Colloids and Surfaces B: Biointerfaces 163 (2018) 175–183

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Smart release of doxorubicin loaded on polyetheretherketone (PEEK) surface with 3D porous structure

Liping Ouyang[a,b], Zhenjie Sun[a,c], Donghui Wang[a,b], Yuqin Qiao[a], Hongqin Zhu[a], Xiaohan Ma[a], Xuanyong Liu[a,*]

[a] State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, PR China
[b] University of Chinese Academy of Science, Beijing 100049, PR China
[c] Department of Chemistry, Shanghai Normal University, Shanghai 200234, PR China

ARTICLE INFO

Article history:
Received 29 August 2017
Received in revised form
21 December 2017
Accepted 23 December 2017
Available online 26 December 2017

Keywords:
Polyetheretherketone
Chitosan
Doxorubicin
Controlled release

ABSTRACT

It is important to fabricate an implant possessing environment sensitive drug delivery. In this work, the construction of 3D porous structure on polyetheretherketone (PEEK) surface and pH sensitive polymer, chitosan, was introduced. The smart release of doxorubicin can be realized on the 3D porous surface of PEEK loading chitosan. We give a feasible explanation for the effect of chitosan on smart drug release according to Henderson–Hasselbalch equation. Furthermore, the intracellular drug content of the cell cultured on the samples with highest chitosan is significantly higher at pH 4.0, whereas lower at pH 7.4 than other samples. The smart release of doxorubicin via modification with chitosan onto 3D porous PEEK surface paves the way for the application of PEEK in drug loading platform for recovering bone defect caused by malignant bone tumor.

1. Introduction

Malignant bone tumor is one of the major causes of bone defect as currently the main therapeutic of bone tumor in clinic is surgical removal [1,2]. However, it is generally difficult to eliminate bone-tumor cells completely through surgical intervention. Consequently, chemotherapy is necessary for malignant bone tumor therapeutic along with debulking surgery. The toxicity on healthy proliferating cells caused by non-specific chemotherapy does have a great threat for patients [3–5]. Tumor-targeting approaches have been developed for improved efficacy and reduced toxicity by using specific tumor extracellular environment to alter biodistribution of drugs [6–8].

Malignant bone tumor therapeutic includes surgery removal and chemotherapy. It is of great importance to fabricate an implant possessing both mechanical properties and environment sensitive drug delivery. Polyetheretherketone (PEEK) is fast emerging as the potential choice for bone defect caused by malignant bone tumor therapeutic. Because PEEK is a semi crystalline polymer with an approximate crystallinity of 30–35%. The glass transition temperature of 143 °C [9] make it easy to molded to fit the bone shape owing to its thermoplasticity [10]. Meanwhile, PEEK exhibits an elastic modulus ranging between 3 and 4 GPa, which can be tailored to closely match cortical bone (~18 GPa) [9]. Furthermore, in our previous experiment, we constructed a three-dimensional (3D) porous structure on the PEEK surface through sulfonation by concentrated sulfuric acid [11], disclosing a good biocompatibility and osteogeneration. Hierarchical porous structure of sulfonated PEEK is beneficial for drug adsorption and delivery, as nano-reservoirs could be used for biological factors or other molecular drugs delivery [12–21]. Consequently, the sulfonated PEEK is a promising material as a drug carrier due to its hierarchical 3D network.

Tumor cells featured by the acidic extracellular environment due to the higher rates of glycolysis appearance. The concentration of extracellular H^+ becomes higher than that of intracellular H^+, which results from the lactate ion and H^+ pumped into the extracellular fluid via the monocarboxylate carrier. The disorganized vasculature of tumors, poor lymphatic drainage and elevated interstitial pressure result in an inefficient clearance of extracellular H^+. Consequently, the extracellular pH is obviously lower than normal tissue [22,23]. It is an effective way to improve efficacy and reduce toxicity by altering release of drugs through pH gradients [24–26]. Chitosan is a solid choice for modified reagent due to its abundant hydroxyl and amino group [27–31]. On the one hand,

* Corresponding author.
E-mail address: xyliu@mail.sic.ac.cn (X. Liu).

https://doi.org/10.1016/j.colsurfb.2017.12.045
0927-7765/

the amino group bodes well for the adhesion of the drugs and the drug delivery. On the other hand, the amino group can enhance the permeability of cell membrane and thus facilitate the drug uptake [32–34].

In this work, the sulfonated PEEK is fabricated via concentrated sulfuric acid etching followed by hydrothermal treatment. The chitosan is immobilized onto the sulfonated PEEK surface to obtain a smart doxorubicin (Dox) release that responding to pH. Through adjusting the chitosan content of the samples, the selected drug release can be accomplished at same pH value as well. The intracellular content of Dox increases with the ascending chitosan content in acidic environment, whereas decreases with the ascending chitosan content in neutral environment. Based on the results of zeta potential, drug release, and Henderson–Hasselbalch equation, we give a feasible explanation for the effect of chitosan on selected drug delivery properties.

2. Experimental

2.1. Materials and methods

2.1.1. Materials preparation

Medical grade polyetheretherketone (PEEK) was machined into wafers with a diameter of 12 mm and thickness of 1.2 mm for surface characterization, immersion tests, and *in vitro* studies on 24-well tissue culture plates. The rectangle samples (20 mm × 10 mm × 1 mm) were used for surface zeta potential measurements. All the samples used *in vitro* test were polished on one side to a near mirror finish. The concentrated sulfuric acid (95–98%) was utilized to fabricate sulfonated samples at room temperature, subsequently hydrothermal treating at 120 °C for 8 h to obtain the sulfonated samples with removed sulfur. Then, the sulfonated samples after removing sulfur were immerged into the mixed solution of doxorubicin and different content of chitosan at 40 °C for 24 h. SP denotes the samples treated with sulfonation and hydrothermal treatment at 120 °C for 8 h. DC0 denotes the samples immerged into the doxorubicin only, and DC1, DC2 and DC3 denote the samples immerged into the mixed solution of 10^{-4}M doxorubicin (fixed) and chitosan with the concentration of 1, 2 and 5 mg/ml respectively.

2.1.2. Material characterization

Field-emission scanning electron microscopy (FE-SEM, Hitachi S-4800, Japan) was used to examine the surface morphology. The surface chemical composition was determined by Fourier transform infrared spectrometer (FT-IR, FTIR-7600, Lambda Scientific, Australia). Ultraviolet and visible spectrophotometer (Uv–vis, Lambada 750, Perkin Elmer, USA) was used to measure the Dox content of the sulfonated samples.

The surface zeta potentials of samples were measured by Surpass electrokinetic analyzer (Anton Parr, Austria). Potassium chloride (KCl, 0.001 M) was used as electrolyte solution. The pH value was adjusted by hydrochloric acid (HCl) and sodium hydroxide (NaOH). The potentials resulting from the motion of ions in the diffusion layer were measured according to Helmholtz–Smoluchowski equation [35].

Contact angle measurement (Automatic Contact Angle Meter Model SL200B, Solon, China) was made to measure the wettability of the samples surfaces. Once the 2 μL deionized water droplet calmed down, the equipped camera system captured the photography immediately. Four samples per group were used.

2.1.3. Dox release measurement

The release content of Dox was tested by Uv–vis spectrophotometer. Several samples were immerged into a 2 mL phosphate buffered saline (PBS) with the pH 7.4, 6.8, and 4.0 respectively. The fluid was collected and measured by Uv–vis spectrophotometer at 480 nm.

2.2. Cell response

2.2.1. Cell viability on samples' surface

Osteosarcoma cells (MG63: provided by Cell Bank of Chinese Academy of Science) were cultured in the dulbecco's modified eagle medium (DMEM, Thermo Fisher Scientific Inc., USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 furnished by an incubator (STIK, China). Every culture dish (Thermo Scientific, USA) containing 9 mL DMEM and was refreshed every three days. The MG63 were seeded on the surface of samples on 24-well plates at a density of 5×10^4 cells per well. Three replicates every group were test by alamarBlue™ (Thermo Fisher Scientific Inc.) assay after culturing for 1, 4, and 7days. At each time point, the culture medium was removed, 1000 μL physiological saline solution (PBS) was used to wash the residual medium, 500 μL fresh medium with 10% alamarBlue™ was transferred to each well. After incubation for 4 h, the amount of reduced alamarBlue™ was determined on a microplate reader (Thermo Fisher Scitnfic Inc., USA). The operation and calculation of cell proliferation according to the instruction of the alamarBlue™ assay [36].

2.2.2. Intracellular Dox concentration

The MG63 were seeded on the surface of the samples (four replicates) at a density of 5×10^4 cells per well in the DMEM with the pH value of 4 and 7.4. 66.7 μM Dox was added to the control cell with the cell density of 5×10^4 cells per well. After incubation for 24 h, the DMEM was removed, and the PBS was used to wash the samples three times. 100 μL of the trypsin-ethylenediaminetetraacetic acid (EDTA) solution (0.25% trypsin, 1 mM EDTA, HyClone, USA) was left on each samples and incubated for 3 min in incubator (STIK, China). Afterwards, 1000 μL PBS was used to detach the cells from the surface of the each sample. And the total 4000 μL cell suspension was collected and centrifuged at 1200 r/min (revolutions per minute for 5 min). Then, the PBS was used to wash the cells three times. Subsequently, 500 μL DAPI (4′, 6-diamidino-2-phenylindole) was used to label cell nuclei for 10 min and followed by washing with PBS three times. A microplate reader was used to measure the fluorescence intensity of Dox (E_x/E_m = 480 nm/570 nm) and DAPI (E_x/E_m = 360 nm/460 nm) in the resuspended cell solution. Where Ex and Em are excitation and emission wavelengths, respectively. The intracellular Dox contents were represented by the following formula:

$$\frac{F_{Dox}}{F_{DAPI}}$$

Where F_{Dox} and F_{DAPI} are the fluorescence emission intensities of Dox and DAPI [24].

2.2.3. Cell imaging

The cell images were obtained by laser scanning microscopy (CLSM, Leica SP8, Germany). The samples were immerged into the 1000 μL DMEM for 24 h and then the leached liquid were collected. MG63 at a density of 5×10^4 cells per well were seeded on the glasses, and the 800 μL leached liquid of each samples was added into the well, and incubated for another 24 h. Then the cells were rinsed by PBS three times, followed by fixed 4% paraformaldehyde (PFA) (Sigma, USA), and the cells were stained with FITC-Phalloidin (Sigma, USA) for 1 h and further stained with DAPI (Sigma, USA) for 5 min. The F-actin, cell nuclei and Dox were examined on a laser scanning microscopy.