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Alkali-treated titanium selectively regulating biological behaviors of bacteria, cancer cells and mesenchymal stem cells

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ABSTRACT

Many attentions have been paid to the beneficial effect of alkali-treated titanium to bioactivity and osteogenic activity, but few to the other biological effect. In this work, hierarchical micro/nanopore films were prepared on titanium surface by acid etching and alkali treatment and their biological effects on bacteria, cancer cells and mesenchymal stem cells were investigated. Gram-positive Staphylococcus aureus, Gram-negative Escherichia coli, and human cholangiocarcinoma cell line RBE were used to investigate whether alkali-treated titanium can influence behaviors of bacteria and cancer cells. Responses of bone marrow mesenchymal stem cells (BMMSCs) to alkali-treated titanium were also subsequently investigated. The alkali-treated titanium can potently reduce bacterial adhesion, inhibit RBE and BMMSCs proliferation, while can better promote BMMSCs osteogenesis and angiogenesis than acid-etched titanium. The bacteriostatic ability of the alkali-treated titanium is proposed to result from the joint effect of micro/nanotopography and local pH increase at bacterium/material interface due to the hydrolysis of alkali (earth) metal titanate salts. The inhibitory action of cell proliferation is thought to be the effect of local pH increase at cell/material interface which causes the alkalosis of cells. This alkalosis model reported in this work will help to understand the biologic behaviors of various cells on alkali-treated titanium surface and design the intended biomedical applications.

1. Introduction

Due to the simplicity and flexibility, chemical surface modification is considered as a promising strategy for metallic biomaterials, including hydrogen peroxide, acid, alkali treatments, etc., which chiefly depends upon the chemical reactions at the interface of biomaterials/reagents [1]. Among these varieties of chemical modification methods, alkali treatment has been widely used for the surface modification of Ti and Ti alloys for biomedical purposes [2–6]. Alkali-treated Ti surfaces possess excellent in vitro bioactivity [7], osteogenic activity [3] and in vivo osseointegration [2,5], as well as good bonding strength of modification layer with substrate. However, previous in vitro works found a phenomenon, that is, the proliferation of cells seemed to be inhibited on the alkali-treated Ti surfaces [8–12]. The intrinsic mechanism behind this adverse effect remains unclear.

In order to shield this adverse effect caused by alkali treatment, many efforts have been made. On the whole, two types of ways are adopted, including surface topography regulation [4,10] and element doping modification [6,13]. With regard to the topography regulation, appropriate surface topography can better simulate natural bone structures and enable cell functionality [14–16]. As for the chemical component modification, numerous attentions have been paid to the bivalent elements, such as magnesium, calcium, strontium, etc. Among these bioactive elements, great importance has been attached to the trace element strontium owing to its excellent promotion of osteogenesis [17] and angiogenesis [18,19]. Furthermore, strontium ions (Sr^{2+}, ionic radii 0.11 nm) tend to diffuse into the larger interlayer space of titanate film (interlayer distance 0.98 nm) to replace sodium ions (Na^{+}, ionic radii 0.102 nm) and maintain the same geometric [TiO_6] octahedra [20,21], thus not altering the surface topography too much.

On the other hand, everything has two sides. The aforementioned adverse effect can be harmful or beneficial. It depends upon the cell type and the intended use. In regard to normal cells, it is

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harmful; while in cancer therapy or bacterial infection treatment, harm to cells may be good. From this perspective, the beneficial side of the adverse effect caused by alkali treatment can also be rationally utilized for particular biomedical purpose. For instance, considering the emergence of antibiotic-resistant pathogens caused by excess antibiotics administration [22,23], research focusing on self-antibacterial acquisition has shown immense potential, independent on antibiotics, and regulating the surface topography is a facile strategy to interfere with the initial bacterial adhesion and biofilm formation [24,25]. Topographical surfaces with length scale comparable to that of bacteria (mostly between 0.5 μm and 5 μm) are expected to have a stronger effect on bacterial adhesion due to the limited sensing ability of a single bacterium [26].

In this work, hybrid micro/nanoporous films were formed on metallic Ti surface by acid etching and alkali treatment. The influences of alkali-treated Ti surface on Gram-positive Staphylococcus aureus, Gram-negative Escherichia coli and human cholangiocarcinoma (CCA) cell line RBE were evaluated. Meanwhile, the responses of bone marrow mesenchymal stem cells (BMMSCs) to alkali-treated Ti surface were also investigated. The mechanism behind various cell behaviors on alkali-treated Ti surface is discussed and proposed here.

2. Materials and methods

2.1. Samples fabrication and modification

Commercially pure Ti (purity > 99.85%, Grade 1, Baoji Shi Shenghua Non-ferrous Metal Materials Co., Ltd., China) plates with dimensions of 10 mm × 10 mm × 1 mm or 20 mm × 20 mm × 1 mm were ultrasonically cleaned in ethanol and deionized water several times, then pickled in oxalic acid solution (5 wt%) at 100 °C for 2 h to remove the oxide layer and acquire a homogeneous micropit surface. After that, the samples were ultrasonically cleaned in deionized water and dried in ambient atmosphere for further use. The nanopore structure on Ti surface was hydrothermally fabricated. Briefly, each pretreated Ti plate was placed in 10 mL NaOH (5 M) aqueous solution in a Teflon-lined reaction vessel at 80 °C for 24 h. After the reaction vessel naturally cooled to room temperature, the Ti plates were gently rinsed with deionized water and then immersed in 0.1 M $SrCl_2$ aqueous solution for 2 h. After that, the Ti plates were gently rinsed with deionized water and dried in ambient atmosphere, followed by the calcination at 450 °C for 1 h. The obtained samples were denoted as Ti, Na-NT and Sr-NT, respectively.

2.2. Surface characterization

The surface morphology was characterized by field-emission scanning electron microscopy (FESEM; Magellan 400, FEI, USA), equipped with an energy dispersive X-ray spectroscopy (EDS). The surface chemical compositions and chemical states of the samples were determined by X-ray photoelectron spectroscopy (XPS; PHI 5802, Physical Electronics Inc, Eden Prairie, MN) with a Mg Kα (1253.6 eV) source. The Raman spectra analysis was conducted on the samples using a Raman microscope system (HR800, Horiba Jobin Yvon, France) with an Ar-ion laser at 20 mW (514 nm) for excitation. TEM analysis was conducted with field-emission transmission electron microscope (TEM; JEM-2100F, JEOL Ltd., Tokyo, Japan), with an accelerating voltage of 200 kV. Samples for the investigation were scratched off from the substrate and dispersed in ethanol using ultrasound. A droplet of the suspension was placed on a holey copper grid covered with porous carbon film. The surface roughness of the samples was measured by a surface profiler (HOMMEL TESTER T8000, Wave, Germany) with a scan distance of 4.8 mm and a scan rate of 0.5 mm/s. The scan was performed three times at different places on each sample and five samples were tested for each group. The parameters used for comparing the surface roughness of the untreated and treated surfaces were R_a (arithmetical mean roughness), R_z (mean peak-to-valley height) and R_{max} (maximum roughness depth). R_a is the arithmetical average value of all departures and of the profile from the mean line throughout the sampling length. R_z is the average value of the single peak-to-valley heights of five adjoining sampling lengths. R_{max} is the maximum value of the peak-to-valley heights. The R_a, R_z and R_{max} values were expressed as means ± standard deviation (SD).

2.3. Na^+, Sr^{2+} and Ti(IV) ions release

The Na-NT and Sr-NT samples were soaked in 10 mL ultrapure water (18.2 MΩ cm) at 36.5 °C for 1, 2, 3 and 4 weeks, respectively. At the end of incubation, the leaching liquid was collected and the concentrations of Na^+, Sr^{2+} and Ti(IV) ions being released were analyzed by inductively-coupled plasma mass spectrometry (ICP-MS; Nu Instruments, Wrexham, UK).

2.4. pH measurements

To determine pH values, the acid-etched Ti, Na-NT and Sr-NT samples were immersed in 10 mL ultrapure water (18.2 MΩ cm), lying flat in the bottom of a container, and the pH values at the solid/liquid interface were measured after immersion for 24 h by using a pH meter (FE20 – FiveEasy™, METTLER TOLEDO). In detail, as the pH electrode was gently immersed into the water, the pH values at different locations above/on the surface were recorded during which the container was stabilized and the pH electrode was moved slowly and gently in order to minimize the disturbance of the water. Three samples were tested for each group.

2.5. Investigation of bacteria

The antibacterial effect of the samples was evaluated by bacteria counting method using Gram-negative *Escherichia coli* (*E. coli*,

Table 1
Primers for real-time polymerase chain reaction (PCR).

Gene	Prime sequence (F, forward; R, reverse)	Product size (bp)	Accession number
GAPDH	F: GGCAAGTTCAACGGCACAGT R: GCCAGTAGACTCCACGACAT	76	NM_017008.3
OPN	F: CCAAGCGTGGAAACACACAGCC R: GGCTTTGGAACTCGCCTGACTG	165	NM_012881.2
OCN	F: GCCCTGACTGCATTCTGCCTCT R: TCACCACCTTACTGCCCTCCTG	103	NM_013414.1
HIF-1	F: CGATGACACGGAAACTGAAG R: CAGAGGCAGGTAATGGAGACA	122	NM_024359.1
VEGF	F: TTGAGTTGGGAGGAGGATGT R: TGGCAGGCAAACAGACTTC	115	NM_001110333.1