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# Effects of an Er : YAG laser and the Vector® ultrasonic system on the biocompatibility of titanium implants in cultures of human osteoblast-like cells

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**Date:**
Accepted 28 October 2002

**To cite this article:**
Schwarz F, Rothamel D, Sculean A, Georg T, Scherbaum W, Becker J. Effects of an Er : YAG laser and the Vector® ultrasonic system on the biocompatibility of titanium implants in cultures of human osteoblast-like cells.
*Clin. Oral Impl. Res.* **14**, 2003; 784–792



**Key words:** cell adhesion, human osteoblast-like cells, lasers/therapeutic use, ultrasonic devices/therapeutic use, titanium surfaces

**Abstract:** The aim of the present study was to investigate the effects of an Er : YAG laser (ERL) and the Vector® ultrasonic system (VS) on the biocompatibility of titanium implants in cultures of human osteoblast-like cells (SAOS-2). One hundred and sixty-eight titanium discs with four different surfaces (sand-blasted and acid-etched, titanium plasma-sprayed, machine-polished, and hydroxyapatite-coated) were used to evaluate cell attachment. The samples were equally and randomly assigned to the following groups: (1) an ERL at an energy level of 100 mJ/pulse and 10 Hz using a special application tip, (2) the VS using carbon fibre tips, or (3) untreated control (C). The discs were placed in culture plates, covered with a solution of SAOS-2 cells, and incubated for 7 days. The specimens were then washed with phosphate buffer to remove cells not attached to the surface, and the adherent cells were stained with hematoxilin–eosin. Cells were counted using a reflected light microscope and the cell density per $mm^2$ was calculated. Additionally, cell morphology and surface alterations of the titanium discs after treatment were investigated using scanning electron microscopy (SEM). All titanium discs treated with ERL demonstrated nearly the same cell density per $mm^2$ as the untreated C surfaces. There was a significant decrease in the number of cells that attached to the implant surfaces treated with VS. The SEM examination showed no visible differences between lased and C titanium surfaces. All surfaces treated with VS showed conspicuous surface damage and debris of the used carbon fibres. The results of the present study indicate that (i) ERL does not damage titanium surfaces and subsequently does not influence the attachment rate of SAOS-2 cells, and (ii) VS, used with this type of carbon fibre tip, does not seem to be suitable for the instrumentation of titanium surfaces.

Today, oral rehabilitation by means of endosseus dental implants has gained importance in clinical practice. Various surface characteristics ranging from relatively smooth machined surfaces to more roughened surfaces (created by coatings, blasting by various substances, acid treatments, or by combinations of the treatments) are available (Cochran 1999). Results from animal and *in vitro* experiments provide clear evidence that rough implant surfaces have increased bone-to-implant contact and require greater forces to break the bone–implant interface compared to smooth surfaces (Carlsson et al. 1988; Deporter et al. 1990).

Although the clinical results during the first decade are promising, about 10% of the osseointegrated implants are lost after loading (Adell et al. 1990). Several factors have been implicated in the pathogenesis of implant failures. One of them is related to the presence of pathogens around the collar of the dental implants (Mombelli et al. 1988; Becker et al. 1990; Alcoforado et al.

1991). This presence may lead to an inflammation of the peri-implant mucosa, and, if left untreated, the inflammation spreads apically and results in bone resorption, which has been named peri-implantitis (Mombelli et al. 1987; Mombelli & Lang 1994). Therefore, the principal objective of treatment is the complete removal of all calcified and bacterial deposits from the implant surfaces in order to stop disease progression. Ideally, the bone-to-implant contacts should increase and the implant undergoes re-osseointegration.

Both mechanical and chemical methods have been recommended for cleaning and decontamination of implant surfaces (Parham et al. 1989; Fox et al. 1990; Mombelli & Lang 1992; Ruhling et al. 1994; Ericsson et al. 1996; Schenk et al. 1997; Augthun et al. 1998). Among the mechanical methods, metal curettes and ultrasonic scalers induce surface alterations in implants and their use is therefore contraindicated (Augthun et al. 1998; Thomson-Neal et al. 1989). The application of plastic curettes is insufficient in the elimination of bacteria on roughened implant surfaces (Fox et al. 1990; Augthun et al. 1998).

Air-powder flow may be used for implant surface decontamination (Parham et al. 1989; Augthun et al. 1998). However, there are limitations in its application because they can be associated with an increased risk of emphysema (Van de Velde et al. 1991). Among the chemical methods, adjunctive subgingival irrigation with local disinfectants or local antibiotic therapy had a beneficial effect in patients suffering from peri-implantitis (Mombelli & Lang 1992; Schenk et al. 1997). Furthermore, surgical therapy in combination with systemic antibiotics resulted in a resolution of the peri-implantitis lesion (Ericsson et al. 1996; Persson et al. 1996).

In addition to these conventional tools, the use of lasers has been proposed for cleaning and for the detoxification of implant surfaces (Oyster et al. 1995; Romanos et al. 2000; Kreisler et al. 2002a, b). The results from recently published studies have indicated that among all lasers used in the field of dentistry, only the carbon dioxide ($CO_2$) laser, the diode laser and the Er : YAG (erbium-doped : yttrium, aluminium and garnet) laser (ERL) may be useful for the instrumentation of implant surfaces because the implant body temperature does not increase significantly after laser irradiation (Oyster et al. 1995; Ando et al. 1996; Kato et al. 1998; Romanos et al. 2000; Kreisler et al. 2002a, b). In contrast, the use of an Nd : YAG laser (neodymium-doped : yttrium, aluminium and garnet) resulted in extensive melting and damage of the porous titanium surface and coating (Pick & Colvard 1993; Romanos et al. 2000). Close attention has been paid to the clinical applicability of the ERL with its wavelength of 2.94 μm in the near-infrared spectrum. This wavelength is well absorbed by water because the peak is close to the absorption coefficient of water. Recently, the ERL has been reported to be the most promising laser for periodontal treatment (Aoki et al. 1994; Ando et al. 1996; Folwaczny et al. 2001; Schwarz et al. 2001a,b). Its excellent ability to ablate hard tissue and dental calculus effectively without producing major thermal side effects to adjacent tissue has been demonstrated in numerous studies (Aoki et al. 1994; Folwaczny et al. 2001; Schwarz et al. 2001a).

Moreover, the results from a controlled clinical trial have indicated that non surgical periodontal treatment with an ERL leads to a significant gain of clinical attachment level (Schwarz et al. 2001b). However, there are only a few reports evaluating the influence of this laser on the characteristics of implant surfaces (Rechmann et al. 2000; Kreisler et al. 2002a). In this context, the effects of ERL irradiation on the biocompatibility of titanium surfaces are of major importance. Therefore, the purpose of the present study was to investigate the effects of an ERL, used with a special application tip, on the biocompatibility of titanium implants in cultures of human osteoblast-like cells in comparison to the recently introduced Vector® ultrasonic system (VS), used with carbon fibre tips. Untreated titanium surfaces served as a control (C). Additionally, cell morphology and surface alterations of the titanium discs after treatment were investigated using scanning electron microscopy (SEM).

## Material and methods

### Titanium discs

Four different types of titanium test discs (10 mm in diameter and 2 mm thick) made of commercially pure titanium with original surface patterns similar to those of sandblasted and acid-etched (SLA), titanium plasma-sprayed (TPS), machine-polished (MP) (IMZ-TwinPlus, Friadent Co., Mannheim, Germany; ITI, Straumann, Waldenburg, Switzerland), and hydroxyapatite (HA)-coated (IMZ-TwinPlus, Friadent Co., Mannheim, Germany) were used. A total of 168 discs were equally and randomly assigned to the following test and C groups: (1) an ERL (test group 1), (2) the VS (test group 2), or (3) untreated C. Each group had 16 SLA, 16 TPS, 16 MP, and eight HA-coated titanium discs. Additionally, 21 titanium discs were assigned to the test and C groups and prepared for SEM examination.

### Treatments

An ERL laser device (KEY II®, KaVo, Biberach, Germany) emitting a pulsed infrared radiation at a wavelength of 2.94 μm was selected for laser treatment. Laser parameters were set at 100 mJ/pulse (12.7 J/cm²), 10 Hz, and the pulse energy at the tip was approximately 85 mJ/pulse. The laser beam was guided onto the implant surfaces under water irrigation with a specially designed periodontal handpiece (2056, KaVo, Biberach, Germany) and a cone-shaped glass fibre tip emitting a radial and axial laser beam (Fig. 1). Prior to the irradiation of the individual samples, the actual pulse energy was measured (Filed Master GS, Coherent, Dieburg, Germany). For the treatment of test group 2, a specially designed ultrasonic system (Vector®, Dürr, Bietigheim-Bissingen, Germany) with a straight carbon fibre and a polishing fluid (HA particles <10 μm) was used (Fig. 2). In both groups, the fibre tips were guided parallel to the titanium surfaces in contact mode. Each titanium surface was only scanned once to standardize the treatment time. The required amount of time for treatment in the laser and Vector® groups was, on average, 2 min per titanium disc.

### Cell cultures

The titanium discs were placed in 24-well plates (Lap Tek Chamber Slide, Nalge Nunc, Naperville, IL, USA), covered with a solution of SAOS-2 cells (ATCC, No. HTB 85, Manassas, VA, USA) (fourth

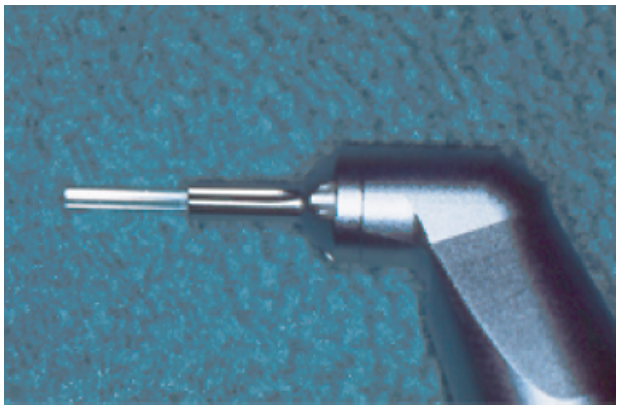

*Fig. 1.* Laser handpiece with a cone-shaped glass fibre tip emitting a radial and axial laser beam.

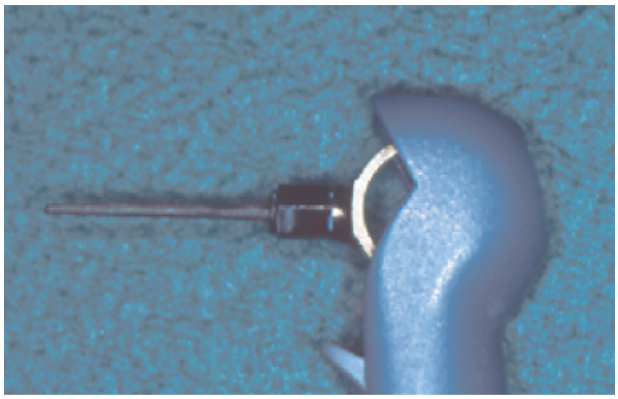

*Fig. 2.* Straight carbon fibre of the Vector® ultrasonic system.

passage, $2 \times 10^4$ cells suspended in 2 ml of McCoy's 5A medium (Gibco No. 21017-025, Life Technologies GmbH, Karlsruhe, Germany) supplemented with 1% penicillin/streptomycin and 15% fetal bovine serum), and incubated for 7 days. Culturing was set at 37°C in a humified atmosphere of 95% air and 5% $CO_2$. The medium was changed every 2–3 days. After incubation, the specimens were gently washed with phosphate-buffered saline (PBS) to remove cells not attached to the surface. Finally, the adherent cells were fixed with paraformaldehyde (3%) and stained with hematoxilin–eosin.

## Microscopic analysis

Cells were counted using a reflected light microscope (Leitz Orthoplan, Leitz, Wetzlar, Germany) (original magnification $\times 200$) and a counting grid. An area of eight fields selected at random (exactly 8 mm²) was scanned and the stained cells were counted out. All samples were investigated independently by two blinded examiners. Intraobserver reliability was evaluated by counting out two discs of each surface ($n = 14$) 10-fold by one examiner. One sample of each surface ($n = 7$) was analysed by two investigators to determine interexaminer differences. The results were expressed as a coefficient of variation.

### SEM observation

Following incubation, the discs were gently washed with PBS to remove cells not attached to the surface and fixed for 30 min with 4% glutaraldehyde in 0.15 M PBS (pH = 7.4) at room temperature and then washed in 0.15 M PBS for 15 min. The specimens were dehydrated in increasing concentrations of acetone (from 40% to 100%, 10% steps). After drying in hexamethyldisilazane, the specimens were sputter-coated with gold and examined using SEM (Scanning Microscope DSM 950, Zeiss, Germany). All samples were investigated independently by two blinded examiners.

## Statistical analysis

A software package was used for the statistical analysis (SPSS 11.0, SPSS Inc., Chicago, IL, USA). The number of cells on an area of 8 mm² was divided by 8 to indicate the cell density per mm² for each titanium disc. Mean values and standard deviations were then calculated for each group. Group comparison was performed by the Wilcoxon test and differences were considered to be significant when $P<0.05$.

## Results

### Assessment of cell numbers

Intraobserver variability was between 1.3% and 2.4% and lower than the interobserver variability (maximum 3.1%). In all test and C groups, the highest number of cells per mm² was seen on the SLA surfaces, followed by the TPS and MP surfaces. The HA-coated surfaces showed the least cell density per mm² (Fig. 3a–c). All titanium discs treated with the ERL demonstrated comparable cell densities per mm² as the untreated C surfaces (Fig. 3a and c). There was, however, a statistically significant decrease in the number of cells that attached to the implant surfaces treated with the VS ($P<0.001$) (Fig. 3b). In the laser-treated and C groups, the mean cell number per mm² was statistically higher than in the Vector®-treated groups ($P<0.001$). The comparison between the lased and C groups revealed no significant differences ($P>0.05$).

### Surface pattern and cell morphology

The SEM examination showed no visible morphologic differences between lased and C titanium surfaces. In the laser-treated groups, no thermal side effects, such as melting or loss of porosity, were observed (Figs 4–7: a, b). However, all surfaces treated with the Vector® system showed conspicuous surface damages (scratches) and deposits of the used carbon fibres (Figs 4–7: c). On the MP titanium surfaces, cells had started to spread, with complete

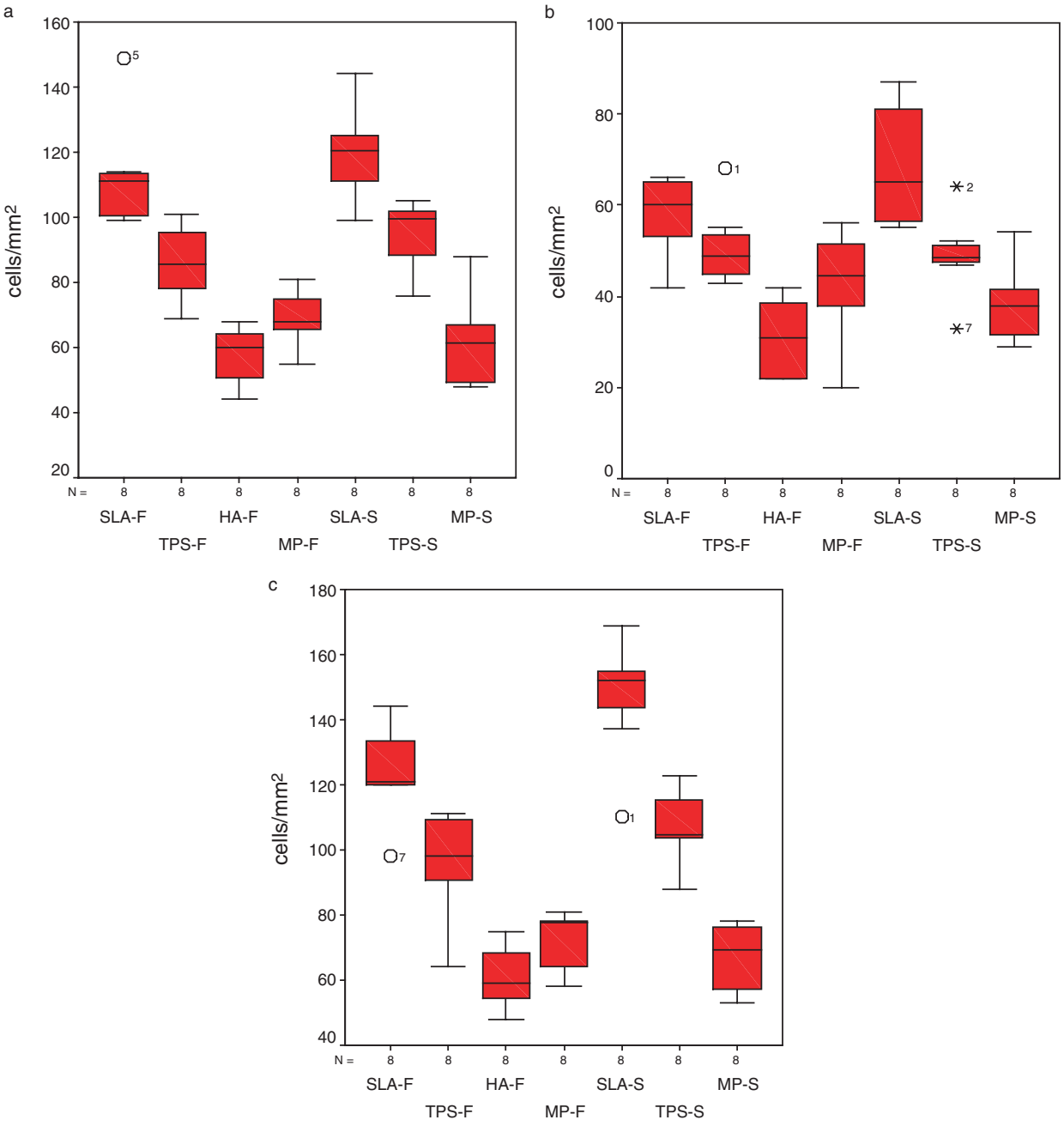


*Fig. 3*. Box plots with outliners for the medians and Q1–Q3 quartiles of cell density (cells/mm²) on different implant surfaces, evaluated after 7 days incubation in an osteoblast-like cell suspension (SLA = sand-blasted and acid-etched; TPS = titanium plasma-sprayed; MP = machine-polished; HA = hydroxyapatite-coated; F = Friadent; S = Straumann). Lines below and above box plots = min, max. (a) Er : YAG Laser. (b) Vector® ultrasonic system. (c) Untreated control surfaces.

cytoplasmatic extension of the cell body on the titanium surface. The cells were oriented in one direction parallel to the linear grooves on the surface (Fig 4a–c). On the SLA and TPS titanium surfaces, cells had assumed a spindle shape. The cell bodies spanned grooves and pits, although some adaptation to the irregularities of the underlying surface was commonly observed. The cells did not display any orientation (Figs 5 and 6: a–c). In contrast to this, the HA-coated surface was heterogeneous, consisting of areas with a smooth, rounded profile, like that of the TPS surface, and areas made up of clusters of small grains of incompletely melted or unmelted particles. Cells were prevailing rounded and attached to all areas (Figs 7a–c). No differences were observed in the morphology of the cells between test and C groups.

## Discussion

In the present study, human osteoblast-like SAOS-2 cells were shown to respond differently to implant surfaces treated with either an ERL or the VS. All titanium discs treated with laser demonstrated comparable cell densities per mm² as the untreated C surfaces, whereas the cell number per mm² was reduced on all implant surfaces

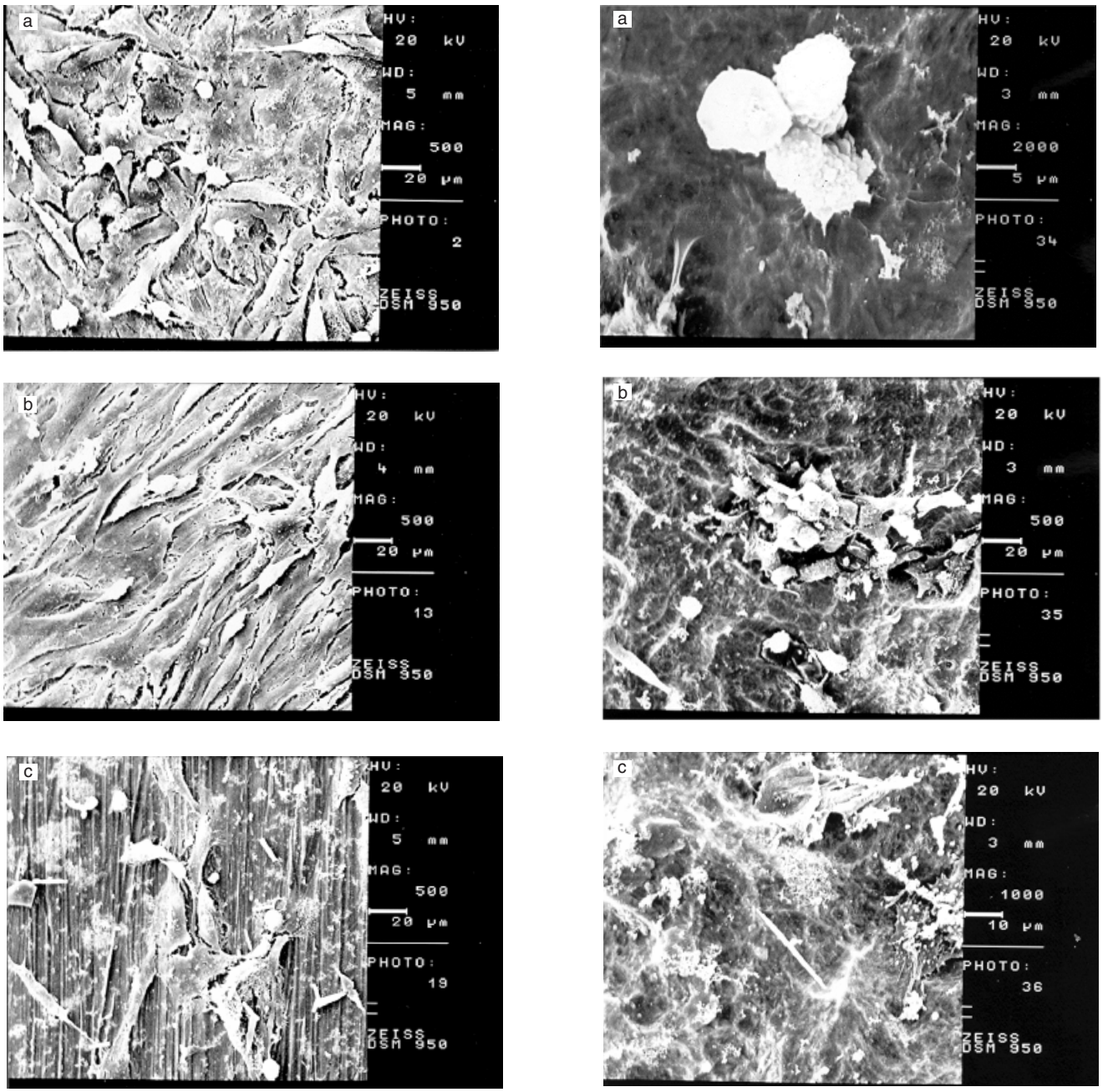


*Fig. 4*. Machine-polished titanium surfaces: cells had started to spread with complete cytoplasmatic extension of the cell body on the substrate surface. (a) Control (C) surface (ITI, 1 : 500). (b) Er : YAG laser treated surface (ITI, 1 : 500) showing no difference in the surface pattern compared to the untreated control. (c) Vector®-treated surface (ITI, 1:500) showing scratches and debris of the used carbon fibre.




*Fig. 5*. Sand-blasted and acid-etched titanium surfaces: cells had assumed a spindle shape. The cell bodies spanned pores, grooves, and pits. (a) Control (C) surface (IMZ, 1 : 2000). (b) Er : YAG laser-treated surface (ITI, 1 : 500) showing no difference in the surface pattern compared to the untreated C. (c) Vector®–treated surface (ITI, 1 : 1000) showing debris of the carbon fibre. The attachment of SAOS-2 cells was totally inhibited.

treated with the Vector® system. These differences were found to be statistically significant ($P$<0.001). No differences were observed in the morphology of the cells between test and C groups. Furthermore, the results have indicated that in the test and C groups the highest number of cells per mm² was seen on the SLA surfaces, followed by the TPS and MP surfaces. The HA-coated surfaces showed the least cell density per mm². These findings are consistent with results from previous studies, which have shown a significant increase in cell number attachment on irregular, sand-blasted surfaces compared to that on regular surfaces (Bowers et al. 1992; Martin et al. 1995). Since HA coating is considered to be rough surface, it is important to point to the results of previous studies to discuss potential biological explanations as to why titanium discs coated with HA showed the least cell density per mm². Previous investigators have noted dissolution of HA coatings in physiologic conditions (Gross et al. 1997). There appeared to be fewer cells on the HA-coated implants, which could possibly reflect a lack of stability of

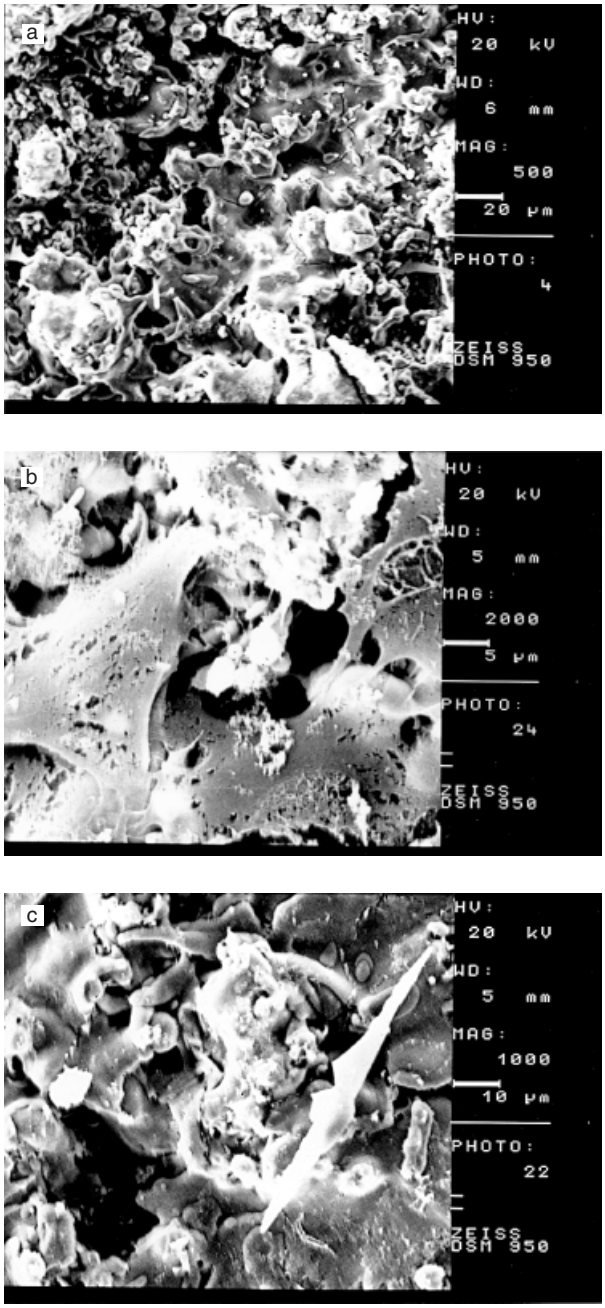


*Fig. 6.* Titanium plasma-sprayed titanium surfaces: cells had assumed a spindle shape. Some adaptation of the cell bodies to the irregularities of the underlying surface was commonly observed. (a) Control (C) surface (ITI, 1 : 500). (b) Er : YAG laser-treated surface (IMZ, 1 : 2000) showing no difference in the surface pattern compared to the untreated C. (c) Vector®-treated surface (ITI, 1 : 1000) showing debris of the carbon fibre.

the HA-coated surface (Anselme et al. 1997). In this respect, it cannot be overlooked as to what extent the cell culture medium might have led to a dissolution of the HA coating and subsequently to a reduction of cell attachment to HA surfaces. In this context, the question of the limits of *in vitro* investigations on bioactive ceramics, such as HA coatings susceptible to modification by simple immersion in aqueous solutions (i.e. cell culture medium or physiologic saline), has to be raised.

Surface topography plays an important role in the adhesion of cells (Inoue et al. 1987; Brunette 1988). Rough or textured porous surfaces have been considered to promote cell attachment. It has been shown that osteoblast-like cells attach more to rough surfaces, allowing increased mineralization, while fibroblasts prefer smooth or finely textured surfaces (Bowers et al. 1992; Kononen et al. 1992; Martin et al. 1995). The cell attachment assay used in our study was similar to the techniques described by previous workers to compare cellular responses to implants of different surface characteristics (Bowers et al. 1992; Chang et al. 1999). In the present study, we used human osteosarcoma-derived SAOS-2 cells that have been well characterized as osteoblast-like cells (Murray et al. 1987; Rodan et al. 1987). However, transformed cell lines have their own limitations as some of the cell characteristics are different from those of primary cells. Nevertheless, in long-term *in vitro* mineralization studies, normal human osteoblast cultures responded in a similar way to implant surfaces such as SAOS-2 cells, but with approximately two-thirds less calcification (Ahmad et al. 1999).

The results of the present study have also shown that the ERL, used with a special application tip, does not damage titanium surfaces. So far, there are only few data available describing the effects of an ERL on the surface characteristics of differently coated titanium discs (Rechmann et al. 2000; Kreisler et al. 2002a). In a recent study, Kreisler et al. (2002a) reported surface alterations, such as melting and glazing, at energy densities of 8.9 J/cm² in TPS surfaces, 11.2 J/cm² in SLA surfaces, 17.8 J/cm² in HA-coated surfaces, and 28 J/cm² in smooth titanium surfaces. The laser was used in noncontact mode without water cooling and the angle of irradiation was 90°. In a similar study, first micro-morphological changes in SLA and TPS titanium surfaces occurred at an average energy density of 7 J/cm² (Rechmann et al. 2000). However, in the present study no alterations were detected in any of the investigated surface patterns at energy densities of 12.7 J/cm². This discrepancy might be explained by the fact that the fibre tip was guided in contact mode, parallel to the titanium surfaces under permanent water cooling. In this context, it is important to point to the results of a previous study, which showed that the angulation of the application tip has a strong influence on the amount of root substance removal using ERL radiation for periodontal treatment (Folwaczny et al. 2001). Furthermore, it should be pointed out that permanent water cooling might cause less damage than irradiation without water irrigation. The interaction between laser light and metal surfaces is mainly

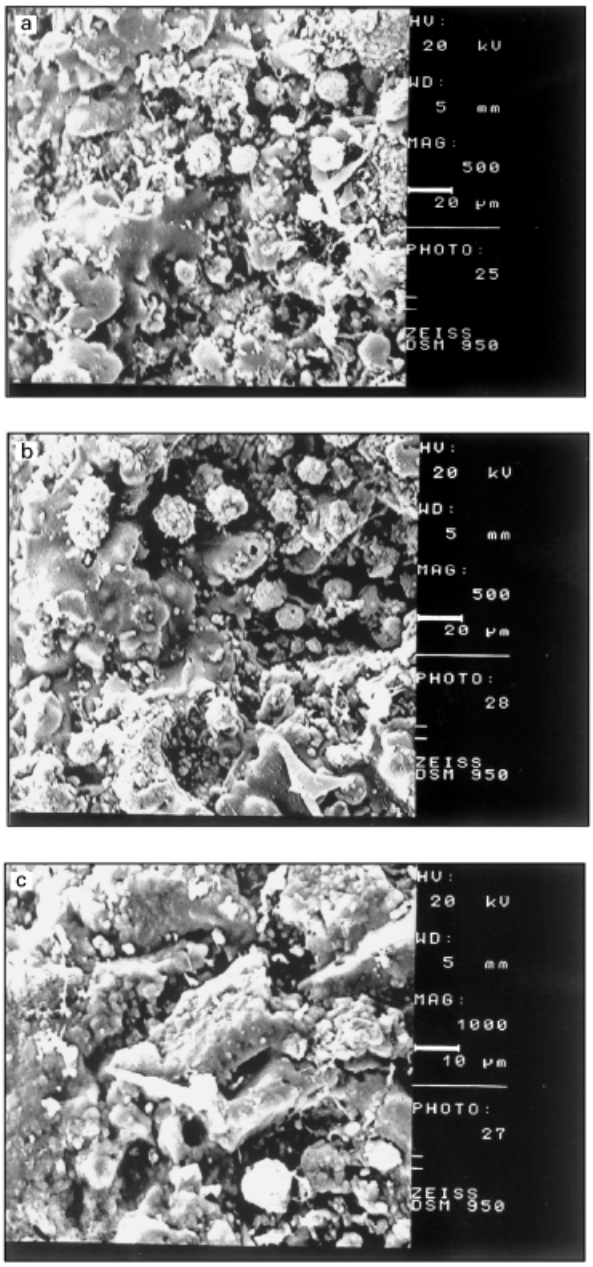


*Fig. 7.* Hydroxyapatite-coated titanium surfaces: cells were prevailing rounded and attached to all areas. (a) Control (C) surface (IMZ, 1 : 500). (b) Er : YAG laser-treated surface (IMZ, 1 : 500) showing no difference in the surface pattern compared to the untreated C. (c) Vector®-treated surface (IMZ, 1 : 1000) showing debris of the carbon fibre.

determined by the degree of absorption and reflection. Each metal features a certain spectral reflection capacity that is dependent on the specific wavelength of the laser. The reflection capacity of titanium for the ERL with its wavelength of 2940 nm in the near-infrared spectrum is 71% and rises up to 96% for the $CO_2$ laser at 10,000 nm (Lide 2002). In this situation, the implant surface does not absorb the irradiation and subsequently there is no temperature increase, which would damage the implant surface.

As mentioned above, the ERL has been reported to be the most promising laser for periodontal treatment (Aoki et al. 1994; Folwaczny et al. 2001; Schwarz et al. 2001a,b). Further *in vitro* studies on the antimicrobial effects of the ERL radiation provided clear evidence for bactericidal effects against periodontopathic bacteria (Ando et al. 1996; Folwaczny et al. 2002). In this context, it is important to point to the results of previous studies, which have shown that the bacterial infection around dental implants is similar to periodontal disease (Mombelli et al. 1987; Lang et al. 1993). To the best of our knowledge, there are no other data investigating the effects of the VS on titanium implants. One possible explanation for the reduced cell numbers in the Vector®-treated group may be due to a cytotoxic effect of the debris of the used carbon fibres. Nevertheless, it must be pointed out that one limitation of the present study was the lack of contamination of the titanium discs with bacterial deposits known to be involved in the peri-implantitis process. Therefore, the hypothesis that the two treatment devices may have an effect on cell attachment rate after bacterial surface decontamination could not be tested.

However, the aim of the present study was not to simulate treatment of peri-implantitis, but to investigate the effects of an ERL and the VS on the biocompatibility of differently coated titanium implants in cultures of human osteoblast-like cells. On the other hand, as mentioned above, previous studies have shown that other forms of therapy (i.e. surgical therapy in combination with systemic antibiotics) have proved successful without the addition of laser therapy. Therefore, further studies using controlled experimental *in vivo* models are needed in order to show that no damage to titanium implants occurs following ERL irradiation and to simulate treatment of peri-implantitis and the process of re-osseointegration.

In conclusion, within their limits, the results of the present *in vitro* study indicate that an ERL, used with a special application tip, does not damage titanium surfaces and subsequently does not influence the new attachment rate of human osteoblast-like cells. Based on these findings, the VS used with this type of carbon fibre tip does not seem to be suitable for the instrumentation of titanium surfaces.

## Résumé

Le but de l'étude présente a été de vérifier les effets d'un laser Er: YAG et d'un système ultrasonique Vector® sur la biocomptabilité d'implants en titane dans des cultures de cellules humaines ressemblant à des ostéoblastes (SAOS2). Cent soixante-huit disques de titane avec quatre surfaces différentes (sablées et mordançées, titane plasma-spray, usinées, recouvertes d'hydroxyapatite) ont été utilisés pour évaluer l'attache cellulaire. Les échantillons étaient répartis de manière randomisée et égale dans un des

groupes suivant : 1) laser Er: YAG (ERL) à un niveau d'énergie de 10mJ/pulsation et 10Hz en utilisant un insert d'application spécial ou 2) le système ultrasonique Vector® (VS) en utilisant des inserts en fibre de carbone ou 3) un contrôle non-traité (C). Les disques ont été placés dans des cultures, recouverts avec une solution contenant des cellules SAOS2 et incubés pendant sept jours. Les spécimens ont ensuite été rincés avec un tampon phosphate pour enlever les cellules non-attachées à la surface et les cellules adhérentes ont été colorées avec de l'hématoxiline-éosine. Les cellules ont été comptabilisées à l'aide d'un microscope optique et la densité cellulaire par mm2 a été calculée. De plus la morphologie cellulaire et les altérations de surface des disques en titane après traitement ont été analysées en utilisant le MEB. Tous les disques en titane traités avec ERL possèdaient presque la même densité cellulaire par mm2 que sur les surfaces contrôles non-traitées. Il y avait une diminution significative du nombre de cellules attachées sur les surfaces implantaires traitées avec VS. L'examen au MEB n'a montré aucune différence visible entre les surfaces traitées. Toutes les surfaces traitées avec VS accusaient des lésions de surfaces évidentes et des débris des fibres carbones utilisées. Les résultats de l'étude présente indiquent que 1) ERL n'abme pas les surfaces en titane et subséquemment n'influence pas le taux d'attache des cellules SAOS2 et 2) VS, en utilisant ce type d'insert en fibre de carbone, ne semble pas adéquat pour l'instrumentation des surfaces en titane.

## Zusammenfassung

*Der Effekt eines Er:YAG Lasers und des Vector® Ultraschallsystems auf die Biokompatibilität von Titanimplantaten in Kulturen von menschlichen osteoblastenähnlichen Zellen*

Das Ziel der vorliegenden Untersuchung war, den Effekt eines Er:YAG Lasers und des Vector® Ultraschallsystems auf die Biokompatibilität von Titanimplantaten in Kulturen von menschlichen osteoblastenähnlichen Zellen (SAOS2) zu untersuchen. 168 Titanscheiben mit 4 verschiedenen Oberfächen (sandgestrahlt und säuregeätzt, titanplasmabeschichtet, maschinell poliert und hydroxyapatitbeschichtet) wurden für die Auswertung der Zellanhaftung verwendet. Die Proben wurden gleichmässig und zufällig den folgenden Gruppen zugeteilt: (1) Er:YAG Laser (ERL) mit einem Energienieau von 100mJ/Impuls und 10 Hz angewendet mit einer speziellen Aplikationsspitze, oder (2) Vector® Ultraschallsystem (VS) mit Arbeitsspitzen aus Carbonfasern, oder (3) unbehandelte Kontrolle (C). Die Scheiben wurden in Kulturplatten gelegt, mit einer Lösung von SAOS2 Zellen abgedeckt und während 7 Tagen inkubiert. Danach wurden die Proben mit einem Phosphatpuffer gewaschen, um nicht an der Oberfläche anhaftende Zellen zu entfernen. Die anhaftenden Zellen wurden mit Hämatoxilin-Eosin eingefärbt. Die Zellen wurden unter einem Reflektionslichtmikroskop gezählt und die Zelldichte pro mm2 wurde berechnet. Zusätzlich wurden die Zellmorphologie und die Oberflächenveränderungen auf den Titanscheiben nach der Behandlung mittels SEM untersucht. Alle Titanscheiben, welche mit ERL behandelt wurden, zeigten annähernd die selbe Zelldichte pro mm2 wie die unbehandelten Kontrolloberflächen. Es bestand eine signifikante Reduktion der anhaftenden Zellen auf den Implantatoberflächen, welche mit VS behandelt worden waren. Die Untersuchung unter dem SEM zeigte keine sichtbaren Unterschiede zwischen den laserbehandelten und den Kontrolloberflächen. Alle Oberflächen, welche mit dem VS behandelt worden waren, zeigten verdächtige Beschädigungen und Reste der verwendeten Carbonfasern. Die Resultate der vorliegenden Untersuchung zeigen, dass (I) ERL die Implantatoberfläche nicht beschädigt und in der Folge auch nicht die Anhaftungsrate von SAOS2 Zellen beeinflusst und dass (II) VS nicht für die Instrumentierung von Titanoberflächen geeignet zu sein scheint, wenn Arbeitsspitzen aus Carbonfasern verwendet werden.

## Resumen

La intención del presente estudio fue investigar los efectos de un láser Er:YAG y el sistema de ultrasonidos Vector® en la biocompatibilidad de implantes de titanio en cultivos de células humanas tipo osteoblastos (SAOS2). Se usaron 168 discos de titanio con 4 superficies diferentes (pulverizadas con arena y gravadas con ácido, pulverizadas con plasma de titanio, pulidos a máquina, y cubiertos por hidroxiapatita) para evaluar la adherencia celular. Las muestras se asignaron por igual y aleatoriamente a los siguientes grupos: (1) Láser Er:YAG (ERL) con un nivel de energía de 100 mJ/pulso y 10 Hz usando una punta de aplicación especial, o (2) Sistema ultrasónico Vector ® (VS) usando puntas de fibra de carbono, o (3) control sin tratamiento (C). Estos discos se colocaron en las placas de cultivo, se cubrieron con una solución de células SAOS2, y se incubaron durante 7 días. Los especimenes se lavaron entonces con un búfer de fosfato para retirar las células no adheridas a la superficie, y las células adheridas se tiñeron con hematoxilina-eosina. Las células se contaron usando un microscopio de luz reflejada y se calculó la densidad celular por mm2. Además, se investigó la morfología celular y las alteraciones de la superficie de los discos de titanio usando SEM. Todos los discos de titanio tratados con ERL mostraron casi la misma densidad celular que las superficies de control no tratadas. Hubo un descenso significativo del número de células adheridas a las superficies de los implantes tratado con VS. El examen con SEM no mostró diferencias visibles entre las superficies tratadas con láser y las de control. Todas las superficies tratadas con VS mostraron daños superficiales llamativos y residuos de las fibras de carbono usadas. Los resultados del presente estudio indican que (I) ERL no daña las superficies de titanio y subsecuentemente no influye en la tasa de adherencia de las células SAOS2 y (II) VS usado con este tipo de punta de fibra de carbono no parece ser adecuada para la instrumentación de las superficies de titanio.

要旨

本研究は、ヒト骨芽細胞様細胞（SAOS2）培養において、Er:YAG レーザーと Vector® 超音波システムがチタン・インプラントの生体適合性に及ぼす効果を調べることを目的に行った。4種類の異なる表面（サンドブラストと酸エッチング、チタン・プラズマ溶射、機械研磨とハイドロキシアパタイト・コーティング）を備えた６８本のチタン・ディスク上で、細胞付着を評価した。標本を等しく、無作為に以下の群に割り付けた：１）、エネルギー・レベル１００mJ/ﾊﾟﾙｽと１０Hz で、特殊なアプリケーション・チップを用いた Er:YAG レーザー（ERL）群、２）カーボン・ファイバー・チップを用いた Vector® 超音波システム（VS）群及び３）未処理の対照群（C）。ディスクを培養皿に入れて、SAOS2 細胞の溶液で覆い、７日間培養した。次に標本を燐酸緩衝液で洗浄して表面に付着していない細胞を除去し、付着している細胞はヘマトキシリン－エオジンで染色した。反射光顕微鏡で細胞を数えて、1 mm2 あたりの細胞密度を計算した。さらに処理後の細胞の形態とチタン・ディスクの表面変化を SEM で調べた。ERL で処理したディスクは全て、未処理の対照群の表面とほぼ等しい 1 mm2 あたりの細胞密度を示した。VS で処理したインプラント表面に付着した細胞数は、有意に少なかった。SEM の検査では、レーザー処理と対照群のチタン表面の間に視認できる差異はなかった。VS で処理した表面は全て顕著な損傷を受けており、使用したカーボン・ファイバーの残渣を示していた。本研究の結論として、１）ERL はチタン表面を損なうことはなく、SAOS2 細胞の付着率に影響を及ぼさない、２）VS はこの種のカーボン・ファイバー・チップを用いる場合は、チタン表面での使用には適さないと思われる。

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