DENTAL MATERIALS 32 (2016) 1374–1384

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Proliferation, behavior, and differentiation of osteoblasts on surfaces of different microroughness

Oleh Andrukhov[a], *Rebecca Huber*[b], *Bin Shi*[a,c], *Simon Berner*[d], *Xiaohui Rausch-Fan*[a], *Andreas Moritz*[a], *Nicholas D. Spencer*[b], *Andreas Schedle*[a,*]

[a] *Division of Conservative Dentistry and Periodontology, School of Dentistry, Medical University of Vienna, Vienna, Austria*

[b] *Department of Materials, Laboratory for Surface Science and Technology, ETH Zurich, Zurich, Switzerland*

[c] *Department of Oral and Maxillofacial Surgery, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China*

[d] *Institut Straumann AG, Basel, Switzerland*

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form
16 August 2016
Accepted 16 August 2016

Keywords:
Titanium surface
Osteoblasts
Micrometer-scale roughness
Osteogenesis

ABSTRACT

Objectives. Titanium surface roughness is recognized as an important parameter influencing osseointegration. However, studies concerning the effect of well-defined surface topographies of titanium surfaces on osteoblasts have been limited in scope. In the present study we have investigated how Ti surfaces of different micrometer-scale roughness influence proliferation, migration, and differentiation of osteoblasts *in-vitro*.

Methods. Titanium replicas with surface roughnesses (Ra) of approximately 0, 1, 2, and 4 μm were produced and MG-63 osteoblasts were cultured on these surfaces for up to 5 days. The effect of surface micrometer-scale roughness on proliferation, migration in time-lapse microscopy experiments, as well as the expression of alkaline phosphatase, osteocalcin, vascular-endothelial growth factor (VEGF), osteoprotegerin (OPG), and receptor activator of nuclear factor kappa-B ligand (RANKL) were investigated.

Results. Proliferation of MG-63 cells was found to decrease gradually with increasing surface roughness. However, the highest expression of alkaline phosphatase, osteocalcin and VEGF was observed on surfaces with Ra values of approximately 1 and 2 μm. Further increase in surface roughness resulted in decreased expression of all investigated parameters. The cell migration speed measured in time-lapse microscopy experiments was significantly lower on surfaces with a Ra value of about 4 μm, compared to those with lower roughness. No significant effect of surface roughness on the expression of OPG and RANKL was observed.

Significance. Thus, surfaces with intermediate Ra roughness values of 1–2 μm seem to be optimal for osteoblast differentiation. Neither proliferation nor differentiation of osteoblasts appears to be supported by surfaces with higher or lower Ra values.

* *Corresponding author at:* Division of Conservative Dentistry and Periodontology, School of Dentistry, Medical University of Vienna, Vienna, Austria.
E-mail address: andreas.schedle@meduniwien.ac.at (A. Schedle).

http://dx.doi.org/10.1016/j.dental.2016.08.217

1. Introduction

Dental implantation is a safe and long-term approach for restoring edentulous patients [1–3]. The formation of a bone-implant interface—a process known as osseointegration—is a key requirement for stable implant anchoring and clinical success. Titanium is largely used as a material for dental implants, because it causes no tissue reaction and exhibits the ability to fuse with bone [4]. Promoting the rate and extent of osseointegration immediately after dental implantation is crucial for the success of implant therapies. The modification of titanium surfaces is a key instrument to enhance osseointegration and improve the clinical outcome of implant therapy. Titanium (Ti) implant surface characteristics, such as chemical composition, surface topography, roughness, surface energy, nanostructures as well as coating of Ti implants with different bioactive materials are considered to have a substantial impact on the osseointegration process during initial wound healing after implantation [5–8].

The micrometer-scale roughness of Ti surfaces is one of the key parameters influencing osseointegration [9]. The roughness of Ti surfaces can be increased by several methods, such as sand blasting, acid etching, laser etching, and anodic oxidation [9]. Most studies describe surface micrometer-scale roughness in terms of either Ra or Sa parameters, which represent mean arithmetic profile roughness and mean arithmetic 2D roughness, respectively. Nowadays, surfaces with moderate roughness with Sa parameters of about 1–2 μm are widely used in implant dentistry and exhibit improved osseointegration and clinical outcome [6]. Arithmetic roughness, however, does not provide information about spatial characteristics of surface and roughness profiles. These topographical surface features are characterized by parameters such as average width of profile elements (R_{Sm}), profile asymmetry parameter surface skewness (R_{Sk}), and surface kurtosis describing peaks' sharpness. These parameters are differently affected by different roughening procedures [10] but their influence on osseointegration is not known. Furthermore, different roughening protocols might influence surface structures not only at the micrometer scale, but also at the nanometer scale. Therefore, further studies that can discriminate the effect of different surface characteristics on osseointegration and specify the relationship between mean arithmetic roughness and osseointegration are required.

Improved osseointegration of Ti surfaces with microscale roughness could be related to its positive effect on osteoblasts, which are directly involved in the process of bone formation. Previous *in vitro* studies show that Ti surfaces with moderate microroughness inhibit proliferation and promote differentiation of osteoblasts [11,12]. This effect of surface microroughness on osteoblasts seems to be associated with activation of α2β1 integrin signaling in osteoblasts [13,14]. Nevertheless, the effect of a large range of microscale roughness values on osteoblast behavior still remains to be investigated. Most previous studies have compared two Ti surfaces with defined microscale roughness values, whereas studies with controlled micrometer-scale roughness are rather rare. Notably, Kunzler et al. showed, in a study with titanium-coated microroughness gradient replicas with Ra values in the range 0–4 μm, that the proliferation of rat calvarial osteoblasts increases with increasing surface roughness [15]. Another study with controlled microroughness shows that osteogenic differentiation of bone marrow mesenchymal stem cells is promoted by surfaces with Ra values of about 2–3 μm [16]. A relationship between surface micrometer-scale roughness and osteoblast differentiation is investigated in two studies with controversial results: one study suggests an improved osteoblast differentiation with an increased surface roughness [17]; the other study suggests a biphasic relationship, where surfaces with moderate roughness better support osteoblast differentiation compared to very smooth or very rough surfaces [18]. Therefore, additional studies on the relationship between the controlled micrometer-scale roughness and osteoblast response would be highly desirable. Therefore, in the present study, we have investigated the behavior, proliferation, and differentiation of osteoblast-like MG-63 cells grown on Ti-coated replicas with Ra parameters of about 0, 1, 2, and 4 μm. The workflow of the present study is shown in Fig. 1.

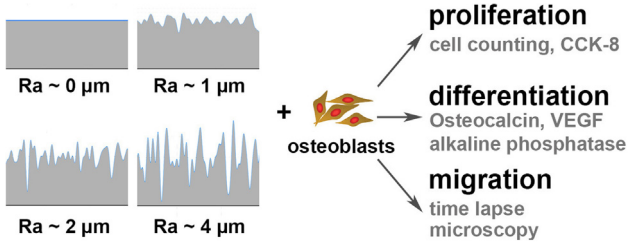

Fig. 1 – Workflow of the study.

2. Material and methods

2.1. *Production of Ti surfaces with different micrometer-scale roughness values*

2.1.1. *Micrometer-featured roughness masters*

Micrometer-featured masters with different roughness values were prepared in a similar way as described previously [19]. A two-step process was used to create micrometer-scale-featured roughness masters. Rolled aluminum sheets (purity 99.5%, dimensions: 20 mm × 40 mm × 2 mm (Metall Service Menzikon AG, Switzerland)) were sand-blasted with corundum particles (81500-826-074, Sablux, Switzerland) to achieve a uniformly rough surface morphology. In a second step, the roughened substrate was fully immersed into a chemical polishing solution (77.5% (v/v) phosphoric acid, 16.5% (v/v) sulfuric acid and 6% (v/v) nitric acid), which preferentially removes small and sharp features as a function of time. The substrates were exposed to the chemical polishing solution for 33, 12 or 2 min. The different polishing times result in homogenous roughness masters with a Ra value of approximately 1, 2 or 4 μm, respectively (referred as surfaces **1**, **2** and **4**). A flat silicon wafer (⟨1 0 0⟩ orientation, Si-mat, Germany) was also used as a master with an Ra value of approximately 0 μm (referred as surface **0**).