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Construct Scaffold-like delivery system with poly (lactic-co-glycolic) microspheres on micro-arc oxidation titanium

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ABSTRACT

In this paper, we present the first report about constructing a scaffold-like delivery system with poly (lactic-co-glycolic) (PLGA) microspheres on micro-arc oxidation titanium (MAO-Ti). The results show that this system could be stable on the porous MAO-Ti surface up for 6 weeks. Not only the system could control the release of model protein BSA, but also the MAO film could regulate the pH value of the solution which would decrease by the degradation of PLGA microspheres. In addition, compared to MAO-Ti, this system loaded with BSA could improve the proliferation of HBMSCs after 3 or 7 days culture.

1. Introduction

As important material for orthopedic implants, titanium is excellent due to its advantages in biocompatibility, corrosion resistance, mechanical properties, and light weight [1]. However, biomaterial-associated complications like low bioactivity or bacterial infection still limit the application of titanium in daily clinical practice. One strategy to solve these problems is to construct a stable and long-term effective delivery system which could load protein/peptide/drug on the titanium surface [2–4].

As a delivery system on titanium surfaces, polymer-based materials have been used widely. For example, delivery systems has been constructed by preparing biodegradable polymer films, such as poly (D, L-lactic acid) or poly (lactic-co-glycolic acid) which shows excellent biocompatibility and biodegradability through natural pathways [5,6], on titanium surface [7–9]. After loading with growth factors in these polymer films, accelerated bone integration of the implants has been observed in various orthopedic applications [8,10,11]. And after loading with antibiotics in the films, reduction of bacterial colonization was achieved on the implant surfaces [12]. Furthermore, these polymer films show excellent biomechanical properties on the titanium surface [13].

As is known, Poly (lactic-co-glycolic acid) (PLGA) microspheres based materials are reported to have broader applications for delivery system than PLGA film [14,15]. Particularly, these microspheres could be used for fabricating sintered microsphere scaffold, which could also be excellent controlled release carriers to hold ideal loading efficiency and allow the drug dosage optimization [16,17]. However, its application as delivery system on titanium-based substrates has not been disclosed.

Herein we construct a scaffold-like delivery system with PLGA microspheres on the micro-arc oxidation titanium (MAO-Ti). It has been well-established that this porous MAO-Ti surface with calcium phosphate can greatly enhance the implant osseo-integration [2,18,19]. So constructing such a delivery system on this surface is of great interest. We present the study of the stability and control release of model protein BSA of this system. Finally, we also report the biocompatibility of the system with human bone mesenchymal stem cells (HBMSCs).

2. Experiment

2.1. Materials

Titanium substrates (grade 2, Baotai Co. Ltd., Shanxi, China) in square shape were used in this study. PLGA (lactic/glycolic 1:1; Mw 31,000 Da; inherent viscosity 0.30dl/g in chloroform at 30°C) was purchased from Daigang Biomaterials Inc. (Jinan, China). Bovine

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Serum Albumin (BSA) was obtained from Sigma-Aldrich (America), and polyvinyl alcohol (PVA124) from China. Dichloromethane and all the MAO-related reagents were purchased from Guanghua Chemical Factory Co. Ltd. (Guangdong, China).

2.2. MAO process

The preparation of the MAO-Ti sample was described in ref.[18]. Briefly, titanium plates were cleaned by treatment with 10% HF for 20s, and washed in ultrasonic bath successively with acetone, ethanol and deionized water, each for 20 minutes. The cleaned Ti plates were placed in an electrolysis cell and served as the anode, and a stainless steel as the cathode. The Ti plates and stainless steel were immersed into an aqueous solution of 82 mM EDTA as chelating agent, 60 mM $Ca(OAc)_2$, and 20 mM $Ca(H_2PO_4)_2$, pH = 11 (adjusted with 2 mol/L NaOH solution). The MAO process was immediately started by applying a pulsed 450 V, 100 Hz DC field with a duty cycle of 30% to the specimens for 5 min. The samples were rinsed roughly with acetone, ethanol and deionized water, and dried by a stream of N_2.

2.3. Microsphere fabrication

PLGA microspheres were prepared via W/O/W method [29]. Briefly, 30 mg BSA was dissolved into 1.5 ml PBS solution as water phase, and 1.5 g PLGA was dissolved into 15 ml of the dichloromethane as oil phase. The water phase and the oil phase were mixed at 1000 rpm for 15 min to form water-in-oil (w/o) of first emulsion. This first emulsion (w/o) was added to 300 ml of the second water phase of 0.1% (w/v) of polyvinyl alcohol (PVA) at 500 rpm to form the second emulsion (w/o/w). Then this second emulsion was stirred at 500 rpm for 4 h to drive off dichloromethane and solidify the microspheres. The achieved microspheres were sieved to select the ones smaller than 100 μm and bigger than 35 μm in diameter, then washed with distilled water and dried at room temperature for 24 h.

2.4. Encapsulation efficiency and loading dosage of the PLGA microspheres

To determine the encapsulation efficiency and loading dosage of BSA in the microspheres, 10 mg PLGA microspheres were immersed into the mixture of 5 ml of PBS (0.01 M, pH 7.4) and 1 ml of dichloromethane. The solution was stirred at 37 °C for 2 h to dissolve the PLGA microspheres. Then the solution was mixed thoroughly with a vortex mixer to extract BSA to the water phase and was centrifuged (20 min at 9000 rpm) to accelerate phase separation. The aqueous supernatant was analyzed with a BCA™ Protein Assay Kit (Thermo, the USA). The encapsulation efficiency (EE%) and loading dosage (LD%) were calculated as follows:

$$EE\% = \left(\frac{\text{actual BSA loading}}{\text{theoretical BSA loading}}\right) \times 100\% \quad (1)$$

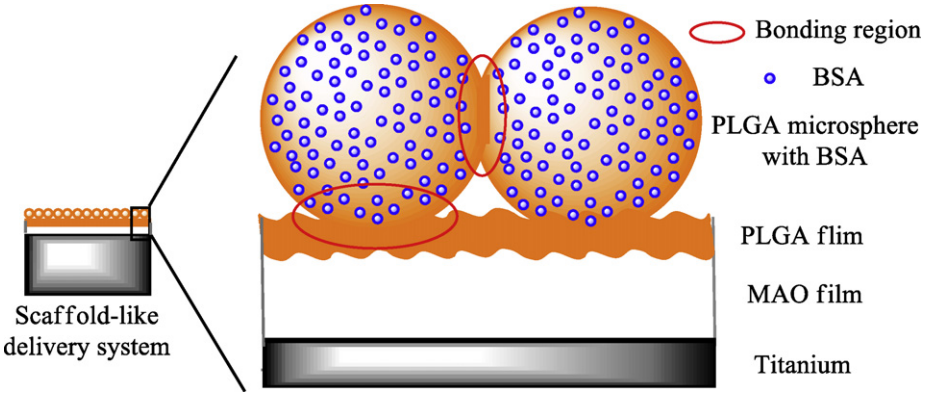
$$LD\% = \left(\frac{\text{BSA concentration} \times \text{PBS volume}}{\text{total weight of PLGA microspheres}}\right) \times 100\% \quad (2)$$

2.5. Construct the scaffold-like delivery system with PLGA microspheres on MAO-Ti samples

The MAO-Ti samples were further subjected to PLGA microsphere coupling to construct the scaffold-like delivery system, which was performed as follows: MAO-Ti samples were immersed in PLGA solution (1 g in 20 ml dichloromethane) for 15s to form a PLGA film on it. Then the samples were removed from the solution and characterized with thin-film X-ray diffraction analysis (TF-XRD; X'Pert Pro, PANalytical B.V., The Netherlands, with Cu Kα, λ=0.15418 nm) and energy dispersive spectroscopy (EDS; INCA, Oxford, England). 10 mg PLGA microspheres were put on the MAO-Ti surface with PLGA film homogenously. The scaffold-like delivery system was then fabricated via solvent sintering method[15]. Briefly, the MAO-Ti sample with PLGA microspheres was transferred into a mold (1 × 1 × 2 cm). After that, acetone/ethanol solution with different concentrations and volumes was added into the mold gently to make the microspheres and the PLGA membrane on the MAO surface fuse together. Finally, the sample was separated from the mold, washed with deionic water, and dried at room temperature for 24 h. The schematic diagram of the scaffold-like delivery system was shown in Scheme 1. The morphological characterization of the system was conducted using scanning electron microscopy (SEM, 30XLFEG, Philips, The Netherlands).

2.6. In vitro degradation of the scaffold-like delivery system on MAO-Ti samples

The degradation of the scaffold-like delivery system on MAO-Ti samples were evaluated under in vitro physiologic conditions in PBS. The microspheres with the same weight served as the control group. All the samples were placed in separate plastic bottles with 2 ml of PBS solution (pH = 7.4) and incubated at 37°C. At scheduled time points (7, 14, 21, 28, 35 and 42 days), the samples were washed with deionized water and freeze-dried to get a constant weight. The degradation profiles of the microspheres were denoted by mass loss with a microbalance and morphology with the SEM. The pH value of the degradation solution was also characterized by a pH meter.



Scheme 1. Schematic diagram of the scaffold-like delivery system on MAO-Ti surface.