BIO-INSPIRED MICROSCALE TOPOGRAPHIES ON DRIE DEFINED TITANIUM SURFACE FOR SOFT TISSUE REGENERATION IN IMPLANT DENTISTRY

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ABSTRACT

Different patterns on Ti substrate which mimic the extracellular matrix (ECM) and cell morphology were designed to study the interaction of fibroblast (L929) and bacteria (Porphyromonas gingivalis) on the surface in vitro system. Patterned titanium samples were prepared by chlorine DRIE (Deep Reactive Ion Etch) to obtain expected microstructures. Patterned titanium samples were seeded with L929 and P. gingivalis for a period of 1-4days. It was revealed that the cell adhesion rates were more than doubled with promoted cell proliferation on the ECM-mimicked titanium surfaces. In addition, the bacterial adherence rates were greatly reduced on ECM surface. The bio-inspired titanium interface provides an excellent solution for soft tissue regeneration around the dental abutment.

KEYWORDS

Titanium; Surface topography; ECM; Cell adhesion; Bacteria adhesion;

INTRODUCTION

The excellent biocompatibility of titanium for interfacial bone formation makes it a prevailed material in clinical dental implantations; nevertheless, soft tissue regeneration around the dental abutment remains a hassle in clinical therapy. To solve this problems, reports have been concentrated on embellishing dental implant surfaces with microscale topographies [1]: simple patterns, i.e. micro-groove, island and holes have been explored for a better cell growth environment[2]. There are many method to fabricate these microscale patterns on titanium, such as sandblasted, acid-etched, sandblasted combined with acid-etched and micro-arc oxidation. However, none of them are able to produce complicated microscale topographies on titanium surface in a well controlled way.

The nature's intrigue plans to create structures from nano-micro to mesoscale inspired researchers to design artificial object with novel and extra ordinary properties. The extracellular matrix (ECM) have been reported to affect the cell growth, however, ECM mimics on dental implants surface are rare for the limitation of titanium fabrication technologies. Recent developments of titanium DRIE offers a new way of interacting with relevant medical processes. High-aspect-ration grooves with arbitrary patterns can be generated on titanium substrate with an accuracy of 1μm with a vertical sidewall profile.[3]

This paper reports on the design, fabrication and characterization of several bio-inspired titanium interfaces. Specifically, ECM mimicked topographies have been realized and compared with micro-groove and cell morphology mimicked patterns. The fibroblast short-term adhesion, proliferation assays and bacterial adhesion experiments are carried out to evaluate the surface bio-activities.

EXPERIMENT

The gaps between the fibers are from hundreds of nanometer to micro meters wide and are not-uniform arranged. In this consideration, different patterns imitating the extracellular matrix(ECM, ECM2) were designed as well as those following cell morphology(Cell1~Cell4). Parallel micro groove pattern(T) and smooth surface(C) were also fabricated for comparison, as shown in Fig.1. The width of the ECM type patterns varied from 5μm to 100μm and two cell morphologies were designed of different sizes. According to the Hansson's hypothesis, the best topographies on the surface is 1.5μm deep when the patterns is 3~5um wide[4], the depth of the groove was set to 2μm.

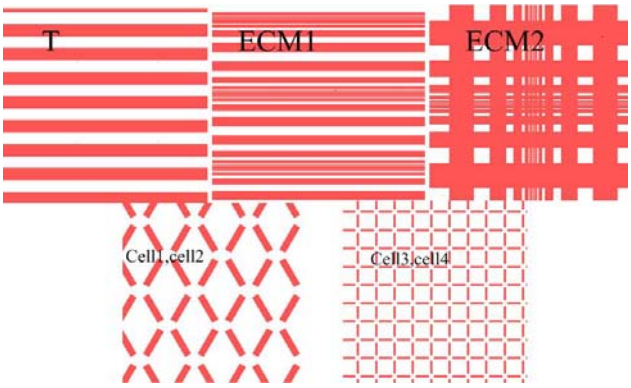

Figure.1: (T) represented traditional regular groove with width of 5μm; (ECM1) represented non-uniform and parallel groove with width of 5-100μm; (ECM2) represented non-uniform and interaction groove with width of 5-100μm; (Cell1,Cell2) represented uniform and interaction groove with length of 30μm and width of 6μm(Cell1),length of 15μm and width of 3μm(Cell2); (Cell3,Cell4) represented uniform and interaction groove with length of 20μm and width of 3μm(Cell3), length of 40μm and width of 6μm(Cell4).

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Fabrication starts with a 4' 400μm thick chemical pure(99.99%) Titanium wafer provided by Xi'An Catalyst Chemical Inc. Both sides of the wafer are chemical mechanical polished. Surface roughness(Ra) was 91.17±5.57 within the range of 0.4μm, which was the preferred surface for implant[5]. The titanium wafer was cleaned with ultrasonic agitation for 5min each in acetone, 2-propanol and de-ionized(DI) water and blown dry with nitrogen. Then, the wafer was baked on hotplate at 150°C about 15min (Fig2.a).

SU-8 photo resist was employed as the etch mask, which could be easily removed without any residue. SU-8 photo resist(PR) of 6μm was deposited on the titanium wafer and patterned by UV photolithography(Fig2.b). After that, titanium is patterned by anisotropic ICP etching of titanium with SU-8 as the mask(Fig2.c). ICP etching was carried out in the STS Multiplex ICP etcher, Cl_2 was employed as the etching agent. The etching parameters were set: 400W ICP coil power, 100W platen power, 60sccm Cl_2 flow rate, 3mT chamber pressure. The etch rate of Ti was 0.7μm/min, while the etch rate of SU-8 PR was 0.56μm/min. The SU8(Fig2.d) were stripped in fuming nitric acid, followed by immersing in ultrasonic agitation for 5min each in acetone, 2-propanol and de-ionized(DI) water. Finally, chips (3mm*3mm) were cut by laser machining.

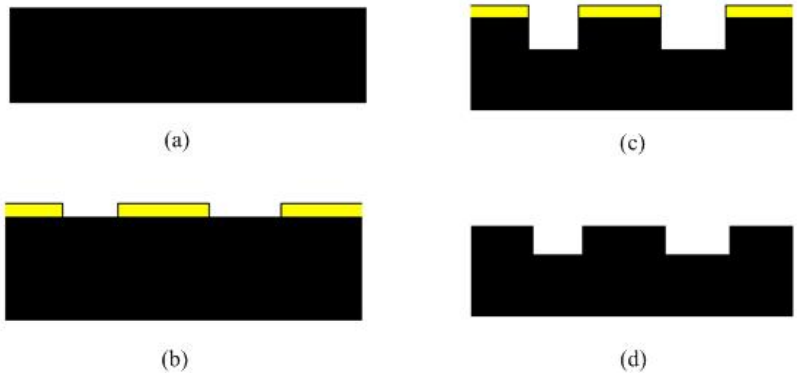

Figure.2 fabrication process of patterned titanium samples:(a)Wafer preparation (b)SU-8 Patterning (c) Titanium DRIE (d) SU-8 stripping

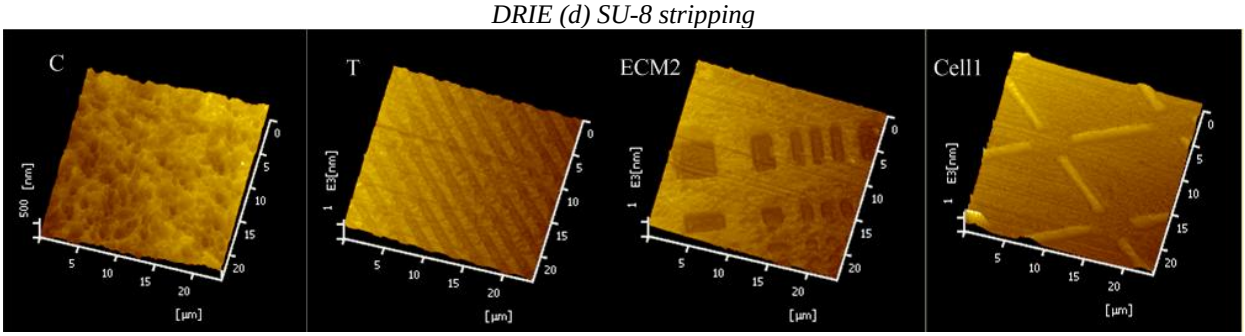

Figure.3: AFM of patterned titanium samples: (C) smooth surface, the control group (T) represented traditional regular groove with width of 5μm; (ECM2)represented non-uniform and interaction groove with width of 5-100μm; (Cell1) : represented uniform and interaction groove with length of 40μm and width of 6μm.

The patterned titanium samples were seeded with fibroblast (L929) and bacteria (Porphyromonas gingivalis) respectively for a period of 1-4days. A cell-counting kit was used for the measurement of L929 adhesion in 4hr and proliferation in 4 days, based on CCK-8 measure method. Samples seeded with P. gingivalis were incubated for 6 hr at 37°C under anaerobic condition. The number of adhered P. gingivalis was evaluated using a colorimetric microbial viability assay kit (Microbial Viability Assay Kit-WST). L929 were seeded for a period of 24hr to observe its attachment on different micro-groove surface by scan electron microscope (SEM). As shown in Fig.4, L929 were well attached on all surface and cells followed the patterned grooves in terms of shape and orientation.

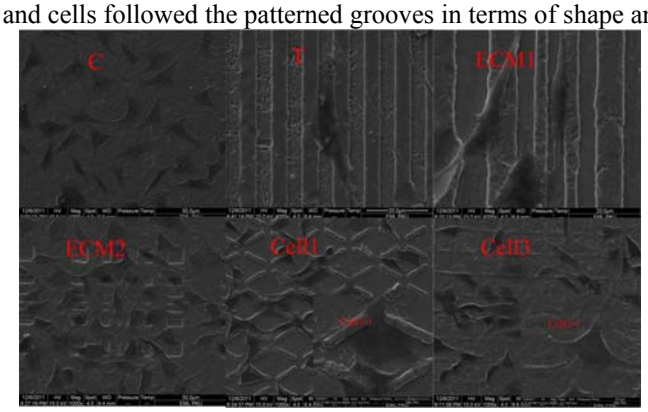

Figure.4: Microscale topography - cell interactions. (C),(ECM2),(Cell1),(Cell3) Mag(1000×) image of adherent cell, and (T),(ECM1),(Cell1-1),(Cell3-1) represent Mag(4000×) images of the same adherent cell. (C)represented control group - smooth titanium; (T)represented traditional regular groove patterns.

The effects of different micro-groove surfaces on the attachment and proliferation of fibroblast were shown in Fig.5. The cell numbers were detected with the cell counting kit-8. This indicated that the bio-mimic patterns greatly promoted fibroblast adhesion. Compared with the uniform parallel groove pattern and smooth surface, the non-uniform topographies(Fig.1 ECM1, ECM2), which were mimicking the extracellular matrix, also conducive to cell proliferation. However, the cell like designs didn't promote cell proliferation. Particularly, the Cell3 pattern, which morphology was similar to the shape of L929, had a negative impact on L929 proliferation, although cell adhesion was significantly favored.

The bacteria numbers were tested with Microbial Viability Assay Kit-WST. As shown in Fig.6, there was no significant change with Cell2, Cell4 and C group($p<0.005$), while others were apparently lower. It is concluded that the bacterial adherence rates were greatly reduced on ECM like surfaces.

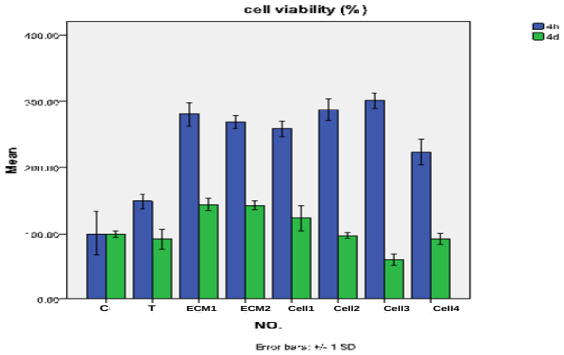

Figure.5. Influence of different micro-groove surfaces on attachment and proliferation of L929 cells($p<0.005$), Values of 4 hour's adhesion on patterned Ti surfaces were significantly higher than the control group, as tested by ANOVA. values of 4day's proliferation ECM1, ECM2 were higher and Cell3 was lower than C (the control group).

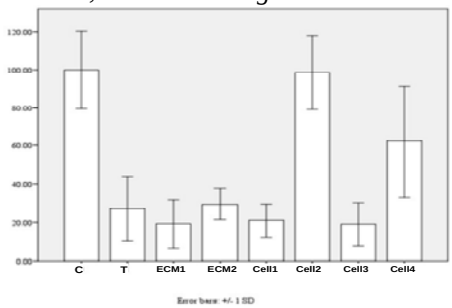

Figure.6.Influence of different micro-groove surfaces on attachment of Porphyromonas gingivalis($p<0.005$).

Compared with the uniform groove patterns, enhanced cell activity and reduced bacterial adherence were revealed on the ECM-mimicked topographies, which are favored for soft tissue regeneration around the dental abutment. While reports showed that surfaces that stimulate desirable mammalian cell adhesion, spreading, and proliferation also enable microbial colonization[1][6], none of these bionic topographies boosted bacterial adhesion, which lead to lower occurrence of peri-implant inflammation. Learning lessons from efficient natural processes to design titanium surface topography for soft tissue regeneration, mimicking natural phenomena could revolutionize the surface modification of medical implants.

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