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Nanostructured medical device coatings based on self-assembled poly(lactic-*co*-glycolic acid) nanoparticles

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ABSTRACT

Here we present a new method for providing nanostructured drug-loaded polymer films which enable control of film surface morphology and delivery of therapeutic agents. Silicon wafers were employed as models for implanted biomaterials and poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles were assembled onto the silicon surface by electrostatic interaction. Monolayers of the PLGA particles were deposited onto the silicon surface upon incubation in an aqueous particle suspension. Particle density and surface coverage of the silicon wafers were varied by altering particle concentration, incubation time in nanoparticle suspension and ionic strength of the suspension. Dye loaded nanoparticles were prepared and assembled to silicon surface to form nanoparticle films. Fluorescence intensity measurements showed diffusion-controlled release of the dye over two weeks and atomic force microscopy (AFM) analysis revealed that these particles remained attached to the surface during the incubation time. This work suggests that coating implants with PLGA nanoparticles is a versatile technique which allows drug release from the implant surface and modulation of surface morphology.

1. Introduction

Polymeric coatings of biomedical devices are among the popular and efficient strategies to enhance biocompatibility and effectiveness of these devices and deliver therapeutic cargos. Drug-loaded polymer films, comprised of biopolymers such as poly(lactic acid) (PLA) or poly(lactic-co-glycolic acid) (PLGA) and their derivatives [1–5], chitosan and its blends [6–10] or other synthetic polymers [11,12], can act as a reservoir for local or systemic drug delivery from the implant surface. Furthermore, film coatings can be specifically designed to modulate implant surface properties in order to regulate biological responses. Numerous studies have shown that physiochemical properties of medical devices at interfaces influence the biological responses. For example, modification of device surfaces with films of polymers can potentially reduce undesired interactions associated with the implantation. Surface topography can be controlled by fabrication of micro- and nanostructured polymer surfaces, which has been shown to reduce protein adsorption, cell adhesion [13–16] and bacterial adhesion [16–19].

Colloidal lithography is a low cost, high throughput, versatile method for surface patterning based on self-assembly of colloidal particles onto surfaces. This method has many advantages as alternative methods commonly used for producing patterned surfaces. In addition to the low cost, it enables the fabrication of a well-defined patterned surface over a large surface area [20]. Through manipulation of particle size and surface coverage it can also be used to easily modify the surface morphology [21]. In comparison to continuous matrix film coatings, colloidal lithography coatings can achieve a controllable surface coverage, have the ability to release biomolecules from the particles on the surface, and are not as sensitive to forces of stress and strain.

Examples of implantable systems coated using colloidal lithography can be found in the literature. Kunzler et al. [22] demonstrated the fabrication of a gradient of negatively charged silica nanoparticles, which were electrostatically adsorbed onto positively charged poly(ethylene imine) (PEI)-coated silicon wafers. After particle sintering, cell experiments with rat calvarial osteoblasts showed that surface coverage with the nanoparticles considerably reduced cell attachment, proliferation and spreading on the nanoparticle coated surfaces. Indeed, at seven days post-seeding, the number of cells on the particle-free surfaces was eight times higher compared to wafers with maximum nanoparticle coverage. Gradient coatings made from microparticles were also investigated by Li et al. [23]. In their work they used an electrospray technique to construct density gradient of PLGA microparticles onto glass slides. Following deposition, the coated slides exhibited variations in surface roughness, which enabled investigation of the effect of physical cues on

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neurite outgrowth from dorsal root ganglia. By optimizing the surface roughness, the neuron adhesion and neurite extension were promoted.

Release of biomolecules from medical device surfaces is another application of self-assembling particle coatings. Lo et al. [24,25] presented a new method for the coating of neural devices based on electrostatic attachment of negatively charged PLGA nanoparticles onto poly(L-lysine) (PLL) coated silicon surfaces. This particle coating showed potential to release multiple agents simultaneously in addition to the high efficiency to deliver therapeutic agents and plasmid DNA.

Since the success of implantable devices is dependent upon reducing undesired foreign body responses, an ideal implant should be carefully designed. Two strategies have shown promising results in reducing foreign body responses: Surface patterning at the nanoscale and implant coating with drug loaded polymer. The combination of these two strategies can provide more advantages and promote the implant compatibility.

To the best of our knowledge, no previous work has focused on the construction of highly ordered nanostructured polymer coatings, which control the release of drugs or biomolecules. This work aims to present novel coating based on polymer nanoparticles assembled onto silicon surface for surface topography modulation and active agent release. Therefore, we investigated surfactant-free, anionic PLGA nanoparticles which self-assembled onto the surface of positively charged silicon surfaces. To control surface coverage with the particles, ionic strength and particle concentration in the aqueous suspension were tuned. To demonstrate the effectiveness of this coating for implants which are in contact with body fluids, morphological characterization of the films was investigated over time in phosphate-buffered saline (PBS) solution. A fluorescent dye representing a model drug was loaded into the particles to test the dye release from the films.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA), Type Resomer® RG 752H, lactide/glycolide ratio 75:25 was purchased from Boehringer Ingelheim, Ingelheim, Germany. 5-Aminofluorescein (AF) and (3-aminopropyl) triethoxysilane (APTES), ≥98% and phenyltrimethoxysilane (PTMS) were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Germany). All other chemicals and solvents used in this study were of high analytical grade and commercially available.

2.2. Nanoparticle preparation

AF loaded nanoparticles were formed according to the method described elsewhere [26]. Briefly, 160 mg PLGA was dissolved in 20 ml acetone at 25 °C under continuous stirring. The desired amount of AF was dissolved in 5 ml acetone and the solution was mixed vigorously with the polymer solution. The resulting solution was slowly added to 50 ml of filtered and double distilled water at constant flow rate of 10 ml/min and under magnetic stirring (360 rpm). For this purpose a syringe with injection needle (Neopoint® 0.90 × 70 mm; Servopharma GmbH, Wesel, Germany) was used. The resulting colloidal suspension was stirred for 4 h under reduced pressure to evaporate off the organic solvent. The PLGA nanoparticle suspension was centrifuged at 10,000 rpm and resuspended in 50 ml double distilled water. The washing procedure was repeated a second time prior to direct use for coating. Nanoparticles of 1% and 2% AF theoretical loading were prepared as described above by adding 1.6 mg and 3.2 mg AF to the polymer/acetone solution, while blank nanoparticles were prepared by omitting the AF.

2.3. Nanoparticle characterization

2.3.1. Particle size measurement

The mean size and the size distribution of the nanoparticles were determined by photon correlation spectroscopy (PCS) using a Zetasizer NanoZS/ZEN3600 (Malvern Instruments, Herrenberg, Germany) at 25 °C as described previously [26,28]. To avoid multiscattering, the particles were suspended in filtered, double distilled water to yield a concentration of 32 μg/ml. Particle mean diameter (Z-Ave) and also the width of the fitted Gaussian distribution, which is displayed as the polydispersity index (PDI) were calculated using the DTS V. 5.02 software. Each size measurement was carried out with at least 10 runs for more accuracy.

2.3.2. ζ-Potential measurement

The ζ-potential was measured by the use of NanoZS/ZEN3600 (Malvern Instruments, Herrenberg, Germany) at 25 °C. Each sample was diluted in 1 M and 0.3 M PBS solution and the ζ-potential was measured in these solutions of different ionic strength. The DTS V. 5.02 software was used to calculate the average ζ-potential values obtained from the data of 100 runs. All ζ-potential measurements were carried out in triplicate.

2.3.3. Encapsulation efficiency

The encapsulation efficiency is defined as the percentage of drug associated with the nanoparticles relative to the total amount of drug added during the nanoparticle preparation:

$$\text{Encapsulation efficiency} : \frac{\text{Drug associated with the nanoparticles}}{\text{Total amount of the drug}}$$

while the drug loading is defined as the mass of drug associated with the particles relative to the total mass of the nanoparticles (polymer + drug) [27]:

$$\text{Drug loading} : \frac{\text{Drug associated with the nanoparticles}}{\text{Total mass of nanoparticles(polymer+drug)}}.$$

The encapsulation efficiency of the model drug, AF, was determined by calculating the mass of drug associated with 5 mg nanoparticles (experimental loading) by dissolving 5 mg of the AF-loaded particles in 5 ml DMSO at room temperature and diluting the solution in DMSO and then measuring the fluorescence absorbance. A fluorescence spectrometer equipped with a plate reader (Saphire II; Tecan, Austria) was employed to determine the fluorescence intensities of the AF in the DMSO solutions at the wavelengths: 353 nm excitation/426 nm emission. A glass plate was used for this purpose. The concentration of AF in the diluted solutions and the mass of AF in 5 mg AF loaded nanoparticles were calculated. The theoretical loads of the particles used in this study were 2% (w/w) (high-dose loaded nanoparticles) and 1% (w/w) (low-dose loaded nanoparticles).

2.4. Silicon surface modification

Silicon wafers were washed with acetone, isopropanol and a large amount of double distilled water and dried in nitrogen flow prior to surface modification. Two distinct surface modifications were prepared and examined regarding their effectiveness to produce self-assembly coating of PLGA nanoparticles. The first surface modification used APTES and was achieved by incubation of the clean silicon wafers in 2 μl/ml APTES/chloroform solution for 1 h at room temperature. The wafers were then washed with chloroform to remove loosely physisorbed APTES, followed by heating at 110 °C for 1 h and storage at 4 °C until further use. The second surface modification