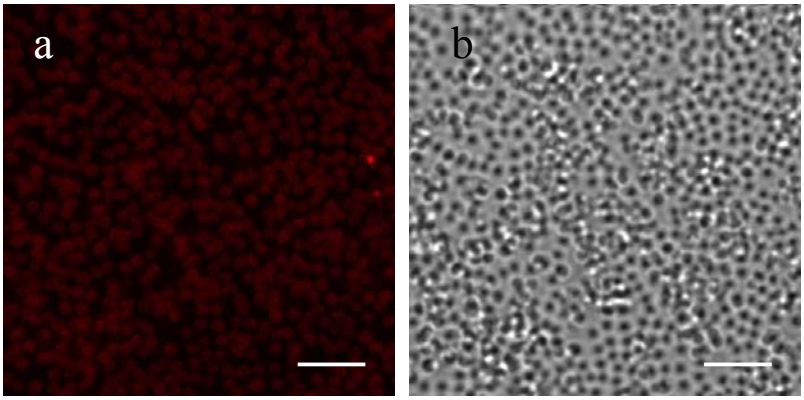

Figure S3. Fluorescence (a) and transmission (b) CLSM images of small SiO_2 nano-containers (0.5 μm) coated with cationic PAH labeled with tetramethylrhodamine-isothiocyanate (PAH-TRITC). CLSM micrographs of small particles were taken with a 100x oil immersion objective, while the 63x dry objective which was used for visualization of films with embedded anisotropic constructs does not allow visualization of small particles on top of large containers embedded into films. The scale bars correspond to 10 μm.

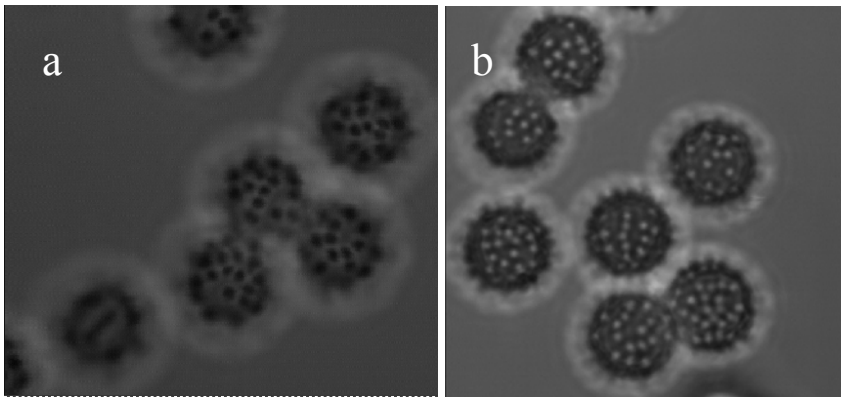

Figure S4. Transmission CLSM images of 4.8 μm SiO_2 particles functionalized with 0.5 μm SiO_2 particles before (a) and after (b) treatment with 0.1 M NaOH. Assemblies presented in Figure S4 were constructed without embedding of the larger particles in films. A uniform coverage of smaller particles can be clearly seen in this Figure.

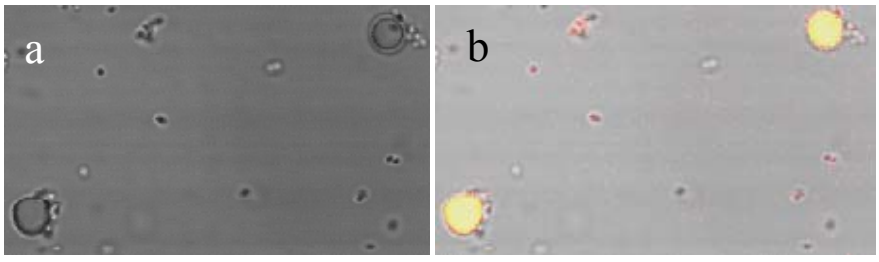

Figure S5. Transmission (a) and overlay (transmission, red and green fluorescence) CLSM images of SiO_2-templated capsules loaded with AF488-dextran (green fluorescence) functionalized with 0.5 μm SiO_2 particles labelled with Rhodamine (red fluorescence).