

Laser-modified titanium implants for improved cell adhesion

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Abstract Concerning dental implant systems, a main problem is the adhesion of peri-implant mucosa in the cervical region. The aim of the present study was to use a laser for modifying titanium implants to promote mucosal adhesion, which is indispensable as a biological barrier against bacterial infection. By the use of a KrF excimer laser, it was possible to induce a holey structure on the polished area of the implant surface, which was analysed by a scanning electron microscope. In addition, the attachment of fibroblast cells to the created structures was investigated with the aid of an environmental scanning electron microscope. It turned out that the cells preferentially attach to the holey structure. Thereby, the cells form bridges inside, leading to a complete covering of the hole. In this way, a more effective biological barrier against bacteria can be created.

Keywords Implant dentistry · Excimer laser · Surface modification

Introduction

In dentistry, endosseous implants are becoming increasingly important. While osseointegration provides the implant with stability, the conditions of the marginal soft tissues adjacent to the implants appear to be an important factor in the long-term success of the implantation.

There are many differences between gingival and peri-implant tissues. While at the teeth, the sub-epithelial collagen fibre bundles are oriented perpendicular to the root cementum; in the case of an implant the fibres are oriented parallel to the implant surface [1]. Moreover, connective tissue in the mucosa around implants has a significantly higher content of collagen and a lower density of fibroblasts than the corresponding compartment of gingival tissue [2]. Therefore, plaque-related infections result in tissue destruction, which is more pronounced at implants and extends rapidly into the bone marrow, unlike teeth [3].

The reason for this difference may be found in the lack of junction epithelium at the base of the soft tissue–implant interface and in the lack of cementum with inserted collagen fibres. This facilitates the more rapid expansion of plaque downward implants compared to the growth downward teeth. The inability of the peri-implant tissue to heal after “sub-gingival” infection results in progressing infections and loss of infected implants. To avoid this situation, surgical techniques have been developed for eliminating mobile mucosa, which is more vulnerable to inflammatory changes [4]. However, it is reported that, unlike natural periodontal tissue, even keratinised peri-implant epithelium lacks hemi-desmosomes [5] and perpendicular fibre bundles [6].

Therefore, it is apparent that coronal implant surfaces need biological improvements, which will enable collagen

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fibres and vessels to grow perpendicular into the body of the implant. Such biological approach also requires new methods of surface modification. Conventional techniques like etching or sand blasting are not suitable for generating complex 3-D canal-like structures [7, 8].

Most recent implant surface modifications have been related to the endosseous part, not to the mucosal aspect. Some of these studies have dealt with the influence of laser irradiation on the endosseous titanium surface [9–13] or with re-osseointegration [14, 15]. For this reason, the purpose of this study was to modify the coronal parts of dental implants by irradiating them by use of an excimer laser. In addition to previous experiments [16], cell attachment behaviour is investigated.

Materials and methods

Dental implants

Standard 15-mm plasma-sprayed implants, each with a diameter of 4.5 mm (Frialit 2, Friadent AG, Mannheim, Germany), were removed from their containers and fixed at their endosseous aspects in a sample holder (see below). The implants then were lased on their coronal polished surface areas.

Experimental setup

The experimental setup is shown in Fig. 1. A KrF excimer laser (1) (248 nm, 30 ns pulses, 50 Hz) was used (LPX 305, Lambda Physics, Göttingen, Germany) in this study. The laser light passes a lens first (2) ($f=10$ m) to reduce the divergence of the light. Then, the cross-section of the light is reduced by an aperture (3) (3×3 mm²) and split into three beams by a triple spot lens (4). In addition, using two lenses [(5) $f=0.1$ m/(6) $f=0.15$ m], the light is focussed onto the coronal part of the implant mounted on a rotatable table. The laser light is absorbed well in titanium so that most of the pulse energy (≈ 15 – 17 J/cm²) is used in the ablation process, resulting in limited thermal influence on the bulk of the metal. In this way, three holes with a diameter down to 50 μ m (separation distance < 100 μ m) are created at the implant.

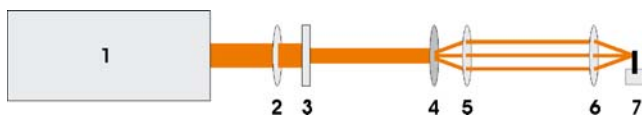


Fig. 1 The experimental setup

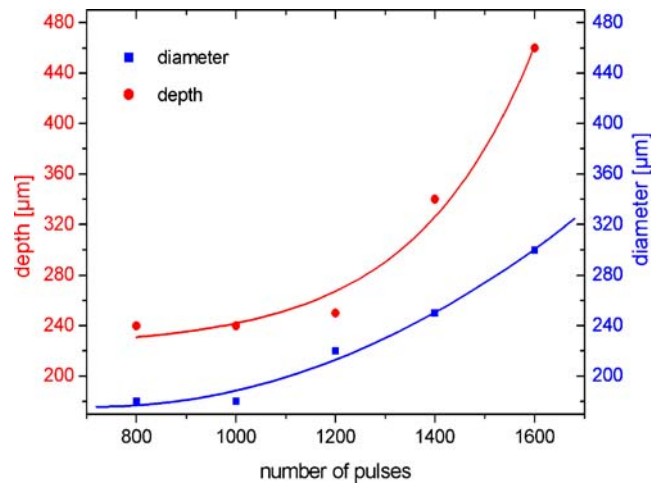


Fig. 2 Dependence of the hole depth and hole diameter on the number of laser pulses investigated at a constant laser energy

Cell adhesion

At first, the implants are cleaned in isopropanol and autoclaved at 134°C for 20 min. Afterwards, they are placed into a fibroblast cell culture for several hours. This procedure is performed as short as possible to see the initial and preferential cell adhesion area.

Characterisation

The samples are examined in an environmental scanning electron microscope (ESEM; XL 30, FEI Company) to visualise the entire surfaces of the lased implants and the adhesion behaviour of the cells. Here, two different modes can be used: For topological investigations, the secondary electrons are detected (SE detector). Nevertheless, a higher contrast is gained if the backscattered electrons are measured (BSE mode). In all cases, the investigations are operated at a water background pressure of 1.4 Torr.

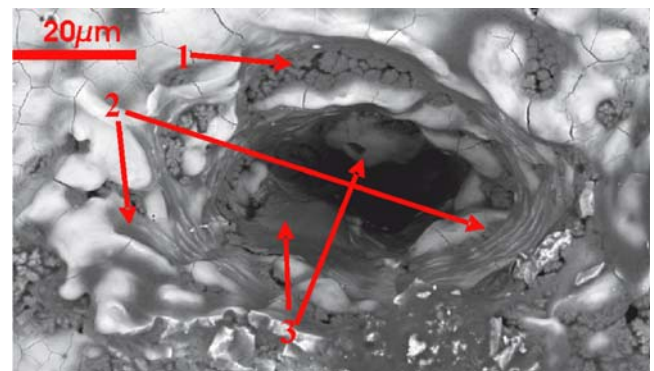


Fig. 3 A typical image of a hole

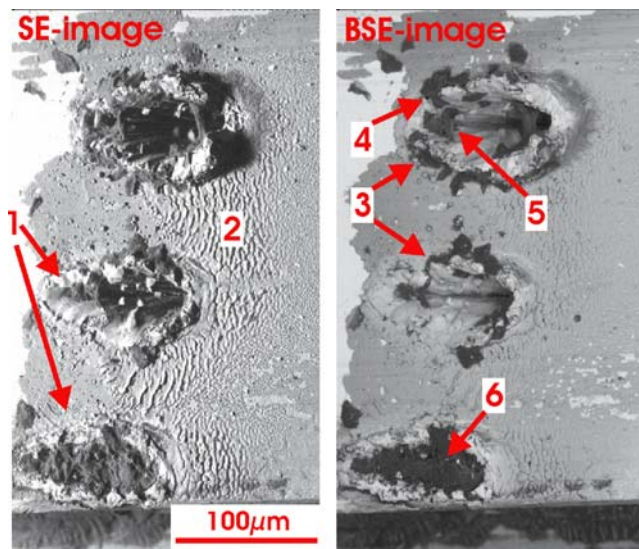


Fig. 4 SE and BSE images of the hole

Results

First, the dependence of the hole depth and hole diameter on the number of laser pulses was investigated at a constant laser energy (see Fig. 2). One can clearly recognise a potential increase of the depth with the number of pulses and a simultaneous increase of the hole diameter.

Afterwards, the implants were immersed into the cell culture and cell adhesion was investigated using an ESEM. A typical image of a hole is shown in Fig. 3. In principle, there are ascertainable bright areas, which correspond to the titanium implant, and darker regions. To distinguish whether the latter one corresponds to the fibroblasts, energy dispersive X-ray analysis was used. It turned out that it is important to differentiate between (1) salt crystals, (2) the solution itself and (3) the fibroblasts.

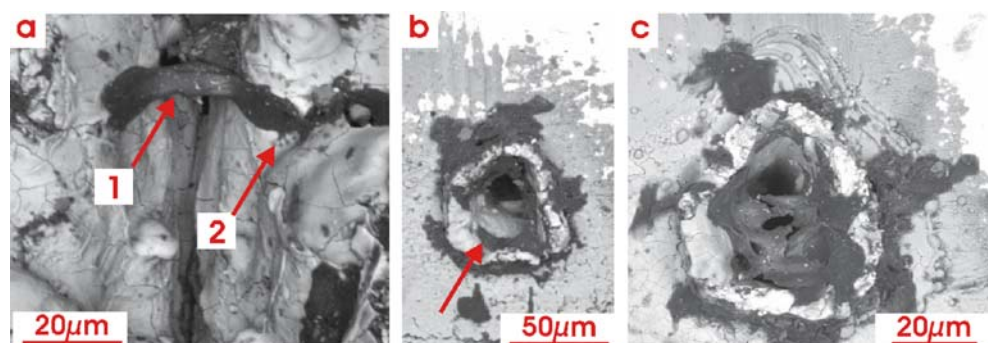
In Fig. 4 (SE and BSE image), cell adhesion on three holes created by the triple spot are shown. The advantage of the SE image (left) is its clear illustration of the surface structure. In evidence, each hole is surrounded by a bead (1), which is due to accumulation of the ablated material. In

addition, a wave-like surface structure formed in-between the holes (2), which is distinctive for melted and re-solidified metal. Thus, the laser light is not completely confined to the hole area. Fibroblast stuck especially around the hole bead (3), seen better at the BSE image (right). In-between the holes, cell adhesion is hardly ascertainable. (The implant was immersed into the cell solution only for a short time.) Thus, the hole bead is a preferential place for cell adhesion. Moreover, cells can overlap the bead and attach to the inner sidewall of the hole (4). Another possibility is the formation of bridges inside the hole (5) or covering the hole completely (6).

Discussion

This study represents ESEM investigations of cell adhesion on holes drilled into implants by the use of a laser. First, we observed an increase of the hole diameter and depth with the number of laser pulses at a constant laser energy (see Fig. 2). In this context, it is to be kept in mind that the ablation rate depends on the spot size or energy density at the sample. Thus, an increase of the hole depth with the number of pulses correlates with an increase of the drill velocity and consequently a higher energy density at the sample. Due to the fact that during the experiment the optical setup does not change, the higher ablation rate or energy density results from the modification of the surface. At the very beginning the surface is flat. Within the first pulses, material is ablated and partially removed. The hole structure starts to develop; that is, a cylindrical hole is formed within the implant. Now, not only laser light directly hitting the bottom of the hole ablates material. Light is reflected from the side wall to the bottom as well. Thus, the energy density at the bottom increases by building up a cylindrical side wall, leading to a higher drill velocity. Certainly, this light and light reflected from the bottom of the hole to the side wall enlarges the hole diameter as well. This is how the increase in diameter of the holes with the number of pulses can be interpreted.

Fig. 5 The implant is surrounded by cells. **a** Cells reach the interior of the hole and start to form bridges (cell, 1; lamellipodium, 2). **b** When the diameter of the hole is too large, cells inside the hole attach along the sidewall. **c** Because of continuous cell adhesion, the hole is capsulated with an empty space underneath



As shown in Fig. 4, cells start to attach especially around the brink of the hole. A flow of the culture medium as a reason can be excluded because the experiment was performed without any setup movement. Thus, the increased surface roughness induced the cells to attach at these certain areas. Starting from this, the development of cell adhesion is to be described as follows.

Within the culture media the implant is surrounded by cells. They start to attach especially at rough areas because the lamellipodium of the cells can stick more easily there. Cells also reach the interior of the hole and start to form bridges (see Fig. 5a). But while forming a bridge, there is a maximum distance which cannot be exceeded. If the diameter of the hole is too large, cells inside the hole attach along the sidewall, as shown in Fig. 5b. It is interesting to note that cells do not attach to the ground of the hole. This phenomenon might be due to the tendency of cells to stretch as wide as possible. Thus, there might be a minimum diameter of the hole as well. Because of continuous cell adhesion, the hole is capsulated with an empty space underneath, like the one shown in Fig. 5c.

Conclusion

Laser irradiation of titanium implants allows to create 3-D surface modification. By dint of the experimental setup, three holes with a diameter down to 50 μm and distanced 100 μm apart were simultaneously drilled into standard dental implants. Thereby, the ablation rate strongly depended on the shape of the sample. It increased during the process due to formation of the hole structure.

Using an ESEM, the initial and preferential attachment of the cells were investigated. It clearly turned out that the fibroblasts attach at the hole brink first because of the increased surface roughness. Inside the hole, cells form bridges and capsulate the hole completely with an empty space underneath.

This experiment showed that an improved cell attachment on implants can be gained by holey structures. In this way, a growth of plaque downward implants can be avoided, which is an important factor in the long-term success of the implant.

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