Acute Inflammatory Response to Laser-Induced Micro- and Nano-Sized Titanium Surface Features

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ABSTRACT

Background: The inflammatory process induced by implant surfaces is an important component of the tissue response, where limited knowledge is available regarding the role of surface topography. With laser ablation, a combined micro- and nanoscale surfacemodification could be created, which have been shown to enhance bone growth and biomechanical stability in vivo.

Purpose: The aim of this article was to evaluate the early in vivo inflammatory response to laser-modified titanium disks, with machined titanium disks and sham operation sites serving as controls.

Materials and Methods: Circular disks were installed in a subcutaneous rat model for 24 and 72 hours, where the cell number, cell types, and cytokine levels were evaluated.

Results: The results revealed that significantly fewer inflammatory cells (mononuclear and polymorphonuclear) were attracted to the sites with the laser-modified implants compared with the machined titanium implants. Similar concentrations of pro-inflammatory cytokines (TNF-a and MCP-1), together with slightly higher cell viability, were observed around the laser-modified surface compared with the machined surface.

Conclusions: The results in the present study suggest that the combination of surface micro and nano features of the laser-treated surface contributes to the downregulation of early inflammatory events.

KEY WORDS: cytokines, inflammation, laser modification, nanotopography, titanium

INTRODUCTION

Bone-anchored implants are widely used in reconstructive surgery, both in orthopedics and dentistry. The original surface of titanium dental implants was machined[1] and exhibited excellent clinical performance in healthy patients with good bone quality and quantity.[2] However, there are a number of clinical situations that compromise the outcome of osseo- and soft-tissue integration. These conditions include diseases and deficiencies that reduce bone quality (osteoporosis) and quantity (loss of bone after trauma, bone resorption), or cause an adverse inflammatory and immunological response.

In order to enhance bone growth toward implant surfaces and scaffolds, several strategies have been developed. One principle for surface modifications is to mimic the natural environment by using biomimetic materials, embedded growth factors, 3-D printing, the mineralization of surfaces and scaffolds and bioreactors.[3,4] Several studies have revealed the importance of substrate micro- and nanotopography, independently of surface biochemistry, for cell behavior.[5] Nanotopography has been shown to exert strong effects in vitro on cell attachment through the activation of focal adhesion complexes and the cytoskeleton, cell morphology, proliferation and gene expression.[6,7] However, different cell types display different behavior patterns in vitro.

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A large number of oral implant surface modifications have been introduced onto the market. The majority of surfaces have been roughened by blasting, etching, and anodic oxidation.[8] Large differences can be observed, with respect to surface topography, oxide thickness, and phase distribution.[9] A recently reported surface modification was laser treatment, where site-specific surface alterations could be performed on implants combining macro-, micro-, and nano-sized surface features.[10] The laser melts the surface material locally, inducing a material transfer and resolidification process, which changes not only the surface oxide layer but also the subsurface layer, thereby increasing the hardness and wear resistance of Ti6Al4V implants[11] and resulting in increased biomechanical anchorage in bone.[12] Fewer stresses in the adjacent tissue around laser-grooved implants have been observed using finite element analysis, which reduces micromotions.[13] Further, cells have a tendency to align along the grooves, thereby reducing scar tissue formation.[14] However, the early inflammatory in vivo response to laser-modified surfaces is unknown.

The healing process consists of different phases: inflammation (early and late), granulation tissue formation and matrix formation, and remodeling.[15] The inflammatory response to implant materials has been identified as an important factor in the healing process around implants[16] where it has multiple purposes, such as removing debris from the surgical trauma and controlling the shift from inflammation to repair and regeneration by providing the appropriate signals.[17] The early inflammation is characterized by the presence of neutrophils (polymorphonuclear neutrophils, PMN) recruited from the vascular system via chemokines and cytokines; they include tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1). The neutrophils are replaced by mononuclear cells (macrophages, monocytes and lymphocytes) in the late stage of inflammation, which normally occurs after 3–48 hours in rat soft tissue in the case of titanium disks.[18] The recruitment of monocytes is stimulated, for example, by monocyte chemoattractant protein-1 (MCP-1), which is therefore a marker of inflammation.

The aim of this study was to evaluate critical in vivo events during the acute inflammatory response following the insertion of laser-modified titanium disks in an experimental rat model. This model makes it possible to discriminate between materials that promote a transient inflammation and materials that stimulate a persistent inflammation and an enhanced peri-implant fibrous response.[18,19]

MATERIALS AND METHODS

Implants

Thirty-two circular disks, with a diameter of 10 mm and a height of 1 mm, were machined from commercially pure titanium grade I (cp-Ti). Sixteen disks were modified with a laser treatment (Test), while the other 16 were left as-machined (control). The laser used for the surface modification (Q-switched Nd:YAG laser; Rofin-Sinar Technologies Inc., Plymouth, USA) operated at a wavelength of 1,064 nm and a spot size of 100 µm. The implants were cleaned in buthanol and ethanol prior to dry and steam sterilization.

Surface Characterization

The surface characterization of the disks was performed by scanning electron microscopy (SEM) to evaluate the surface topography. A focused ion beam (FIB) (FEI strata 235 DB FIB/SEM) was used for the sample preparation of ultrathin sections of the implant surface in a cross-section using an in situ lift-out.[20] Transmission electron microscopy (TEM; FEI Technai 300 STEM, equipped with a GATAN energy filter) analysis was subsequently performed on the ultrathin sections in order to evaluate the morphology, oxide thickness and phase composition.

Animal Model

The surgical procedure is described in detail in an earlier study.[19] In short, 16 female Sprague-Dawley rats, weighing 225–275 g, fed on a standard pellet diet and water, were used. The rats were anesthetized with a mixture of 2.7% isoflurane and air (Univentor 400 Anesthesia Unit, Univentor, Malta) and 0.01 mg of Temgesic (Schering-Plough AB, Stockholm, Sweden) was given as an analgesic s.c. preoperatively. The dorsum of the rat was shaved and cleaned with 5% chlorhexidine (5 mg/mL Pharmacia AB, Stockholm, Sweden) before 10-mm long incisions were made through the skin about 15 mm lateral to the midline, followed by the creation of subcutaneous pockets by careful blunt dissection. Three surgical sites was used, one for the test implant, one for the control implant and a sham operation site (control site for evaluating the tissue response to the surgical trauma),

following a randomized schedule and the skin at the sites was closed with three sutures of non-resorbable Suturamid® 5–0 Fs-2 (Ethicon Inc., Sommerville, NJ, USA). No surgical pockets were in contact with one another. All the surgical procedures were performed in an aseptic manner with sterilized instruments. Experiments were approved by the Local Ethics Committee, University of Gothenburg.

Twenty-four hours or 72 hours postoperatively, the rats were anesthetized, cleaned with 5% chlorhexidine, and sacrificed using an overdose of pentobarbital i.p. The sutures were removed and the pockets were re-opened by gently pulling them apart. The implants were removed with tweezers and placed in separate polystyrene wells with 400 µL of lysis buffer (Reagent A100, Nucleocounter™ system, ChemoMetec A/S, Allerød, Denmark) and 400 µL of stabilizing buffer (Reagent B, Nucleocounter™ system, ChemoMetec A/S). The exudates in each pocket were collected separately using repeated aspirations (×5) with a total of 300 µL of sterile Hank's balanced salt solution (HBSS; with Ca2+ and Mg2+, Gibco, UK) and were kept on ice. For subsequent cell analysis, 10 µL of exudate per site were saved separately, while the remaining exudates were centrifuged for 5 min at 400 g. The resulting supernatants were saved for the analysis of lactate dehydrogenase (LD), TNF-α and MCP-1 and the cell pellets were prepared with 50 µl of lysis buffer (Reagent A100, Nucleocounter™ system, ChemoMetec A/S) and 50 µl of stabilizing buffer (Reagent B, Nucleocounter™ system, ChemoMetec A/S) to count the total number of exudate cells. The number of cells was counted using the NucleoCounter™ system (ChemoMetec A/S).

Cell Analysis

The total cell concentration and percentages of PMN and Mono cells in the exudates were calculated by staining the cells with the nucleostaining Gentian violet and the number of PMN and mononuclear cells were counted by light microscopy using a Burker chamber. The lactate dehydrogenase (LD) concentrations in the exudates were determined in an enzymatic reaction; NAD (nicotinamide adenine dinucleotide) was added to a mixture of exudates together with N-metylglucamine and Li-lactate. The LD activity directly correlates to the amount of the formed colorimetric product read at 340 nm. The analysis was performed by Sahlgrenska University hospitals accredit laboratory for Clinical Chemistry.

Cytokine Determination

To determine the content of secreted TNF-α and MCP-1, the Quantikine® rat TNF-α Immunoassay (R&D systems, UK) and [(r) MCP-1] Biotrak ELISA system (Amersham Biosciences, UK) were used. All exudate supernatant samples were frozen at –70°C until analysis. The assays were prepared according to the instructions provided by the suppliers and the optical density was determined in an ELISA plate reader (SpectraMAX plus, Molecular Devices, Crawley, UK) by subtracting readings at 540 nm from readings at 450 nm. HBSS (with Ca2+ and Mg2+, Gibco, UK) were analyzed as a negative control to normalize the possible negative interactions from buffer salt in the exudate samples. Standard curves run in parallel with the samples were used for the determination of the cytokine concentrations in ng/mL. The detection limit for the two tests was 5 ng/mL.

Statistical Evaluation

The statistical tests that were used were the Friedman test for evaluating significant differences between the test, control and sham groups within the time points, followed by Wilcoxon's signed rank test. No correction for mass significance was made.

RESULTS

Surface Characterization

SEM images of the surface structure (Figure 1) revealed large differences in both the micro- and nanotopographies between test and control implants, where the laser modification created spherical globules with surface irregularities in the nanoscale. Further, TEM analyses of the surface layer in cross-sections revealed differences in the surface oxide thickness, where test implants had an oxide layer of 200–300 nm, while the machined surface had an oxide layer of about 10 nm (Figure 2). Rutile phase was identified in both oxides by high resolution TEM.

Cell Analysis

The results showed that the control implant attracted significantly more cells initially (at 24 hours) compared with both the test implant and the sham operation site,

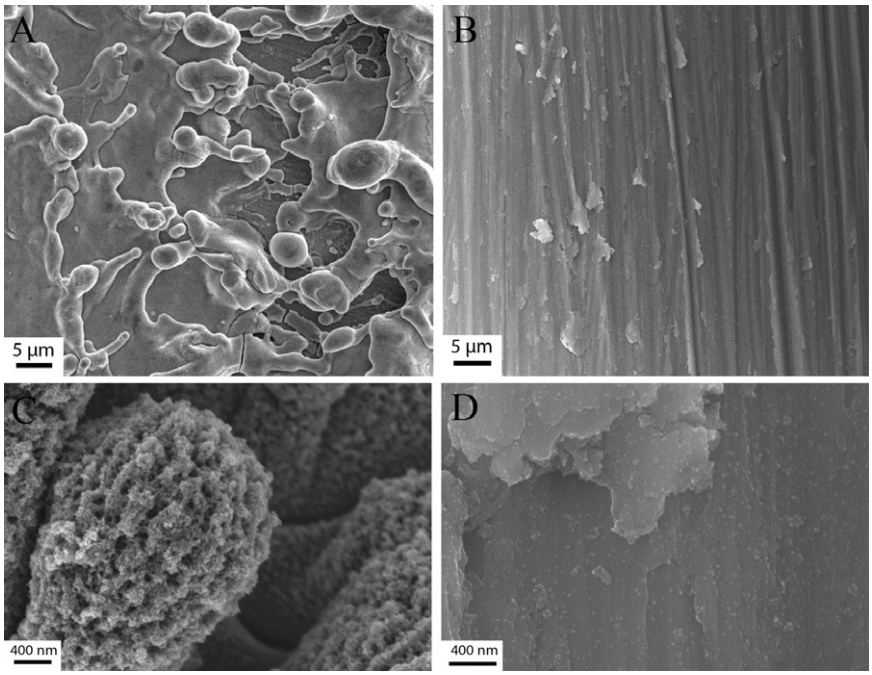

Figure 1 Scanning electron microscopy images of the implant surfaces. *A* and *C* show the laser-modified surface; *B* and *D* show the machined surface.

while the test implant attracted significantly more cells compared with the sham site (Figure 3A). No significantly difference between the implant types and sham was observed after 72 hours. Cells attached to the implants showed no difference between the materials, test and control, while a significant reduction in cell number was observed for the test implant going from 24 to 72 hours (Figure 3B). The cell viability was high on both implants, as well as in the sham sites (Figure 4), as indicated by the low LD values that were obtained. No significant differences were observed, although the test implant displayed a tendency toward higher cell viability compared with the control implant at both time points, 24 and 72 hours. The relative amount of PMN in the exudates showed a significant decrease going from 24 to 72 hours for both the test and control implant, while no significant difference was observed for the sham site (Figure 5).

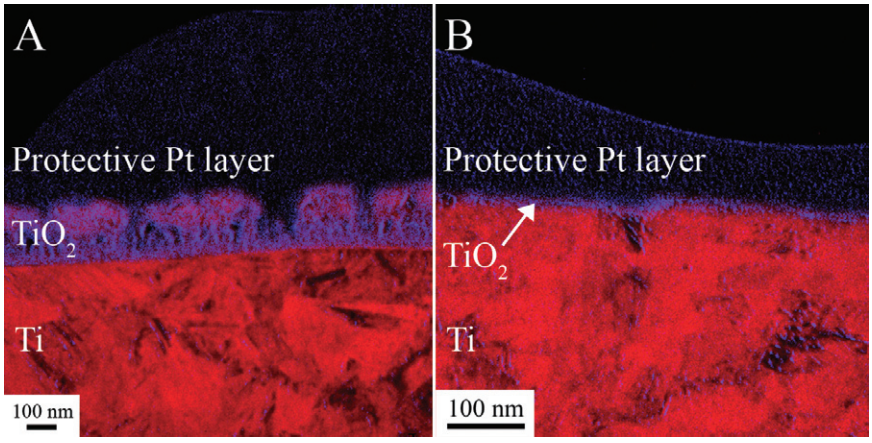

Figure 2 Energy-filtered transmission electron microscopy. Red represents titanium and blue represents oxygen. Purple is therefore the surface titanium oxide layer. (A) Laser-modified surface in a cross-section with an oxide thickness of 200–300 nm. (B) Machined surface in a cross-section with an oxide thickness of ~10 nm.

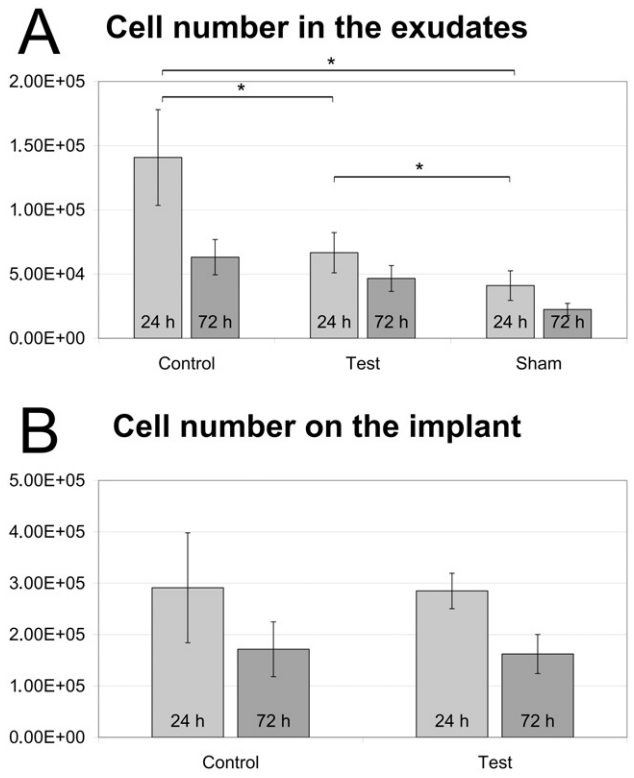

Figure 3 (A) The number of cells retrieved from the surgical pockets around the implants and from the sham site. (B) The number of cells attached to the implants. *Indicates a significance level of $p < .05$.

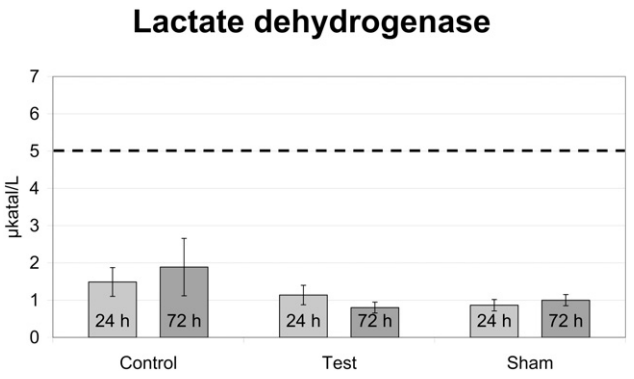

Figure 4 The cell viability measured by lactate dehydrogenase, where lower values indicate the higher viability of the cells.

Cytokine Concentrations

The concentrations of pro-inflammatory cytokines showed no significant difference between the different sites, test implant, control implant, and sham operation site, at the two time points. The MCP-1 secretion displayed a slightly higher concentration around the control surface compared with the test surface, which in turn showed a slightly higher concentration compared with the sham operation site. The concentration of TNF-α was often below the detection level of 5 ng/mL for all sites and could therefore only be characterized as very low (Table 1).

DISCUSSION

In the present study, the laser-treated titanium disks were compared with a conventional machined titanium surface in the rat subcutaneous model. Further, in order to determine the influence of the surgery on the early inflammatory reactions in the soft tissue, a sham operation site was created.

Both sites with implants, irrespective of surface treatment, attracted more cells initially compared with

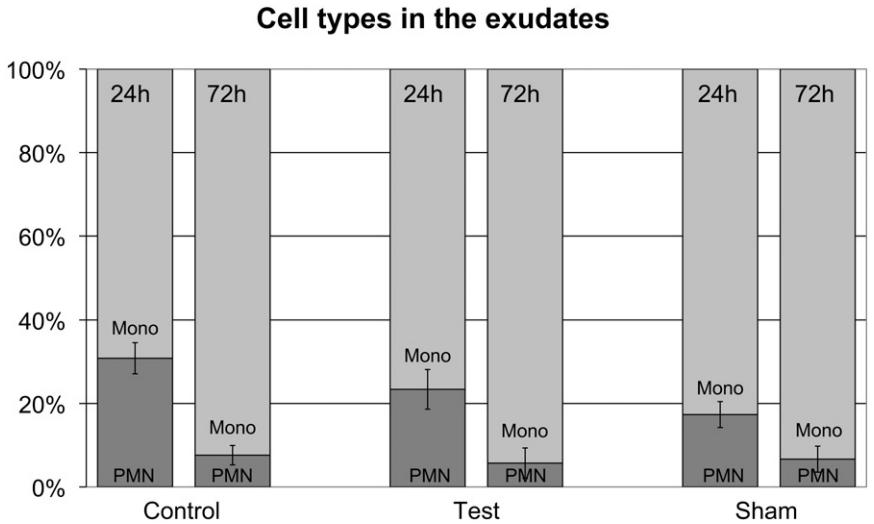

Figure 5 The relative numbers of polymorphonuclear neutrophils (PMN) and mononuclear cells (Mono) in the exudates.

TABLE 1 Cytokine Concentrations (ng/mL) in Exudates Retrieved from the Surgical Pockets

	TNF-α		MCP-1	
	24 Hours	72 Hours	24 Hours	72 Hours
Control	2.00 (0.72)	2.07 (0.61)	3,234 (1,144)	8,156 (4,752)
Test	0.99 (0.39)	1.72 (0.76)	1,863 (482)	1,843 (863)
Sham	0.10 (0.10)	1.87 (0.85)	439 (212)	220 (54)

MCP-1 = monocyte chemoattractant protein-1; TNF-α = tumor necrosis factor alpha.

the sham operation site. However, the initial recruitment of the inflammatory cells was significantly lower to the test surfaces compared with the machined titanium surfaces. No differences were found when the cell number attached to the surfaces was counted, showing that the chemical and topographic specificity of laser-treated surfaces influenced inflammatory cell recruitment but not attachment. One possible explanation of this reduction in cellular recruitment could be the laser surface-specific nanotopography. Extracellular matrix (ECM) proteins, basement membranes and individual ECM molecules exhibit a nanometer-scale structure present natively in the cells surrounding tissues. Surfaces with similar nano features may mimic the natural environment of the cell and minimize the production of signals, which are crucial for cell chemotaxis. One of the possible mechanisms for an effect of this kind could be differences in complement activation at the surface or qualitative and quantitative differences in the coagulation system and the amounts of fibrin that formed on the implant.[21–23] Fibrinogen and complement factors have been shown to be important chemotactic signals.[24,25] Further, cellular membrane in contact with the nanostructured surface will be exposed to tensile and relaxation mechanical forces that will rearrange membrane components, thereby mediating signal transduction leading to changes in gene expression. As a result, cells sensing a laser-treated surface during the first hours after implantation may produce signals, such as anti-inflammatory cytokines, downregulating the subsequent recruitment of the inflammatory cells, or reducing the production of chemokines and pro-inflammatory cytokines. One of the chemotactic factors mediating the recruitment of the blood-borne precursors of macrophages, the monocytes, is MCP-1. MCP-1 belongs to a chemokine family (CC chemokines, or β-chemokine), which have been suggested to influence macrophage activation, periprosthetic granuloma formation and osteolysis.[26–28] MCP-1 is a chemokine that is found in granulomatous tissue surrounding loosened prosthetic implants.[29] For this reason, the measurement of MCP-1 levels in the inflammatory exudates around materials is of great interest when it comes to predicting the integration outcome. Interestingly, our study indicated that the secretion of MCP-1 was lower to the test surfaces compared with untreated titanium.

Although there are a number of in vitro studies showing that the cellular response to the nano-scale geometry of implants is strongly dependent on cell type,[5,30,31] very few observations have been made in vivo.[32–34] In the present study, the test material recruited fewer PMN, cells characteristic of the acute phase of the inflammatory response, compared with the control material. Combined with the total number of cells in the exudates at 24 hours, this revealed that there were a significantly larger number of PMN around the control implant than the test implant and sham operation site. These findings indicate that laser-treated implants exhibited features that reduced the specific attraction of leucocytes to the interface.

It has previously been shown that the LD values around 5 μkat/L are a reference value for clinically well-tolerated materials.[35] In the present study, all the sites displayed high cellular viability, with slightly higher viability for the test surface compared with the control surface. The degree of inflammatory response to the control surface and sham sites was similar to that described previously in the same model.[35]

Another possible explanation of the improved inflammatory response of laser-modified implants could be the thickness of the oxide layer (300 nm compared with 10 nm on untreated surfaces). Titanium oxide has been associated with very good inflammatory properties, especially with regard to contact with

reactive oxygen species.[36–38] It has previously been shown that the surface treatment of Ti will influence the pattern of protein adsorption.[39–41] Host plasma protein adsorption to the implanted material is the first event in the inflammatory cascade that influences the recruitment, adhesion, and activation of inflammatory cells.[42] Albumin has been shown to passivate the surface, while fibrinogen initiates the acute inflammatory response.[43,44] Nygren and coauthors have demonstrated that serine proteases were the dominant proteins adsorbed onto annealed titanium in dry heat (39 nm oxide layer), while the amount of fibrinogen was higher on the acid-oxidized surface (4 nm oxide layer).[45] Further, the thicker oxide was associated with a smaller number of adhered platelets.[46] The recruitment and activation of the inflammatory cells appears to be associated with the thickness of TiO_2. Platelet activation, PMN priming, and monocyte adhesion were significantly increased on the surfaces with a thicker oxide layer.[47,48] The authors explained the influence of oxide thickness on protein adsorption behavior through electrostatic and van der Waal interactions.

Yet another possible explanation of the reduced number of inflammatory cells around the test implant compared with the control implant could be relative movement. It is known from clinical situations with percutaneous implant systems that relative movement between the soft tissue and the implant increases the inflammatory response. With a rougher surface, less relative movement would be expected, as mechanical interlocking occurs between the soft tissue and implant surface, thereby reducing the number of inflammatory cells. Greater connective tissue attachment and thinner fibrous encapsulation were observed for rougher implant surfaces compared with smoother ones in a subcutaneous rat model.[49]

To summarize; the results that were obtained revealed that the laser treatment of titanium surfaces did not enhance and even reduced the degree of the early inflammatory reaction approaching machined titanium at the empty surgery site without implants. The range of the cell numbers in the exudates outside disks, the percentage of PMN numbers, cellular viability, and the secretion of MCP-1 and TNF-α were lower around test surfaces than machined titanium (even if the statistical significance could not always be proved). Because all these parameters are thought to influence the integration outcome,[35] it can be concluded that the laser treatment of titanium enhances its biocompatible properties. Taken together with previous findings in bone tissue, demonstrating increased biomechanical retention compared with machined titanium,[50] it is suggested that laser-treated implants look promising for clinical practice.

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REFERENCES

1. Brånemark PI, Hansson BO, Adell R, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Suppl 1977; 16:1–132.
2. Adell R, Lekholm U, Rockler B, Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981; 10:387–416.
3. Stevens B, Yang Y, Mohandas A, Stucker B, Nguyen KT. A review of materials, fabrication methods, and strategies used to enhance bone regeneration in engineered bone tissues. J Biomed Mater Res B Appl Biomater 2008; 85:573–582.
4. Variola F, Vetrone F, Richert L, et al. Improving biocompatibility of implantable metals by nanoscale modification of surfaces: an overview of strategies, fabrication methods, and challenges. Small 2009; 5:996–1006.
5. Martinez E, Engel E, Planell JA, Samitier J. Effects of artificial micro- and nano-structured surfaces on cell behaviour. Ann Anat 2009; 191:126–135.
6. Dalby MJ, Riehle MO, Sutherland DS, Agheli H, Curtis AS. Use of nanotopography to study mechanotransduction in fibroblasts – methods and perspectives. Eur J Cell Biol 2004; 83:159–169.
7. Yim EK, Darling EM, Kulangara K, Guilak F, Leong KW. Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells. Biomaterials 2010; 31:1299–1306.
8. Albrektsson T, Wennerberg A. Oral implant surfaces: part 1 – review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2004; 17:536–543.
9. Jarmar T, Palmquist A, Branemark R, Hermansson L, Engqvist H, Thomsen P. Characterization of the surface properties of commercially available dental implants using SEM, FIB and HRTEM. Clin Implant Dent Relat Res 2008; 10:11–22.

10. Kurella A, Dahotre NB. Review paper: surface modification for bioimplants: the role of laser surface engineering. J Biomater Appl 2005; 20:5–50.
11. Singh R, Kurella A, Dahotre NB. Laser surface modification of Ti–6Al–4V: wear and corrosion characterization in simulated biofluid. J Biomater Appl 2006; 21:49–73.
12. Cho SA, Jung SK. A removal torque of the laser-treated titanium implants in rabbit tibia. Biomaterials 2003; 24:4859–4863.
13. Alexander H, Ricci JL, Hrico GJ. Mechanical basis for bone retention around dental implants. J Biomed Mater Res B Appl Biomater 2009; 88:306–311.
14. Soboyejo WO, Nemetski B, Allameh S, Marcantonio N, Mercer C, Ricci J. Interactions between MC3T3-E1 cells and textured Ti6Al4V surfaces. J Biomed Mater Res 2002; 62:56–72.
15. Henson PM, Clark RAF. The molecular and cellular biology of wound repair. New York: Plenum Press, 1988.
16. Thomsen P, Gretzer C. Macrophage interaction with modified material surfaces. Curr Opin Solid State Mater Sci 2001; 5:163–176.
17. Larsson C, Esposito M, Haihong L, Thomsen P. The titanium-bone interface in vivo. In: Brunette DM, ed. Proceedings of the Titanium in medicine: material science, surface science, engineering, biological responses and medical applications. Heidelberg, Germany: Springer, 2001:587–648.
18. Suska F, Kalltorp M, Esposito M, Gretzer C, Tengvall P, Thomsen P. In vivo/ex vivo cellular interactions with titanium and copper. J Mater Sci Mater Med 2001; 12:939–944.
19. Suska F, Esposito M, Gretzer C, Kalltorp M, Tengvall P, Thomsen P. IL-1alpha, IL-1beta and TNF-alpha secretion during in vivo/ex vivo cellular interactions with titanium and copper. Biomaterials 2003; 24:461–468.
20. Jarmar T, Palmquist A, Branemark R, Hermansson L, Engqvist H, Thomsen P. Technique for preparation and characterization in cross-section of oral titanium implant surfaces using focused ion beam and transmission electron microscopy. J Biomed Mater Res A 2008; 87A:1003–1009.
21. Hong J, Andersson J, Ekdahl KN, et al. Titanium is a highly thrombogenic biomaterial: possible implications for osteogenesis. Thromb Haemost 1999; 82:58–64.
22. Liu L, Elwing H. Complement activation on solid surfaces as determined by C3 deposition and hemolytic consumption. J Biomed Mater Res 1994; 28:767–773.
23. Thor A, Rasmusson L, Wennerberg A, et al. The role of whole blood in thrombin generation in contact with various titanium surfaces. Biomaterials 2007; 28:966–974.
24. Uhing R, Snyderman R. Chemoattractant stimulus-response coupling. In: Gallin JI, Snyderman R, eds. Proceedings of the inflammation: basic principles and clinical correlates. Philadelphia, PA: Lippincott Williams & Wilkins, 1999:1335s.
25. Loike JD, el Khoury J, Cao L, et al. Fibrin regulates neutrophil migration in response to interleukin 8, leukotriene B4, tumor necrosis factor, and formyl-methionyl-leucyl-phenylalanine. J Exp Med 1995; 181:1763–1772.
26. Taub DD, Oppenheim JJ. Review of the chemokine meeting the Third International Symposium of Chemotactic Cytokines. Cytokine 1993; 5:175–179.
27. Uguccioni M, D'Apuzzo M, Loetscher M, Dewald B, Baggiolini M. Actions of the chemotactic cytokines MCP-1, MCP-2, MCP-3, RANTES, MIP-1 alpha and MIP-1 beta on human monocytes. Eur J Immunol 1995; 25:64–68.
28. Wolpe SD, Cerami A. Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. FASEB J 1989; 3:2565–2573.
29. Nakashima Y, Sun DH, Trindade MC, et al. Induction of macrophage C-C chemokine expression by titanium alloy and bone cement particles. J Bone Joint Surg Br 1999; 81:155–162.
30. Jager M, Zilkens C, Zanger K, Krauspe R. Significance of nano- and microtopography for cell-surface interactions in orthopaedic implants. J Biomed Biotechnol 2007; 2007: 69036.
31. Kriparamanan R, Aswath P, Zhou A, Tang L, Nguyen KT. Nanotopography: cellular responses to nanostructured materials. J Nanosci Nanotechnol 2006; 6:1905–1919.
32. Giavaresi G, Tschon M, Daly JH, et al. In vitro and in vivo response to nanotopographically-modified surfaces of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and polycaprolactone. J Biomater Sci Polym Ed 2006; 17:1405–1423.
33. Bjursten LM, Rasmusson L, Oh S, Smith GC, Brammer KS, Jin S. Titanium dioxide nanotubes enhance bone bonding in vivo. J Biomed Mater Res A 2009; 92:1218–1224.
34. Meirelles L, Melin L, Peltola T, et al. Effect of hydroxyapatite and titania nanostructures on early in vivo bone response. Clin Implant Dent Relat Res 2008; 10:245–254.
35. Suska F. On the initial inflammatory response to variations in biomaterial surface chemistry. PhD, Göteborg University, 2004.
36. Tengvall P, Lundstrom I, Sjoqvist L, Elwing H, Bjursten LM. Titanium-hydrogen peroxide interaction: model studies of the influence of the inflammatory response on titanium implants. Biomaterials 1989; 10:166–175.
37. Tengvall P, Lundstrom I. Physico-chemical considerations of titanium as a biomaterial. Clin Mater 1992; 9:115–134.
38. Tengvall P, Elwing H, Sjoqvist L, Lundstrom I, Bjursten LM. Interaction between hydrogen peroxide and titanium: a possible role in the biocompatibility of titanium. Biomaterials 1989; 10:118–120.
39. Sousa SR, Bras MM, Moradas-Ferreira P, Barbosa MA. Dynamics of fibronectin adsorption on TiO2 surfaces. Langmuir 2007; 23:7046–7054.
40. Sousa SR, Moradas-Ferreira P, Barbosa MA. TiO2 type influences fibronectin adsorption. J Mater Sci Mater Med 2005; 16:1173–1178.

41. Francois P, Vaudaux P, Taborelli M, Tonetti M, Lew DP, Descouts P. Influence of surface treatments developed for oral implants on the physical and biological properties of titanium. (II) Adsorption isotherms and biological activity of immobilized fibronectin. Clin Oral Implants Res 1997; 8:217–225.
42. Norde W, Lyklema J. Why proteins prefer interfaces. J Biomater Sci Polym Ed 1991; 2:183–202.
43. Amiji M, Park H, Park K. Study on the prevention of surface-induced platelet activation by albumin coating. J Biomater Sci Polym Ed 1992; 3:375–388.
44. Kottke-Marchant K, Anderson JM, Umemura Y, Marchant RE. Effect of albumin coating on the in vitro blood compatibility of Dacron arterial prostheses. Biomaterials 1989; 10:147–155.
45. Nygren H, Tengvall P, Lundstrom I. The initial reactions of TiO2 with blood. J Biomed Mater Res 1997; 34:487–492.
46. Takemoto S, Yamamoto T, Tsuru K, Hayakawa S, Osaka A, Takashima S. Platelet adhesion on titanium oxide gels: effect of surface oxidation. Biomaterials 2004; 25:3485–3492.
47. Nygren H, Eriksson C, Lausmaa J. Adhesion and activation of platelets and polymorphonuclear granulocyte cells at TiO2 surfaces. J Lab Clin Med 1997; 129:35–46.
48. Eriksson C, Lausmaa J, Nygren H. Interactions between human whole blood and modified TiO2-surfaces: influence of surface topography and oxide thickness on leukocyte adhesion and activation. Biomaterials 2001; 22:1987–1996.
49. Kim H, Murakami H, Chehroudi B, Textor M, Brunette DM. Effects of surface topography on the connective tissue attachment to subcutaneous implants. Int J Oral Maxillofac Implants 2006; 21:354–365.
50. Palmquist A, Lindberg F, Emanuelsson L, Branemark R, Engqvist H, Thomsen P. Biomechanical, histological, and ultrastructural analyses of laser micro- and nano-structured titanium alloy implants: a study in rabbit. J Biomed Mater Res A 2009; 92:1476–1486.