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Preparation, characterization, and preliminary biocompatibility evaluation of particulate spin-coated mesoporous silica films

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ABSTRACT

Porous, biocompatible supports are interesting substrates for biological applications, as they allow for release of actives with spatial control. Here we report on the synthesis and characterization of spin-coated thin films made of mesoporous silica nanoparticles of different size and shape on microscopy slides. By controlling processing parameters like spinning-speed and particle concentration, homogeneous films can be prepared with controlled thicknesses ranging from bilayers to several multilayers. Different particle diameters and shapes can be used, thus providing further parameters for film thickness and morphology control. The use of particles in the preparation of the spin-coated films has several advantages. Relatively homogeneous thick films can be prepared in one step with thicknesses exceeding those using molecular precursor solutions. Furthermore, as the morphological properties of the surface are known to influence cell attachment, proliferation, and also differentiation of stem cells, the possibility to influence the morphology of the films is highly attractive from a biomaterial perspective. Preliminary *in vitro* cell studies indicate that the particles can be endocytosed by cells cultivated on these films, and that model drugs can be released inside the cells showing the potential of these films for local drug delivery applications. The results are important for further optimization of novel scaffolds exhibiting spatial, and temporal control of the delivery of active cues in, for example, tissue engineering applications.

1. Introduction

Mesoporous or macroporous inorganic materials are interesting supports for tissue engineering applications. Porous scaffolds are attractive for directed cell attachment and differentiation [1], and may also allow for local release of biological cues at the implantation site [2–5]. With respect to controlled release of bioactive factors, mesoporous coatings are of special interest, as the pore size is on the order of the molecular size of the biological cues. There are numerous studies in the literature related to the synthesis of highly ordered, mesoporous inorganic thin films starting from sols containing molecular inorganic precursors and surfactants serving as structure directing agents, and where the ordering is a result of evaporation-induced self-assembly. Typically, such films are prepared by dip- or spin-coating [6–8]. The pore dimension, pore geometry and connectivity can be controlled by changing the sol composition and/or the processing parameters. Other possibilities for preparing meso- or macroporous films are, for example, anodization of metals [9–13], lithographic techniques [14] or reactive ion etching [15,16]. Not surprisingly, such films have been used as a support for cells [17] and are also able to serve as a drug release system [18]. However, there are some limitations related to the use of such films as in cell cultures and as a platform capable of delivering drugs to cells. Depending on the means of preparation, limited film thicknesses can lead to low maximum loading capacities. Furthermore, the release is typically diffusion controlled. There are limited variation possibilities when it comes to film morphology, and, if thicker films are needed, relatively long processing times are required. An alternative film architecture would be films of mesoporous inorganic particles that would serve as the drug reservoirs. The film preparation should be fast and reproducible, and suitable for smaller supports like microscopy slides, but there is no need for a fully developed long-range order as long as the film is homogeneous at least on length-scales corresponding to the size of cells. Such films could overcome several of the limitation discussed above. There are several potential advantages of mesoporous particulate films in relation to drug release

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applications. Thicker films than what normally could be achieved by spin-coating and dip-coating using molecular precursor sols can be prepared using particulate precursors, allowing the absolute amount of drug present in the film to be increased as compared to thinner films. Furthermore, variation of the particle diameter and shape allows for additional tuning of film thickness and morphology, the nanoparticles can easily be functionalized in a step separated from the film preparation, and particle mixtures can be used in order to, for example, deliver two or more cues or create more elaborate surface morphologies. Spin-coating is an attractive, fast and reproducible means for preparation of particulate films, as has already been demonstrated for multilayer films made from non-porous Stöber [19] or small mesoporous spherical silica nanoparticles [20]. Spin-coated films made of non-porous Stöber particles of different size have been shown to be biocompatible supports for cells *in vitro*, and the topological differences of the films originating from the differently sized particles was shown to have an influence on the proliferation rate of the cells [21,22]. However, to the best of the knowledge of the authors, particulate films have to date not been evaluated in relation to drug release applications. In the following, we report results related to the use of spin-coating in order to process films with controlled thicknesses from ethanolic dispersions of mesoporous silica nanoparticles of different size and shape. We also demonstrate that different particle sizes and shapes, and particles having different surface functions can be used without compromising the film homogeneity. The films are biocompatible, cells can internalize particles from the films, and model drugs can successfully be intra-cellularly released after particle internalization. Thus, the results add to the already exhaustive body of literature showing that mesoporous silica nanoparticles are highly promising drug delivery vectors *in vitro* and *in vivo* (For recent reviews see for example references [23–28].) The results can serve as design criteria for further developments related to tissue engineering applications *in vitro*, and if combined with scaffolds, *in vivo*.

2. Experimental

2.1. Particle Synthesis

Mesoporous silica nanoparticles were prepared according to the synthesis published by Rosenholm et al. [28]. A solution containing the structure-directing agent was prepared by adding 0.039 g NaOH and 3.7 g cetyltrimethylammonium chloride, CTAC, to 800 g of a mixture of water and methanol. 1.19 g Tetramethylorthosilicate, TMOS, was mixed with aminopropyltrimethylsilane, APTMS, under inert atmosphere and added to the basic solution. In a typical synthesis the molar ratios were 0.9 TMOS:0.1 APTMS:1.27 CTAC:0.26 NaOH:1439 MeOH:2560 H_2O. All chemicals used were analytical grade. The sol was stirred for 12 h at room temperature and then aged for another 8 h under static conditions. The particles were separated through centrifugation, re-dispersed in ethanol and filtered or centrifuged. The different sizes of the particles were reached by changing the water/methanol ratio. A water/methanol ratio of 50:50 and a stirring speed of 400 rpm lead to mesoporous silica particles with a diameter of about 500 nm, while a water/methanol ratio of 55:45 lead to the formation of corresponding particles with a diameter of 200 nm. The residue was washed with de-ionized water and dried *in vacuo*. The removal of the structure-directing agent was performed either by calcination or by ion-exchange. Calcination was performed in air at 500 °C for 5 h with a heating rate of 1.5 °C/min. Ion-exchange was performed by ultrasonication of particulate dispersions for 20 min in acidic (HCl) ethanol (about 0.12% w/w) or in ethanolic ammonium nitrate solution (6 g/L) for 60 min. This step was repeated three times. Calcined mesoporous silica spheres are denoted MSN-XX-c and spherical amino-functionalized particles MSN-XX-a throughout the text, where XX stands for the mean particle diameter as determined from SEM images based on the analysis of minimum 100 particles.

2.2. Synthesis of cylindrical particles

Cylindrical particles (aspect ratio 1:4) were synthesized according to the procedure of Huh et al. [29]. 480 mL water and 7 mL of a 2 M caustic soda solution were mixed with 2.0 g CTAB and kept at 80 °C for 30 min under stirring. 9.11 g TEOS and 1.22 g APTES were pre-mixed under inert gas and added to the alkaline solution. After a reaction time of 2 h at 80 °C the particles were separated by centrifugation and dried at 60 °C. The surfactant was removed by stirring 2 g of particles in 4 mL conc. HCl in 200 mL MeOH for 2 h at 50 °C, washed with H_2O and MeOH and dried at 60 °C. The cylindrical amino-functionalized rod-like particles are denoted MSR-340a throughout the text. The mean width of the particles was 340 nm and the mean length about 1450 nm.

2.3. Cubic haematite/silica core–shell nanoparticles

The cubic haematite core was synthesized according to Rossi et al. [30] in a 250 mL Pyrex bottle by drop-wise addition of 90 mL of a 6.0 M caustic soda solution to 100 mL of a 2.0 M $FeCl_3 \cdot H_2O$ solution under gentle stirring. 10 mL of water was subsequently added to this solution which was further stirred for another 10 min. Then the mixture was aged at 100 °C under static conditions. The size of the nanoparticles was controlled by the aging time. An aging time of 8 days was used to obtain particles with a mean size of 1335 nm (SEM). The particles were dried at 50 °C.

Polyvinylpyrrolidone (PVP, average MW 25,000) was adsorbed to the particles in dispersion following the procedure in Ref. [24,31].

A layer of silica was deposited onto the haematite particles following the procedure described by Graf et al. [31]. 5 g of the polymer-coated haematite nanoparticles were dispersed in 33 mL ethanol and added to a solution containing 485 mL ethanol, 35 mL water and 5,3 mL tetramethylammoniumhydroxide (1% wt in water) under stirring, ultrasonically treated for 15 min. The deposition was started by adding 1 mL of a solution of 2,7 mL TEOS and 2,1 mL ethanol within a time-frame of 15 min., followed by a further addition of a mixture of 2.7 mL TEOS and 2.7 mL ethanol. A premixed solution of 10.6 g poly(vinyl pyrrolidone) in 106 mL ethanol was subsequently added to the sol, followed by 2 h of ultrasonication and further stirred overnight. The particles were separated be filtration and dried at 60 °C. The so-synthesized truncated cubic haematite (core)-silica (shell) particles are denoted SHC-1300 throughout the text.

2.4. Procedure for attachment of fluorescent dye

Amino-functionalized silica nanoparticles were dispersed in 10 mL dried DMF with ultrasonic treatment (Covaris) and the fluorescent dye tetramethylrhodamineisothiocyanate (TRITC) were added to the solution. For 100 mg silica nanoparticles 66 μL of a DMF solution containing 1 mg/mL fluorescent dye was added, and let to react for 12 h. The particles were isolated by centrifugation and dried in vacuum for 24 h.

2.5. 3,3′-Dioctadecyloxacarbocyanine perchlorate (DiO) loading

DiO, as a lipophilic tracer, was loaded into the particles' pores after drying the films in vacuum overnight. 3% DiO calculated based on the mass of the silica nanoparticles was dissolved in