Applied Surface Science 436 (2018) 11–21

Contents lists available at ScienceDirect

Applied Surface Science

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

Full Length Article

Fabrication of biomimetic resorption lacunae-like structure on titanium surface and its osteoblast responses

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ARTICLE INFO

Article history:
Received 20 September 2017
Received in revised form
28 November 2017
Accepted 30 November 2017
Available online 2 December 2017

Keywords:
Titanium
Biomimetic
Resorption lacunae
Anodic oxidation
Super-hydrophilicity
Osteoblasts

ABSTRACT

Biomimetic specific surface structure could improve biological behaviors of specific cells and eventual tissue integration. Featuring titanium surface with structures resembling bone resorption lacunae (RL) can be a promising approach to improve the osteoblast responses and osseointegration of implants. As a most common used dental implant surface, sandblasting and acid etching (SLA) surface has micro-sized structures with dimensions similar to RL, but great differences exist when it comes to shape and contour. In this work, by anodizing titanium substrate in a novel $HCOONa/CH_3COONa$ electrolyte, RL-like crater structures were fabricated with highly similar size, shape and contour. Compared with SLA, it was much more similar to RL structure in shape and contour. Furthermore, through subsequent alkali-heat treatment, nano-sized structures that overlaid the whole surface were obtained, which further mimic undercuts features inside the RL. The as-prepared surface was consisted of crystalline titania and exhibited super-hydrophilicity with good stability. *In vitro* evaluation results showed that the surface could significantly improve adhesion, proliferation and differentiation of MG63 cells in comparison with SLA. This new method may be a promising candidate for biomimetic modification of titanium implant to promote osseointegration.

1. Introduction

Osseointegration is a complex process occurring at the bone/implant interface and is highly influenced by the implant surface's chemical, physical, mechanical and topographic characteristics [1]. The topography of implant surface has been shown to influence the differentiation and proliferation of osteoblasts, and the up-regulation of transcription factors that are responsible for the expression of bone matrix formation genes [2,3]. From the point of view of biomimics, a promising strategy to design implant topography should be illuminated by natural bone interface between the newly formed bone and the old one [4]. During bone remodeling, osteoclasts form many resorbed lacunae on bone and then osteoblasts migrate into the lacunae and fill it with mineralized matrix, which indicates the bone resorption surface serves as a recipient for new bone apposition [5,6]. Many researchers considered that the bone-resorbing surface with resorption lacunae played a critical role in the establishment of the interface, by providing a three-dimensionally complex surface where the cement line interdigitates and interlocks [4,6]. Boyan et al. found that pretreatment of bone with osteoclasts could improve phenotypic expression and osteocalcin level of osteoblast-like cells, which proved RL structures could enhance osteoblast responses [7]. Spence et al. suggested that carbonate substituted hydroxyapatite, conditioned by osteoclasts, could promote human osteoblast proliferation and collagen synthesis [8]. Thus, fabricating RL-like topography structures on implant surface may be a promising approach to improve the osseointegration.

The resorption lacunae (RL) were excavated by differentiated osteoclasts through acid dissolution and enzymatic degradation

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https://doi.org/10.1016/j.apsusc.2017.11.282
0169-4332/

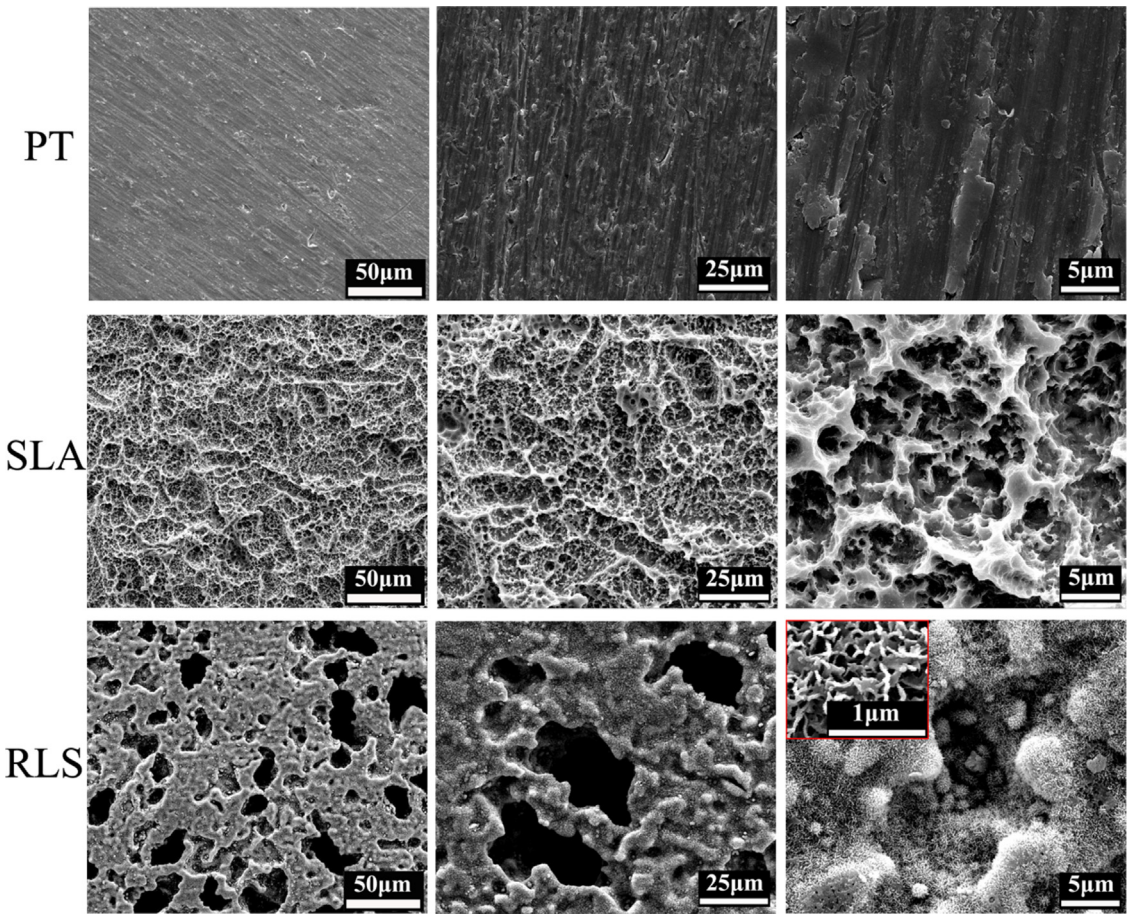

Fig. 1. FE-SEM images of PT, SLA and RLS surfaces at different magnifications.

[5,6]. The resorption lacunae usually have typical crater-like contour with diameter mainly ranging from tens of microns to around one hundred microns, depending on the size of osteoclasts as well as their activity and excavation period [3,4,9–17]. And inside the resorption lacunae, remaining collagen fibres with varying orientation exhibit undercuts structures at nano-scale [2,4–6]. A number of surface treatment strategies have been applied on titanium implants for osseointegration improvement [9–12], among which sandblasting and acid etching (SLA) is the most commonly method. Craters with similar dimensions can be found on both SLA and bone resorption surface, but their shape and contour profile have great differences [18]. Craters of bone resorption surface (i.e. RL) have distinct edges which could distinguish them with surrounding non-resorbing surface, while craters of the SLA surface lack such edges as well as nano-scale undercuts structure [18].

Hence, the objectives of the present study were twofold. First, we tried to develop a new method to treat titanium to obtain RL-like structures and investigate its physicochemical properties. Second, we aimed to explore its superiority to SLA surface in terms of osteoblasts behavior *in vitro*. This study may provide some insights into the design and treatment of dental implant surface.

2. Materials and methods

2.1. Samples preparation

Titanium disks with diameter of 15 mm were machined from 1 mm thick commercially pure titanium (PT) sheets (Grade 1). Titanium disks were polished with No. 600, No. 400, and No. 800 SiC abrasive paper and then ultrasonically washed with acetone, pure ethanol and distilled water for 5 min, sequentially. SLA sample was prepared by sandblasting PT sample with alumina particles sized about 250–300 μm and then acid etched in 18% HCl/48% H_2SO_4 solution at 60 °C for 30 min.

Treatments to fabricate RL-like surface (RLS) are shown below: PT samples were first anodized in an aqueous electrolyte (0.7 M HCOONa and 0.3 M CH_3COONa) under a step-galvanostatic mode, where the start current density was 5 mA/cm^2, elevated by a step of 5 mA/cm^2 until reaching 50 mA/cm^2, with each step remaining for 2 min. Then the samples were anodized in 1 M CH_3COONa electrolyte under potentialstatic mode at 180 V for 1 min. Finally, the samples were respectively soaked in 60 °C NaOH solution (10 M) and 40 °C HCl solution (50 mM) for 24 h in sequence, ending with gentle washing by distilled water. Subsequently the samples were heated up to 600 °C at a rate of 10 °C/min in a muffle furnace under air atmosphere, maintained for 1 h and then naturally cooled to room temperature. The as-prepared samples were named as RLS. All samples were ultrasonically cleaned in distilled water and dried in oven overnight. Samples for cell culture were sterilized in autoclave at 121 °C for 30 min.

2.2. Surface characterization

A field emission scanning electron microscope (FE-SEM; Inspect F, FEI, Netherlands) at an accelerating voltage of 20 kV was used to detect all the surface morphological microstructure and cross-section of RLS samples. The cross-sectional samples were prepared according to our previous study [19]. In brief, the RLS samples were anodized in a glycerol electrolyte solution containing NH_4F(1 wt.%) + H_2O(10 vol.%). Afterwards, the samples were gen-