Laser therapy accelerates initial attachment and subsequent behaviour of human oral fibroblasts cultured on titanium implant material
A scanning electron microscopic and histomorphometric analysis

Low-level laser therapy (LLLT) is being applied extensively in medicine and dentistry to treat both hard- and soft-tissue injuries (Dyson & Young 1986; Glinkowski 1990; Midamba & Haanæs 1993; Glinkowski & Rowinski 1995; Yu et al. 1997). In an animal study, Lyons et al. [1987] demonstrated considerable enhancement of tensile strength of laser-irradiated wounds. Sapera et al. [1986] reported that LLLT increased transcription of mRNA of procollagen types I and III. Collagen production was significantly enhanced compared with the non-irradiated wounds, suggesting a beneficial effect of the helium–neon (He–Ne) laser on wound healing in vivo (Abergel et al. 1984; Balboni et al. 1986; Lam et al. 1986). Several experimental studies have demonstrated the biostimulatory effect of laser therapy on soft- and hard-tissue healing (Boulton & Marshall 1986; Chomette et al. 1987a, 1987b; Pourreau-Schneider et al. 1989, 1990, 1992; Nara et al. 1992; Loewshall & Arenholt-Bindslev 1994; Dörntbudak et al. 2000; Khadra & Ronold 2002). In clinical studies, Neiburger [1997, 1999] showed that He–Ne diode lasers, at low energy level, appear to increase the rate of gingival wound healing in humans, without negative side-effects. To date, however, there have been few large-scale controlled...
studies of the effects of laser biostimulation in human subjects.

For many years, titanium implants have been used with good long-term success to rehabilitate total and partial edentulousness [Adell et al. 1990; Buser et al. 1990; Arvidson et al. 1992; Olsson et al. 1995]. Long-term sustainability of uncompromised function depends not only on the integrity of osseointegration, but also on the health of the epithelium and the quality of attachment of the connective tissue to the titanium surface [Listgarten et al. 1991; Buser et al. 1992; Berglundh 1993]. As in the normal periodontium, the healthy mucosa has an essential role in protecting the peri-implant sulcus from bacterial invasion. It has been proposed that the mucosa surrounding an implant and the free marginal gingiva surrounding a natural tooth have several clinical and histological features in common [Arvidson et al. 1990; Berglundh et al. 1991; Lindhe et al. 1992]. However, peri-implant soft tissue is more fragile than the periodontal tissue of natural teeth [Ericsson et al. 1992; Lindhe et al. 1992]. As there is neither cementum nor fiber insertion on the surface of an endosseous implant, lack of attachment around the implants may lead to peri-implantitis and eventual failure [Kurashina et al. 1984; Lang et al. 1994]. In this context, regeneration of the oral mucosa and establishment of an intact functional barrier at the transmucosal passage of the abutment is one of the major benefits claimed for laser therapy.

Although the effect of LLLT on the biological tissues has been studied quite extensively, there are few studies of the mechanism of action of cell biostimulation by laser or light and the influence of LLLT on implant–tissue interaction. In recent experimental animal studies, we have shown that LLLT enhances the functional attachment of titanium implants to bone and promotes bone healing and mineralization [Khadra et al. 2004a, 2004b]. Cellular reactions at the interface between host and biomaterial are the major determinants of clinical success of osseointegrated implants. In the search for the optimal implant–tissue interaction, the effect of laser therapy on these cells is an important field of investigation.

The purpose of the present study was therefore to investigate the effect of low level laser irradiation on attachment and proliferation of human gingival fibroblasts cultured on titanium implant material.

Material and methods

Implants

Test implant discs measuring 10 mm in diameter and 1 mm in thickness were made from sheets of grade 4 commercially pure titanium. The surfaces of all titanium discs were standardized by polishing with Chem cloth and colloidal silica paste with a grain size of 0.04 μm oxide polishing suspension [OP-S] according to Struers® Metalog Guide (Struels A/S, Ballerup, Denmark). The implants were mirror polished for 1 h.

Prior to use, the discs were treated with trichloroethylene in an ultrasonic bath for 15 min, rinsed with 100% ethanol and then treated with 100% ethanol in an ultrasonic bath for 3 × 10 min. The samples were thereafter autoclaved.

Surface characterization

The surface topography of five titanium samples was characterized qualitatively and quantitatively using a scanning electron microscope [SEM, Philips XL30 ESEM, Eindhoven, Holland] and a confocal laser scanning microscope [Leica TCS 4D, Heidelberg, Germany]. For numerical characterization, the parameters evaluated were the average height deviation value (S₅), the maximum peak-to-valley roughness (S₉) and the developed surface area ratio (S₈). Each sample was measured at three sites. The field area measured was 1000 × 1000 μm. Before use, the titanium surfaces were also examined by energy-dispersive X-ray microanalysis [EDX].

Laser treatment

A photon-plus, gallium–aluminum–arsenide (GaAlAs) diode laser device (Renvig Dental AS, Daugawa, Denmark), was used in this study. This system operates in the near-infrared spectrum at a continuous wavelength of 830 nm and a power output of 84 mw. To ensure uniform exposure of the whole culture dish (35 mm diameter Petri dish, NUNC, Roskilde, Denmark), the beam was specially expanded. The distance from the probe to the cell layer was 9 cm. The culture dish was placed in a standardized position in a milled hollow in anodized metal. Each titanium disc was placed in the center of the culture dish and laser irradiated at dosages of 1.5 or 3 J/cm²: this laser dose has been reported to stimulate fibroblast numbers in vitro. The control dishes were maintained under a laminar flow hood for a period of time corresponding to those of experimental groups, but were not irradiated.

Cell culture

Human gingival fibroblasts [HGF] were grown from biopsies obtained during oral surgery, cultured and maintained in plastic culture flasks [NUNC] according to the technique described by Liu et al. [1991]. The cells were cultured in Dulbecco’s modified Eagle’s medium [DMEM, Sigma, Linz, Austria], supplemented with 5% fetal calf serum [FCS] and penicillin/streptomycin solution. The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Cells for the experiments were harvested between the fourth and eighth passages. For each experiment, cell populations were selected from different subjects, depending on their availability at the time of the scheduled experiments. Exposure to ambient light was avoided as much as possible. The study protocol was approved by the Ethics Committee at Karolinska Institutet, Stockholm.

Cell morphology

Cell morphology on titanium discs and attachment assays at different time-points and different exposure periods were analyzed by a SEM and an automatic image analyzer. The culture medium was removed and the specimens were washed twice with phosphate buffer solution [PBS], fixed in 1% glutaraldehyde and dehydrated with an ascending ethanol series from 30%, 70%, 80%, 95%, 100%. Critical point drying was carried out in a critical point dryer [Bio-Rad, E3100, Watford, UK] using CO₂ as transitional fluid. The specimens were mounted on aluminium specimen holders and coated with a 30 nm conducting layer of gold/palladium using a sputter coater [Bio-Rad SC510 Watford, UK]. SEM photographs of each titanium sample were obtained both before and after incubation with HGF.

Attachment assay

Fibroblasts were released from the culture dishes using 0.025 trypsin in PBS contain-
ing 0.02% EDTA. The cells were washed three times in PBS, centrifuged, resuspended, and then plated in three 35 mm diameter Petri dishes. Two dishes were subjected to laser exposure with 1.5 and 3 J/cm², respectively; the third served as a control. Following irradiation, the cells were resuspended in growth medium and plated at a density of 2 x 10⁴ cells/cm² on 45 titanium discs which were placed on the bottom of 24-multiwell dishes. The titanium discs were divided into three groups. Group I [untreated control] served as a reference for in vitro growth of HGF. Groups II and III received 1.5 and 3 J/cm² laser therapies, respectively. Cells of the control group were not irradiated but were handled in a similar manner as the irradiated ones. Cell attachment was observed after 1, 3 and 24 h. Fifteen titanium discs were observed on each time point. Unattached cells were then pipetted from the surfaces, and washed twice with PBS to collect any unattached cells remaining on the surfaces. The attached cells were then fixed and prepared for SEM.

Cell attachment was analyzed on micrographs taken from randomly selected areas (Mustafa et al. 1998). Thus, the first field of interest was chosen by placing the specimen in a central position, at random, under the microscope. The following consecutive micrographs were then taken in one direction. Hence, 10 micrographs, corresponding to the attachment analysis, were taken for each specimen incubated for 1 and 3 h [final magnification = x 400], and 20 micrographs were taken for each specimen incubated for 24 h [final magnification = x 800]. The cell profile areas were measured using an automatic image analyser. Attachment was calculated by dividing the area of the cell profiles by the area of the total micrograph corresponding to the percentage of attachment.

Growth assays
Thirty titanium discs were placed on the bottom of 24-multiwell dishes and plated with fibroblasts at a density of 500 cells/cm². The titanium discs were randomly divided into three groups [n=10 each]. After 24 h culture, the experimental groups were exposed to laser irradiation using a GaAlAs diode laser for 3 consecutive days, at dosages of 1.5 or 3 J/cm². The remaining 10 samples served as a control. Cells were allowed to grow for 8 and 10 days respectively, with three changes of medium. The titanium discs were then gently washed twice with PBS. Attached fibroblasts were stained with [0.5 mg/ml] Hoechst [Sigma-Aldrich Chemie GmbH, Steinheim, Germany] for 15 min followed by [0.5 mg/ml] propidium iodide [Sigma-Aldrich Chemie GmbH] for another 15 min. The samples were then allowed to dry in the dark (Kelly et al. 2003).

The number of colonies was counted in each of the 10 randomly selected fields from each titanium disc, each colony ≥ 3 cells, using a microscope [Nikon, Eclipse E600, Tokyo, Japan] connected to an image analyser [Soft Imaging System GmbH, Münster, Germany]. All measurements were calculated using a [x 10] magnification objective. The mean colony-forming efficiency (CFE) was calculated as the percentage of colonies divided by the number of seeded cells. For the clonal growth rate (CGR), expressed as mean population doubling per day, PD/D, the number of cells in 10 randomly selected colonies from each titanium disc was determined, and the average of log, of the number of cells per colony was divided by the number of days of incubation.

Cell morphology and attachment
One-hour culture
Fibroblasts were attached on all samples. The cells were round, spreading out radially from the round cell body, which contained the nucleus (Fig. 2).

Cell viability
At the termination of the experiment, viability was assessed by applying the staining technique described above. The uptake of propidium iodide indicates damage to the cell membrane: necrotic cells thus identified were excluded from the cell count.

Statistical analysis
Statistical analysis was performed using SPSS 11 for Windows [SPSS]. Descriptive statistics were used to describe mean and standard deviations. The significance of difference between means of the irradiated and control groups in fibroblasts cultured on titanium was tested by independent t-test. The level of significance was set at $P<0.05$. The data presented are from one of two identical experiments performed in parallel using biopsies from two different donors. Moreover, the experiments were repeated three times.

Results
Surface analyses
SEM examination confirmed that the surfaces of the implant discs were smooth, with no irregularities. Surface analysis by EDX showed that all surfaces were cleaned and consisted of pure titanium. Quantitative analysis by Leica TCS 4D confocal laser microscopy yielded a mean deflection value of ($S_m$) of 0.477 μm, a maximum peak-to-valley roughness ($S_A$) of 14.25 μm and a developed surface area ratio ($S_d$) of 1.01 (Fig. 1).

Cell morphology and attachment
One-hour culture
Fibroblasts were attached on all samples. The cells were round, spreading out radially from the round cell body, which contained the nucleus (Fig. 2).

Cell viability
At the termination of the experiment, viability was assessed by applying the staining technique described above. The uptake of propidium iodide indicates damage to the cell membrane: necrotic cells thus identified were excluded from the cell count.

Statistical analysis
Statistical analysis was performed using SPSS 11 for Windows [SPSS]. Descriptive statistics were used to describe mean and standard deviations. The significance of difference between means of the irradiated and control groups in fibroblasts cultured on titanium was tested by independent t-test. The level of significance was set at $P<0.05$. The data presented are from one of two identical experiments performed in parallel using biopsies from two different donors. Moreover, the experiments were repeated three times.

Results
Surface analyses
SEM examination confirmed that the surfaces of the implant discs were smooth, with no irregularities. Surface analysis by EDX showed that all surfaces were cleaned and consisted of pure titanium. Quantitative analysis by Leica TCS 4D confocal laser microscopy yielded a mean deflection value of ($S_m$) of 0.477 μm, a maximum peak-to-valley roughness ($S_A$) of 14.25 μm and a developed surface area ratio ($S_d$) of 1.01 (Fig. 1).

Cell morphology and attachment
One-hour culture
Fibroblasts were attached on all samples. The cells were round, spreading out radially from the round cell body, which contained the nucleus (Fig. 2).

Cell viability
At the termination of the experiment, viability was assessed by applying the staining technique described above. The uptake of propidium iodide indicates damage to the cell membrane: necrotic cells thus identified were excluded from the cell count.

Statistical analysis
Statistical analysis was performed using SPSS 11 for Windows [SPSS]. Descriptive statistics were used to describe mean and standard deviations. The significance of difference between means of the irradiated and control groups in fibroblasts cultured on titanium was tested by independent t-test. The level of significance was set at $P<0.05$. The data presented are from one of two identical experiments performed in parallel using biopsies from two different donors. Moreover, the experiments were repeated three times.
polarized (Fig. 4, columns on right). At low magnification, SEM micrographs demonstrate more attached cells to the irradiated implant surfaces compared to controls (Fig. 4, columns on left).

The analysis showed higher percentage of cell attachment in the irradiated groups at dosages of 1.5 and 3 J/cm². The difference was statistically significant \( P = 0.002 \) and \( P = 0.013 \), respectively, after 1 h, \( P = 0.003 \) and \( P = 0.013 \), respectively, after 3 h, and \( P = 0.01 \) and \( P = 0.01 \), respectively, after 24 h.

Twenty-four-hour culture

SEM revealed elongated cells, flat and well spread, indicating good attachment on all samples. However, more interspaces could be seen on the control samples, confirming the attachment assay (Fig. 5).

After 24 h of culture, the analysis showed more cells had attached in the irradiated groups at dosages of 1.5 and 3 J/cm². The difference was statistically significant \( P = 0.01 \) and \( P = 0.01 \), respectively, Fig. 3).

Growth assays

At the dosages applied (1.5 and 3 J/cm²), CFE was significantly higher in the irradiated groups than in the control groups after 8 days \( P = 0.017 \) and \( 0.038 \), respectively and after 10 days \( P = 0.036 \) and \( P = 0.013 \), respectively, Fig. 6). CGR was also significantly higher in the irradiated...
respective, and 10 days (P = 0.015 and P = 0.007, respectively), and 10 days (P = 0.001 and P = 0.006, respectively, Fig. 7). However, with respect to both CFE and CGR, the dose-related differences were not statistically significant for either time interval.

**Cell viability**

Cell viability, assessed at the end of the experiments on day 8 and 10, showed no differences between experimental and control groups, exceeding 90% in all cases.

**Discussion**

Attempts to stimulate the rate of gingival regeneration by LLLT have been described in several experimental studies and a few clinical studies (Chomette et al. 1987a, 1987b; Pourreau-Schneider et al. 1989, 1990, 1992; Steinlechner & Dyson 1993; Neiburger 1997, 1999; Almeida-Lopes et al. 2001). One of the critical goals in implant therapy is the achievement of optimal soft tissue integration. The ability of LLLT to promote a favorable-soft tissue healing response around the transmucosal part of an implant is the result of a complicated interaction of a number of cellular functions, regulated by systemic hormones and local factors.

The purpose of the present study was to investigate the effect of low-level laser irradiation on HGF cultured on a relatively smooth titanium implant surfaces. To our knowledge, no such studies have previously been reported.

The main result is that the percentage of attachment of laser-exposed fibroblasts was significantly higher after 1, 3 and 24 h than for the non-exposed cells. Furthermore, the irradiated cells showed significantly higher proliferation rates than the non-irradiated cells. However, the study disclosed no significant differences between the two laser doses, indicating that increasing the dose to the range of 3 J/cm² does not further promote the initial response of HGF. The data clearly showed that application of LLLT stimulated greater spread of HGF over the titanium surface.

Several studies have reported satisfactory tissue healing rates at He–Ne laser exposure levels between 1 and 4 J/cm² (Pourreau-Schneider et al. 1989; Webb et al. 1998, Almeida-Lopes et al. 2001). Laser-enhanced biostimulation has been reported to induce intracellular metabolic changes, resulting in faster cell division, rapid matrix production (increased collagen, myofibroblasts, etc.) and cell movement. However, higher laser energy doses are reported to inhibit cell proliferation (Karu 1989, 1990). The doses used in this study are in the same range as those previously reported to produce biostimulatory responses.

A special expansion of the laser beam was used to ensure uniform exposure of all cells cultured on the titanium discs. The culture dish was placed in a standardized position in a milled hollow in anodized metal. Each titanium disc was placed in the center of 35 mm diameter Petri dish and the laser irradiation was initiated at dosages of 1.5 or 3 J/cm². It should be noted that neither SEM analysis nor the viability test disclosed any apparent damage to the cells by either of the two energy densities used in this study.

The percentages of attached cells on each titanium specimen were determined by SEM and an automatic image analyzer, according to the method previously described by Mustafa et al. (1998), and shown to be appropriate for determining cell attachment in vitro. The significant increase in initial fibroblast attachment supports the hypothesis that the enhanced healing effect is associated primarily with the early, most sensitive stages of the healing process.

**Fig. 5.** Scanning electron micrographs after 24 h of attachment. The cells appeared elongated, flat and well spread, indicating good attachment on all samples. However, more interspaces could be seen on the control samples (C) than on the irradiated samples L1 (1.5 J/cm²) and L2 (3 J/cm²), confirming the attachment assay. Scale bar = 10 μm.

**Fig. 6.** Effect of low-level laser therapy on colony-forming efficiency (CFE) of human gingival fibroblasts cultured on titanium implant material. Each bar represents the mean ± SD. The irradiated groups showed a significantly higher CFE than the control group at dosages of L1 = 1.5 J/cm² and L2 = 3 J/cm² after 8 P = 0.017 and P = 0.036, respectively, and 10 days P = 0.015 and P = 0.013, respectively.

**Fig. 7.** Effect of low-level laser therapy on clonal growth rate (CGR) of human gingival fibroblasts cultured on titanium implant material. Each bar represents the mean ± SD. The CGR was significantly higher in the irradiated groups than in the control group at dosages of L1 = 1.5 J/cm² and L2 = 3 J/cm² after 8 P = 0.013 and P = 0.007, respectively, and 10 days P = 0.001 and P = 0.006, respectively.
After initial attachment, cells spread, proliferate and subsequently form colonies. Therefore, measurements of proliferation rates and colony numbers are useful for evaluating fibroblast proliferation. Kreisler et al. [2003] clearly revealed considerably higher proliferation activity 24 h after laser irradiation, decreasing in an energy-dependent manner over 48 and 72 h, indicating that repeated laser applications are necessary to achieve a positive effect on proliferation. On this basis, in the present study HGF grown on the titanium specimens were lased for 3 consecutive days (24, 48 and 72 h). Both CFE and CGR were significantly higher for the irradiated cells than for the controls.

The greater attachment of fibroblasts in response to laser exposure is in agreement with the results reported by Boulton & Marshall [1986] who investigated the effects of He–Ne laser and monochromatic incoherent light on the attachment and proliferation of human skin fibroblasts and human embryonic foreskin fibroblast cultures. They demonstrated that laser-irradiated cultures exhibited a significant increase in the number of cells grown on plastic substrate in comparison with their respective non-irradiated controls after 24 and 48 h. The differences in cell numbers between irradiated and control cultures in the experiments with the incoherent source were, however, not significant. Bosrat et al. [1984] demonstrated that laser irradiation with 2 J/cm² could produce a remarkable accumulation of fibrillar material in the outer part of the fibroblast cytoplasm. These findings are supported by Karu et al. [1996], who evaluated the adhesion of HeLa cells to glass after irradiation with monochromatic low-intensity light or laser irradiation. They showed increased cell–cell and cell–glass adhesion following laser irradiation. It has been suggested that the intensity of ion fluxes through the cell membrane depends on the intensity of the signal received from the photoacceptor, which in turn depends on the primary reactions occurring in-with photoreceptor during irradiation with light at different wavelengths.

However, conflicting results have also been reported for cellular laser therapy. Some discrepancies may be attributable to differences in the source and species of cells [Wiegand-Steubing et al. 1996], the wave-length [Karu et al. 1984], energy density [Karu & Pyatibrat 1987] and exposure time [van Breugel & Bär 1992].

Several studies have reported that laser therapy stimulates the release of specific growth factors from fibroblasts [Anders et al. 2002]. Yu et al. [1994] demonstrated that fibroblast production of basic fibroblast growth factor (bFGF) can be enhanced by laser irradiation with 2.16 J/cm² at a wavelength of 660 nm and concluded that stimulation of fibroblast proliferation by laser irradiation may be associated with the autocrine production of bFGF from fibroblasts. However, at a dose of 3.24 J/cm², there was no increase in cellular proliferation or production of bFGF.

A stable connection between the titanium surface and surrounding tissue is an important determinant of clinical implant success. Our in vitro data indicate that laser therapy enhances the initial attachment, spreading and proliferation of HGF. Extrapolated to in vivo conditions, these results suggest that LLLT could strengthen and broaden fibroblast attachment to titanium, reduce healing time and accelerate soft tissue–implant interactions.

It is concluded that in vitro, LLLT has beneficial effects on the primary step in establishment of the implant–soft tissue interface. Further study is warranted of the influence of LLLT on proliferation and production of beneficial growth factors of HGF.

Acknowledgements: The authors would like to thank Mr Steinar Stolen for technical assistance with the SEM and Ms Solaf Khadra for excellent laboratory assistance.

Résumé
Le but de l'étude présente a été d'analyser l'effet du traitement par laser à bas niveau (LLLT) sur l'atteinte et la prolifération des fibroblastes gingivales humains (HGF) mis en culture sur du matériel implantaire en titane. HGF ont été exposés à des lasers d'iode GaAlAs à des doses de 1,5 ou 3 J/cm² et ensuite mis en culture sur des disques de titane commercialement purs. Des aires de profils cellulaires ont été mesurées après une, trois et 24 h en utilisant le MEB et l'analyse d'image automatique. Les résultats ont été exprimés en pourcentage d'attache. Afin d'analyser l'effet du LLLT sur la poussée cellulaire après huit et dix jours, HGF ont été mis en culture sur des disques en titane pour 24 h et ensuite exposés à l'irradiation par laser trois jours consécutifs. L'efficacité à former des colonies (CFE) et les taux de croissance de clones (CGR) ont été mesurés. La viabilité cellulaire a été déterminée par la coloration iodure de prodidium et Hoechst. Des cultures sans utilisation du laser ont servi de contrôles. Morphologiquement les cellules s'étaient bien sur toutes les surfaces en titane indiquant une bonne attache des cellules tant irradiées que non-irradiées. Des fibroblastes exposés à l'irradiation laser avaient significativement des pourcentages plus élevés d'attache cellulaire que les cellules non-exposées (P<0.05). Les taux de CFE et de CGR augmentaient également pour les cellules irradiées (P<0.05). La vitalité cellulaire était importante (>90%) dans les groupes irradiés et contrôles sans aucune différence significative. Le LLLT in vitro augmente l'attache et la prolifération des HGF sur le matériel implantaire en titane.

Zusammenfassung
Laser Therapie beeinflusst die initiale Anhaftung und die darauf folgende Proliferation von humanen oralen Fibroblasten, welche auf Implantatmaterial aus Titan gezüchtet wurden. Eine rasterelektronenmikroskopische und histomorphometrische Analyse
Das Ziel dieser Studie war, den Einfluss der Low Level Laser Therapie (LLLT) auf die Anhaftung und Proliferation von humanen gingivalen Fibroblasten (HGF), welche auf Titan Implantatmaterial gezüchtet wurden, zu untersuchen. HGF wurden einem GaAlAs Diodenlaser mit einer Dosis von 1,5 oder 3 J/cm² ausgesetzt und dann auf im Handel erhältlichen Titanimplantaten gezüchtet. Die Zellproliferation wurde nach 1, 3 und 24 Stunden mit einem Rasterelektronenmikroskop und einer automatischen Bildanalyse ausgemessen. Die Resultate wurden als Prozent der Anhaftung ausgedrückt. Um den Effekt der LLLT auf das Zellwachstum nach 8 und 10 Tagen zu untersuchen, wurden HGF auf Titanimplantaten während 24 Stunden gezüchtet und dann an den drei darauf folgenden Tagen der Laserbestrahlung ausgesetzt. Es wurden die Koloniebildungseffizienz [CFE] und die klonalen Wachstumsraten (CGR) gemessen. Die Zelllebensfähigkeit wurde durch die Hoechst und Prodidumiodyl Färbung bestimmt. Als Kontrolle dienten nicht mit Laser bestrahlte Kulturen. Morphologisch breiteten sie sich gut auf den Titanoberflächen aus, was eine gute Anhaftung sowohl für die bestrahlten als auch für die unbehandelten Zellen bedeutete. Fibroblasten, welche der Laser Bestrahlung ausgesetzt waren, zeigten höhere Prozentwerte bezüglich Zellanhaltung als die nicht bestrahlten Zellen (P<0.05). Die Koloniebildungseffizienz und die klonalen Wachstumsraten waren bei den bestrahlten Zellen gesteigert (P>0.05). Die Zelllebensfähigkeit war bei der bestrahlten Gruppe und bei der Kontrollgruppe hoch (>90%). Es bestanden keine signifikanten Unterschiede. Es wird die Schlussfolgerung gezogen, dass LLLT in vitro die Anhaftung und die Proliferation von HGF auf Titan Implantatmaterial fördert.
Resumen
La intención del estudio fue investigar el efecto del tratamiento con láser de bajo nivel (LLLT) en la inserción y la proliferación de fibroblastos humanos (HGF) cultivados en material de implantes de titanio. Se expusieron HGF a láser de diodo GaAlAs en dosis de 1.5 o 3 J/cm² y posteriormente cultivados en ciscos de titanio comercialmente puro. Se midieron los perfiles de las áreas de células tras 1, 3 y 24 h, usando microscopía electrónica de barrido y un analizador de imágenes automático. Los resultados se expresaron en porcentajes de inserción. En orden a investigar el efecto de LLLT en el crecimiento celular tras ocho y diez días, se cultivaron HGF en discos de titanio durante 24 h y luego se expusieron a irradiación láser durante tres días consecutivos. Se midieron la eficiencia en formar colonias (CEF) y los índices de crecimiento clonal (CGR). Se determinó la viabilidad celular por medio de tinción de Hoechst y Prodidium iodide. Cultivos no expuestos al láser sirvieron de control. Morfológicamente, las células se expendieron bien en todas las superficies de titanio, indicando una buena inserción tanto las células irradiadas como las no irradiadas. Los fibroblastos expuestos a la irradiación láser tuvieron unos mayores porcentajes de inserción celular que las células no expuestas (P<0.05). La eficiencia en la formación de colonias y los índices de crecimiento clonal fueron también realizados en las células irradiadas (P<0.05). La viabilidad celular fue alta (>90%) en los grupos irradiados y de control, sin diferencias significativas. Se concluye que en vitro el LLLT realiza la inserción y la proliferación de HGF en material de implante de titanio.

Referencias
Almeida-Lopes, L., Rigau, J., Zangaro, R., Guidugli-Neto, J. & Jaeger, M.M. (2001) Comparison of the low level laser therapy effects on HGF and Prodidium iodide. Cultivos no expuestos al láser sirvieron de control. Morfológicamente, las células irradiadas como las no irradiadas. Los fibroblastos expuestos a la irradiación láser tuvieron unos mayores porcentajes de inserción celular que las células no expuestas (P<0.05). La viabilidad celular fue alta (>90%) en los grupos irradiados y de control, sin diferencias significativas. Se concluye que en vitro el LLLT realiza la inserción y la proliferación de HGF en material de implante de titanio.


