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# The effect of nanometric surface texture on bone contact to titanium implants in rabbit tibia

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ABSTRACT

Designing biomaterial surfaces to control the reaction of the surrounding tissue is still considered to be a primary issue, which needs to be addressed systematically. Although numerous *in vitro* studies have described different nano-metrically textured substrates capable to influence bone cellular response, *in vivo* studies validating this phenomenon have not been reported. In this study, nano-grooved silicon stamps were produced by laser interference lithography (LIL) and reactive ion etching (RIE) and were subsequently transferred onto the surface of 5 mm diameter Titanium (Ti) discs by nanoimprint lithography (NIL). Patterns with pitches of 1000 nm (500 nm ridge and groove, 150 nm depth), 300 nm (150 nm ridge and groove, 120 nm depth; as well as a 1:3 ratio of 75 nm ridge and 225 nm groove, 120 nm depth) and 150 nm (75 nm ridge and groove, 30 nm depth) were created. These samples were implanted in a rabbit tibia cortical bone. Histological evaluation and histomorphometric measurements were performed, comparing each sample to conventional grit-blasted/acid-etched (GAE) titanium controls. Results showed a significantly higher bone-to-implant contact at 4 weeks for the 300 nm (1:3) specimens, compared to GAE ($p = 0.006$). At 8 weeks, there was overall more bone contact compared to 4 weeks. However, no significant differences between the nano-textured samples and the GAE occurred. Further studies will need to address biomechanical testing and the use of trabecular bone models.

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## 1. Introduction

Due to the aging of the population the need of orthopedic and oral bone-anchored implants increases almost exponentially every year [1]. However, the number of revision surgeries also grows [2]. In the last few decades much effort has been placed on optimizing implant lifespan, through methods involving improvement of eg. surface wettability [3], bulk composition [4], and surface topography [5,6]. From *in vitro* and pre-clinical animal studies it is generally accepted that introducing random macro- or micro-roughness on a biomaterial surface positively affects implant integration in the human body [7,8]. Frequently, the combination of grit-blasting and acid-etching (GAE) is used, in which grit-blasting is responsible for creating macro-roughness, while acid-etching is mainly responsible for micro-and nanoroughness [9,10]. Although the mechanism behind the osteophilicity of roughened surfaces is not fully elucidated, an important factor is the initial response towards the implant [11]. Certainly, roughened surfaces give rise to different protein accumulation and subsequent bone cell attachment.

Although surface roughness is reported to enhance the bone-to-implant contact, it can be hypothesized that the currently created roughness patterns are not optimal from a biological point-of-view. For example, in terms of protein accumulation, collagen is one of the key-proteins in all living tissues. The collagen molecules reside in a very organized fashion in the bone extracellular matrix (ECM), forming parallel arrays of fibrils with nanometric dimensions and providing structural cues for cell anchorage. Such organization guides cells towards geometrically and structurally functional differentiated tissues [12,13]. Thus, it could be postulated that mimicking a similar nanostructure, by applying organized grooves on the surface of biomaterials (Fig. 1), might provide a more "natural surface" and may lead to an improved ECM (re)organization, mineral deposition on the implant surface and promotion of tissue integration. Already, many *in vitro* studies have been performed to

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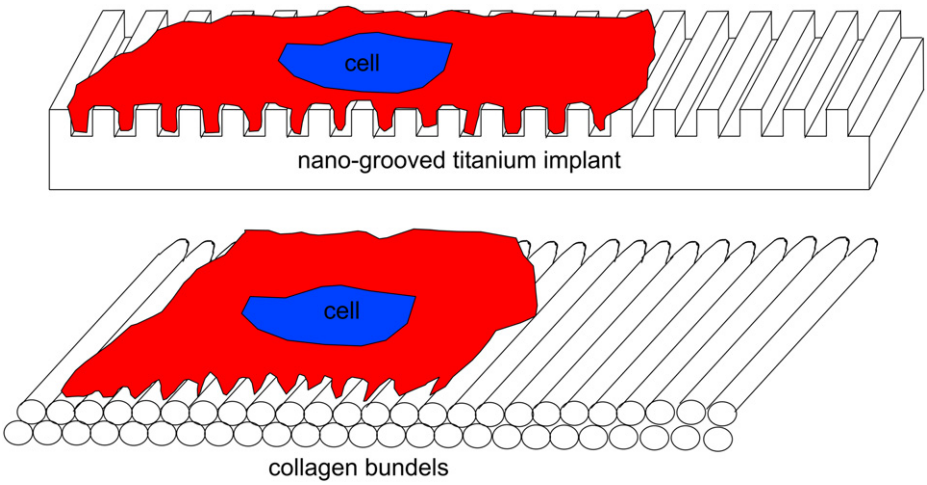


**Fig. 1.** Schematic representation of nano-grooved topographical surface modifications mimicking the structure of the collagen fibrils.

assess the response of bone cells to ordered and semi-ordered nano-textures, showing promising data in terms of osteoblast function [14]. Recently, we also studied *in vitro* the initial behavior and differentiation of osteoblast-like cells on a variety of nano-grooved substrates in the range of 75–1000 nm, and demonstrated that nano-grooves of specific dimensions influenced cellular morphology, motility and differentiation capacity [6,15]. In addition, we also demonstrated *in vitro* and *in vivo* that nano-grooves with pitch sizes in the range of 150 nm–1000 nm can induce an altered inflammatory response [16].

Still as of yet, *in vivo* studies corroborating such findings are scarce. In this study nano-grooved silicon stamps were produced by laser interference lithography (LIL) and reactive ion etching (RIE) and were transferred on the surface of titanium discs by nano-imprint lithography (NIL). Based on our previous results, the aim of the study was to produce nano-patterns in the range of 1000 nm pitch (i.e. 500 nm groove – 500 nm ridge) down to 150 nm pitch (i.e. 75 nm groove – 75 nm ridge) and compare these with conventional grit-blasted and acid-etched roughened implants, in a cortical bone defect model in a large animal.

## 2. Materials and methods

### 2.1. Laser interference lithography on silicon wafers

To create nano-textured substrates as shown in Table 1, single-side polished prime quality 4″ silicon wafers (Okmetic Oyj, Vantaa, Finland) were spin-coated with a tri-layer resist. This resist stack consisted of a 30 nm thick bottom anti-reflective coating (DUV 30; Brewer Science, Rolla, MO), a 120 nm thick negative tone deep UV resist (MA-N 2403; Micro Resist Technology, Berlin, Germany) and a top antireflective coating (AZ®Aquatar; MicroChemicals, Ulm, Germany) of a thickness less than 10 nm (Fig. 2). LIL exposure was performed in a home-built Lloyd's mirror interference setup with a laser wavelength of 266 nm. The defining value for the interference pattern period was the angle of incidence in the Lloyds setup. The angle ($\Theta$) defines the period of structure (P) according to the formula $P = \lambda/2 \sin \Theta$, where $\lambda$ is the wavelength of the light source. Angles were set to 7.6°, 26.3° and 62.4° to give periods of: 1000 nm 300 nm and 150 nm, respectively as described in detail previously [17]. All the silicon NIL-stamps were coated by chemical vapor deposition of (1H, 1H, 2H, 2H)-perfluorodecyltrichlorosilane (FDTS; Sigma–Aldrich Chemie BV, Zwijndrecht, The Netherlands) used for anti-stiction.

**Table 1**
Texture design dimensions, surface assessment, and implant placement.

| Pattern | 1000 nm (1:1) | 300 nm (1:3) | 300 nm (1:1) | 150 (1:1) nm | GAE |
|---|---|---|---|---|---|
| Pitch size (nm) | 1000 | 300 | 300 | 150 | |
| Ridge/groove size (nm) | 500/500 | 75/225 | 150/150 | 75/75 | |
| Depth (nm) | 150 | 120 | 120 | 30 | |
| Actual pitch (nm) | 1100 ± 8 | 317 ± 11 | 312 ± 12 | 148 ± 6 | |
| Placed 4 weeks | 4 | 6 | 6 | 4 | 6 |
| Successfully processed 4 weeks | 4 | 3 | 4 | 3 | 5 |
| Placed 8 weeks | 6 | 6 | 8 | 4 | 6 |
| Successfully processed 8 weeks | 5 | 2 | 3 | 3 | 3 |

### 2.2. Nanoimprint lithography on titanium substrates

Thermoplastic resist MR-I 8020 (Micro Resist Technology, Berlin, Germany) was spin coated onto ASTM-grade 2 bulk titanium (Ti) discs (Bimo Metals, Wroclaw, Poland). To realize nanoimprinting with different pitch sizes, the MR-I 8020 was spin-coated at 3000, 1500 rpm, which resulted in resist thicknesses of 280 nm and 200 nm, respectively. Subsequently, NIL was performed using an Eitre 6 machine (Obducat, Lund, Sweden). The pressure–temperature settings were as follows: heating to 160 °C, no pressure (approximately 2 min); holding the temperature at 160 °C and a pressure of 40 bar for 120 s; cooling down to 100 °C and holding the pressure at 40 bar for 6 min; demolding at 100 °C and no pressure. At the end of the pressure–temperature cycle the titanium was immediately detached from the stamp. The NIL patterns were transferred to the Ti by inductively coupled plasma (ICP) RIE using Oxford 100 ICP 180 dry etching equipment (Oxford Instruments, Oxford, UK). Gas composition of 33 sccm $Cl_2$, 2.3 sccm $CF_4$, 50 sccm Ar, and 2 sccm $O_2$ was applied, with an ICP power of 15:3 W $cm^{-2}$ as well as a radio frequent power of 0:76 W $cm^{-2}$. The operating pressure was 3 Pa and the substrate holder temperature was 40 °C. The etch times were 72 s, 64 s, and 35 s for the pitch sizes of 1000 nm, 300 (1:3) nm and 300 (1:1) nm, respectively. After the pattern transfer etch, a final oxygen plasma step was conducted for 10 min in 500 W r.f. plasma with 55 sccm $O_2$ flow using a TePla 300E apparatus to remove residues of resist (PVA TePla, Westhausen, Germany).

### 2.3. Ti coating on 150 nm silica

The interference of grain boundaries in the titanium with the lithography prevented the production of smaller patterns directly into titanium as explained in [18]. Thus, to achieve 150 nm patterns, silica specimens were made and uniformly coated with an ultrathin layer of titanium by physical vapor deposition in an ESM 100 evaporator (Edwards, Crawly, UK) at a pressure of $5 \times 10^{-3}$ mbar and a power of 100 W for 10 min. This resulted in Ti layer of ~10 nm thickness as determined by AFM (Nanoscope IIIa; Veeco, Santa Barbara, CA).

### 2.4. Control samples

Grade 2 cp Ti (Thyssen Krupp, Veghel, Netherlands) discs with a diameter of 5 mm were used as control. These discs were grit-blasted and acid-etched (GAE) according to the following protocol. The blasting was performed using $Al_2O_3$ (50 μm grit), followed by 90 s of etching in a solution of water, 37% acetic acid, and 96% sulfuric acid (1:1:1). All discs were cleaned ultrasonically in 10% nitric acid (15 min), followed by acetone (15 min) and isopropanol (15 min) and air dried.

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