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Surface optimization of dental implants with laser surface texturing and silver coating

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Abstract
The risk of bacterial inflammation at the interface between implant and tissue exists following the implantation of a dental prosthesis. Nearly half of implants are at risk of colonisation by pathogenic bacteria, which is associated with the occurrence of peri-implant mucositis. This disease can develop into peri-implantitis and thereby trigger a severe inflammatory process. The occurrence of peri-implantitis includes different phases. The initial attachment of microorganisms is only possible by pioneer bacteria, such as the gram-positive *streptococci*. Since the pathogenic cannot form a biofilm unless attached to a surface, the attachment of the pioneer bacteria is crucial for the onset of peri-implantitis. Due to the flexibility and contact free process, laser material processing is used for the surface structuring of several materials. In the biomedical field, laser-based surface texturing enables the production of implants with improved biological reaction surfaces to positively influence protein adsorption and cell adhesion. This paper presents laser texturing and silver coating to reduce initial biofilm formation on Ti6Al4V. The laser processing includes the manufacturing of LIPSS (Laser Induced Periodic Surface Structures), which enables the functionalisation of the surface. Furthermore, the surfaces are coated with silver to act as an inhibitor of biofilm formation. The implant material undergoes an *in vitro* culture of the microorganism Streptococcus salivarius in order to determine the biofilm formation applying both techniques. The analysis was realized by fluorescence microscopy with the application of 4',6-diamidino-2-phenylindole (DAPI) on the adhered biofilm. Results show that the surface modification plays a major role in the inhibition of biofilm formation.

Surface texturing, Biofilm formation, LIPSS, Dental implant

1. Introduction

Bacterial infections are the most common cause of inflammation of the peri-implant tissues. Periprosthetic joint infection (PJI) persists as a major complication in orthopaedic surgery [1]. Regarding odontology, peri-implantitis is characterized as an inflammatory process that affects the tissues around dental implants in different degrees of severity [2].

A natural increase of associated infections is generally expected due to the increasing number of patients requiring an implant. Peri-implant infection are potentially correlated with symptomatic pain, increased risk of bone loss and severe concomitant diseases such as cardiovascular events and systemic infection [3, 4].

Following the surgery for insertion of dental implants, interactions occur between the biological environment of the oral cavity and the metal surface of the implant, such as biological and defense reactions of the organism [5]. Biofilms comprises a complex formation with different bacteria, embedded in a extracellular matrix (biofilm) that provides protection towards the environment. The biofilm provides mechanical and chemical resistance increasing the bacteria chances of survival, resisting to the patient immune defence as well as treatment with antibiotics [6].

Dental implants are directly screwed into the jaw bone. A high risk for starting the infection is the upper surface area, which is in contact with the gingiva and the oral bacterial. After the surgery, the risk of chronic inflammation at the implant-tissue interface threatens 50 % of the implants. Peri-implant mucositis, which develops to Peri-implantitis, lead to the loss of the implant in 12 % to 40 % of the cases [5, 7, 8, 9]. These infections are similar to the gingivitis and periodontitits on natural teeth. However they result in a higher inflammatory infiltrate, an immune response, a higher tissue damage, and higher risk of bone loss. The colonization of the implant starts with gram-positive, facultative anaerobic bacteria, which are present in the healthy oral flora [10]. The so called pioneer bacteria are the responsible to the first attachment on the implant resulting in the formation of the biofilm required for the pathogenic bacteria. *Streptococci* and *Actinomyces* are the oral bacteria mainly responsible for initializing the biofilm.

The implant materials applied in orthopaedic, maxillofacial, and dental surgery include several metals, ceramics, or polymers [11]. Most metals applied are titanium and its alloys, cobalt-chromium-molybdenum, and tantalum [12, 13]. Independent of the material applied, its surface influences the the microorganism attachment [14]. The development of strategies for surface modification were the goal of many research groups. Outcomes include conventional techniques such as sandblasting [14], chemical etching [15] and coatings [16]. The laser processing shows great applicability because it offers a reduced risk of contamination [17]. Ultrashort-pulsed lasers on the structuring of titanium for biomedical implants is promising [18, 19].

The present study aims on investigating the effects of laser texturing and silver coating for reducing the biofilm formation of the early colonizer *S.salivarius* on Ti6Al4V implant material. The

culture of the microorganim within the cultivation time t_c = 24 h and t_c = 72 h allows the assessment of the texturing and coating strategies.

2. Materials and methods

2.1. Material

The material used for the experiments was the Ti6Al4V ELI from HIGH TECH ALLOYS SONDERWERKSTOFFE GMBH, Wuppertal, Germany. It corresponds to an implant material according to the norm ISO 5232-3 [20]. For the biological *in vitro* experiment was required to prepare the samples as disks that fit into the well plates. The samples were manufactured with diameter d = 10 mm and height h = 2 mm by the dental implant manufacturer A.K.TEK MEDIZINTECHNIK GMBH, Hagen. After the machining, all samples were electropolished with ElpoLux TI-Med, ELPOCHEM AG as a standard procedure from the company.

2.2. Laser processing

The laser machine tool applied for the laser processing is the LMBS 3W-015-xy300z200-IA from LASERMIKROTECHNOLOGIE DR. KIEBURG GMBH, Berlin, Germany. It has an average beam power P_L = 3.0 W with laser beam diameter of d_u = 12.0 µm at the focusing position. The wavelength λ_{UV} = 355 nm was applied for the experiments. The parameters applied for the laser processing are presented in Table 1.

Table 1 Processing parameters for laser surface texturing

Processing parameter	Unit	Value
Fluency Φ	J/cm²	0.04
Number of laser pulses N	-	200
Feed rate v_f	mm/s	105
Pulse frequency f_p	kHz	200
Pulse duration t_L	ps	10
Lateral overlap rate Ψ	%	90

2.3. Silver coating

The coating with silver was realized with the high vacuumsputter coater EM SCD500, LEICA MICROSYSTEMS, Wetzlar. This high vacuum film deposition system is capable of producting very thin, fine-grained silver (Ag) coating films on the substrate. The processing parameters for the silver coating are presented in Table 2.

Table 2 Processing parameters for Ag coating

Processing parameter	Unit	Value
Sputtercurrent I_s	mA	50
Sputterpressure p_s	mbar	$1e^{-2}$
Basepressure p_b	Mbar	$1e^{-4}$
Sputterrate v_s	nm/s	0.54

2.4. Surface characterization

The surface characterization was perfomed with scanning electron microscopy (SEM). The atomic force microscopy was applied for the qualitative assessment of the laser texturing. It enables the determination of the profile height h_p and spatical periodicity Λ of the surface textures produced by the laser processing. The fluorescence microscope Axio Scope.A1, CARL ZEISS MICROSCOPY GMBH, Jena was applied for the biological assessment. The images were processed with the software ImageJ with package for biological-image analysis Fiji, NATIONAL INSTITUTES OF HEALTH (NIH), Bethesda, USA.

2.5. Oral bacteria

The microorganism applied for the experiments is the *Streptococcus salivarius* (DSMZ-Nr. 20560). The gram-positive bacteria and facultative anaerobic organism. The bacteria strains for the experimental analysis were obtained from the LEIBNIZ INSTITUTE DSMZ, Braunschweig and stored at the temperature ϑ = - 80 °C.

2.6. Design of Experiments

The samples underwent a cleaning procedure with ultrasound frequency f_U = 80,000 Hz for the time = 5 min. The fluids were first submerged in isopropyl and acetone, which enable the proper removal of possible contamination from the manufacturing processes. After that, PBS was applied in order to remove alcohol residues provenient from the previous cleaning. This ensures that the microorganisms exposed on the surface do not suffer any influence of the cleaning process.

The test specimens applied for the experiment are presented in Table 3. The polished Ti6Al4V samples are applied as a reference. The LIPSS samples were manufactured according to the processing parameters presented in paragraph 2.2. The Ag coating was produced with coating thickness τ = 20 µm on both polished and LIPSS surfaces.

Table 3 Samples applied for the *in vitro* experments

Name	Material	Processing
PO	Ti6Al4V	Polished
LIPSS	Ti6Al4V	Laser texturing
Ag	Ti6Al4V + Ag	Ag with τ = 20 µm
LIPSS+Ag	Ti6Al4V + Ag	Laser texturing + Ag with τ = 20 µm

The sample size for each condition (PO, LIPSS, Ag, LIPSS+Ag) was of n = 5 specimens. The experiment was repeated twice under the same conditions. The bacteria *S. salivarius* pre-culture were established from a frozen stock with ϑ = -80 °C on agar plates. They were cultivaded in trypticase soy yeast (TSY) extract on liquid medium at ϑ = 37 °C. Single colonies were inoculated onvernigth at ϑ = 37 °C, n = 250 rpm in TSY. A biofilm medium (BM) was applied for the *in vitro* experiments.

The fluorescence microscopy was applied to evaluate the biofilm formation on the Ti6Al4V surface. The samples were exposed to the microorganisms for a culture time t_c = 24 h and t_c = 72 h. After the cultivation, the samples were washed with PBS and stained with 4'6-Diamidin-2-phenylindole (DAPI) for the incubation time t = 1 h at the temperature ϑ = 37 °C. The DAPI enters the cell membrane, adhearing to the bacteria DNA, which than reacts to the ultraviolet light. This enables the determination of the biofilm covered area A_B.

3. Results and discussion

3.1. Laser surface texturing

The laser surface texturing aplied to the samples are Laser Induced Periodic Surface Structures (LIPSS). Those correspond to low-spacial-frequency LIPSS (LSFLs), which are perpendicular to the polarization direction of the linearly polarized laser beam. They have spatial periodicity Λ slightly smaller than the wavelength λ, which corresponds to λ = 355 nm from the laser.

The manufacturing of the laser textures require the overlap adjacent laser beam tracks in order to induce the formation of the texture. This feature is represented by the processing parameter lateral overlap rate Ψ.

After the laser processing, the LIPSS presented spatial periodicity 225 nm ≤ Λ ≤ 280 nm and profile height 62 nm ≤ h_P ≤ 93 nm. These results are expected for LSFLs by ultraviolet radiation.

3.2. Silver coating

The coating of the manufactured nanostructures is carried out using the process of magnetron sputtering. The technology enables the coating adhesion without cracking and embrittlement.

The antibacterial function of the silver coating is based on the effect of silver ions over microorganisms. They are killed by membrane damage as well as influencing metabolic processes. Thus, the silver coating leads to an effective antibacterial protection on the surface of the abutments. Nanosilver particles are particularly effective when they are released from the base material in a controlled and slow manner, which leads to a continuous and efficient effect.

In order to evaluate the effects of the ions release in regard to the coating thickness τ, three conditions were developed as presented in Table 1. Furthermore, the coating thickness τ = 20 µm was also applied over the LIPSS structures for combining both antibacterial effects.

3.3. Biofilm formation

The colonization of *S. salivarius* on the Ti6Al4V was investigated with DAPI assay after the culture time t_c = 24 h and t_c = 72 h. The growth of the microorganisms led to the formation of a biofilm, which was measured in relation to the total area A of the sample. Figure 1 presents the results for the four conditions presented in Table 1.

The biofilm covered area A_B on polished surface show significantly higher values for t_c = 24 h and t_c = 72 h. The biofilm formation reduced for the surface treatment with LIPSS and also with silver coating. After t_c = 72 h, both presented similar values for inhibiting the biofilm formation. A substantial reduction was observed on the sample with LIPSS with the silver coating.

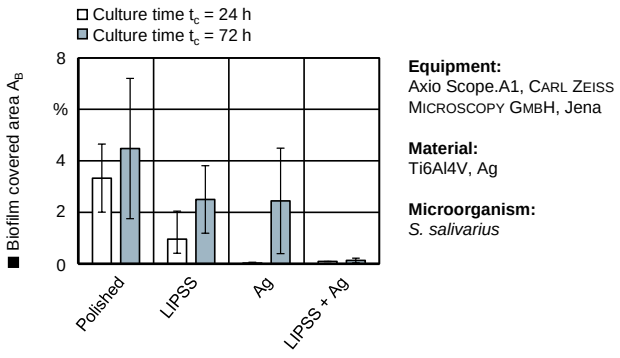

Figure 1. Biofilm covered area A_B

4. Conclusion

This paper presents results on the development of antibacterial surfaces for dental implants in order to reduce the initial biofilm formation. The approach considered the cultivation of the pioneer bacteria *S. salivarius* at the culture time t_c = 24 h and t_c = 72 h.

The proposed surface treatments with laser processing and silver coating showed a successful reduction of the biofilm formation. Their combination proved to be a even stronger inhibition for the bacterial attachment of the *S. salivarius*.

The great advantage of the laser processing is the conservation of the base material altering only its nanostructure. It constitutes a promising solution to provide antibacterial effect to the surface without exposing the patient to any potencial toxic agents. Its effects are mainly correlated with the surface topography and wettability. The size of the structures play an important role since they determine the area A as well as anchor points for the attachment of microorganisms. In the case of the LIPSS manufactured in this work, the spatial periodicity Λ is smaller than the bacteria itself. This avoid their attachment between the ripples.

The silver coating also presented very good results concerning the reduction of the biofilm formation. However, it alone was comparable to the LIPSS results with the disadvantage that an extra material (Ag) added to the surface.

The combination of LIPSS and silver coating presented a very successful strategy for the inhibition of biofilm formation. This strategy is particularly promising for the upper part of the implant, which has direct contact with the oral flora. Reducing the attachment of the bacteria in this region, a considerabe reduction of infection riscs may be achieved. Further approaches shall consider other microtexturing strategies in combination with silver coating to evaluate the full potential of this strategy.

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