Osteoblast Attachment on Titanium Disks After Laser Irradiation

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***Purpose**: Osteoblast attachment on titanium surfaces is necessary to achieve new bone formation and osseointegration. The purpose of this study was to examine osteoblast attachment on irradiated titanium disks. **Materials and Methods:** Machined, hydroxyapatite (HA)-coated, sandblasted, and titanium plasma-sprayed (TPS) surfaces were irradiated with either a carbon dioxide (CO_2) or an Er,Cr:YSGG laser. A control group of nonirradiated disks was also examined. Osteoblast cultures were cultivated on the titanium disks and examined with scanning electron microscopy. **Results:** The findings demonstrated that osteoblasts could be grown on all of the surfaces. Pseudopodia and a spread of cells that demonstrated maturation were observed on the lased irradiated titanium disks. **Conclusions:** The data show that laser irradiation of titanium surfaces may promote osteoblast attachment and further bone formation.* (Basic Science) Int J Oral Maxillofac Implants 2006;21:232–236

Key words: *laser irradiation, osteoblast attachment*

Osseointegrated implants have demonstrated a high success rate after a period of more than 10 years.[1–3] Peri-implant bony complications such as peri-implantitis may lead to implant failure if no treatment can be established. Currently, there are no standard treatment protocols to control peri-implant infections, and the long-term results of peri-implant treatment must be critically assessed.[4] Some articles have presented positive results on the use of laser irradiation to control peri-implant infection; lasers may reduce the bacterial accumulation and affect implant surface decontamination.[5–9] Moreover, previous in vitro microbiologic studies have shown a significant reduction of the periodontopathogenic bacteria *(Porphyromonas gingivalis)* on implant surfaces irradiated with different hard (surgical) lasers[8,9] or with soft lasers using photosensitizers.[10]

Various studies have documented the capacity for the laser wavelength and the laser parameters used to affect an implant's surface.[5,10–15] In addition, laser characteristics are important because of the different reactions they can produce on the implant surfaces. Specifically, continuous-wave carbon-dioxide (CO_2) lasers do not appear to exert adverse effects on the surface chemistry. In contrast, superpulse mode seems to have a significant influence on the surface chemistry, which is not desirable for decontamination of failing implants.[16]

In vivo histologic studies in dogs have shown new bone formation when failing implants are irradiated to decontaminate the implant surface using the CO_2 laser.[17] New bone was observed in close contact with the titanium surface. This means that the laser irradiation of implant surfaces may allow "reosseointegration." In addition, clinical case series have presented long-term results in the treatment of peri-implantitis in cases where the implant surface was irradiated with special laser wavelengths and the bone defect was filled with autogenous or xenogenic bone substitutes.[18–20]

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Cell culture experiments have become more attractive in recent years in an attempt to understand, control, and direct interfacial interactions at biomaterial surfaces. In particular, cultures of osteoblasts, either primary or from tumor lines, are frequently used to evaluate the effect of surface modifications on cell behavior and metabolism. The aim of this study was to examine the attachment of osteoblasts on titanium surfaces after laser irradiation using scanning electron microscopic analysis.

MATERIALS AND METHODS

Titanium Disks

Four different types of titanium disks, 1.5 cm in diameter and 2.0 mm thick, were used: machined, hydroxyapatite (HA)-coated, sandblasted, and titanium plasma-sprayed (TPS). The disks were divided into 3 groups based on their surface pattern and the laser used. All disks were autoclaved before use in the present study. Group 1 was irradiated using the CO_2 laser (10,600 nm, SmartOffice Plus; DEKA, Florence, Italy); group 2, with the Er,Cr:YSGG (2,780 nm, Millennium Waterlase; Biolase, Santa Clemente, CA); and group 3 was nonirradiated as a control.

Laser Irradiation

The CO_2 laser was used with a spot size of 1.5 mm. The power output set used in this experiment varied between 4 and 6 W, with a frequency of 20 Hz and a duty cycle of 6%.

The Er,Cr:YSGG laser was used with a power of 1.25 W, air 42 and water 41.

Cell Adhesion Experiments

The human osteosarcoma cell line SaOS-2 was used for the cell adhesion experiments; it is an immortalized cell line with an osteoblastic phenotype. The experimental cell culture medium (Sigma-Aldrich, Milan, Italy) consisted of McCOYS 5A Medium modified without L-glutamine, 10% fetal bovine serum, streptomycin (100 µg/L), penicillin (100 U/mL), 2.5 g/mL amphotericin B, and 2 mmol/L L-glutamine in a 250-mL plastic culture flask (Corning, Acton, MA). Cells were cultured at 37°C in a humidified incubator equilibrated with 5% CO_2. Cells were harvested prior to confluence by means of a sterile trypsin-EDTA solution (0.5 g/L trypsin, 0.2 g/L EDTA in normal phosphate-buffered saline [PBS], pH 7.4), resuspended in the experimental cell culture medium and diluted to 5×10^5 cells/mL.

For experiments, 5 mL of the cell suspension was seeded into 6-well tissue culture polystyrene plates (9.6 cm^2 of growth area; Falcon; Becton & Dickinson, Franklin Lakes, NJ) containing the samples. After 3 days, the samples were carefully rinsed with PBS and fixed in a 5% glutaraldehyde-PBS. Samples were dehydrated using increasing concentrations of ethanol in water-ethanol solutions up to 100% ethanol. The final dehydration step was performed with hexamethyldisilazane (HMDS, Sigma-Aldrich). Dehydrated samples were gold sputter-coated (AGAR Auto Sputter-Coater; Agar Scientific, Stansted, UK) and observed with a scanning electron microscope (LEO 420; Leica, Cambridge, UK) (SEM).

SEM Analysis

After irradiation the disks were mounted on a sample holder for SEM. All samples, irradiated and control, were introduced into the vacuum chamber of the SEM and photographed at 5 magnification levels (×50, ×200, ×1,000, ×2,500, ×5,000) (Figs 1 through 4). Control and irradiated surfaces were compared.

RESULTS

Machined Surfaces

In the machined disk group, the control (nonirradiated) area presented low cellular density (Fig 1a). The cell morphology present on machined surfaces was typically flat. The lased disk surfaces (both those lased by CO_2 and those lased by Er,Cr:YSGG) presented a higher cellular density than in the control area. This was probably the result of the cleaner effect of the laser on superficial layers (Figs 1b and 1c).

HA-coated Surfaces

In the group of HA-coated disks, a proliferation of osteoblasts was present along the surface (Fig 2a). Both the HA-coated disks lased by the CO_2 laser and those lased by the Er,Cr:YSGG laser presented a spread of osteoblasts with good cellular maturation globular form and pseudopodia (Figs 2b and 2c).

Sandblasted Surfaces

In the control group of sandblasted-surface disks, the osteoblasts were spread over the surface (Fig 3a). The test group surfaces (those irradiated by the CO_2 or Er,Cr:YSGG lasers) presented a spread of osteoblasts with good cell maturation in pseudopodia and globular form (Figs 3b and 3c).

TPS-coated Surfaces

On the surfaces of the TPS-coated control disks, some spreading of osteoblast cells was present (Fig 4a). On the test TPS-coated surfaces, a spread of osteoblasts with good cell maturation in pseudopodia and globular form was found (Figs 4b and 4c).

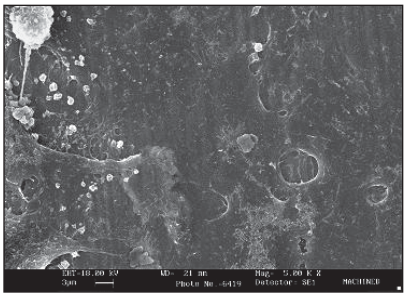

Fig 1a The cell morphology present on machined nonirradiated surfaces (control) was typically flat, and the cell number was low (original magnification ×5,000).

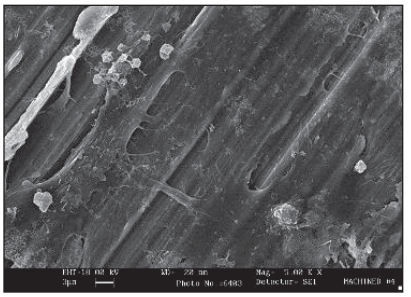

Fig 1b Machined titanium surfaces lased by CO_2 laser presented a higher cellular density in comparison to the control group. However, the cell morphology present on machined surfaces was typically flat (original magnification ×5,000).

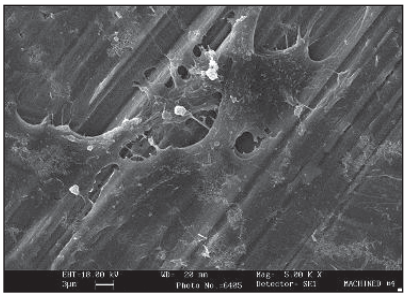

Fig 1c Machined surfaces lased by Er,Cr:YSGG laser presented a spreading of the cells similar to the CO_2 laser–irradiated group (original magnification ×5,000).

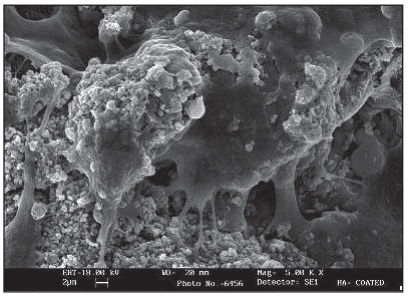

Fig 2a HA-coated disks presented a proliferation of osteoblasts along the titanium surface (original magnification ×5,000).

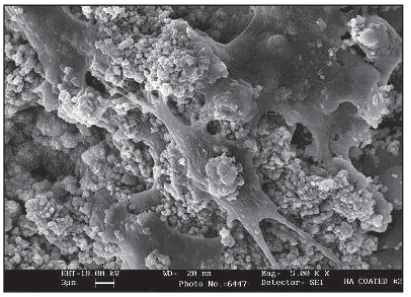

Fig 2b HA-coated disks lased by CO_2 laser presented a spread of osteoblasts with good cellular maturation (original magnification ×5,000).

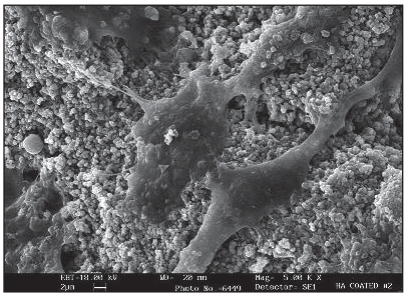

Fig 2c HA-coated disks lased by Er,Cr:YSGG laser presented a spread of osteoblasts with good cellular maturation (original magnification ×5,000).

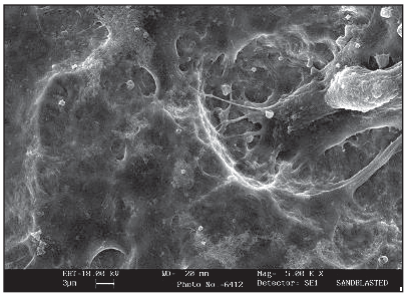

Fig 3a In the sandblasted nonirradiated (control) surfaces, osteoblasts were spread over the surfaces (original magnification ×5,000).

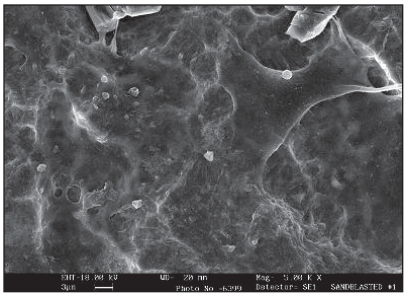

Fig 3b Sandblasted disks lased by CO_2 laser presented a spread of osteoblasts with good cell maturation (original magnification ×5,000).

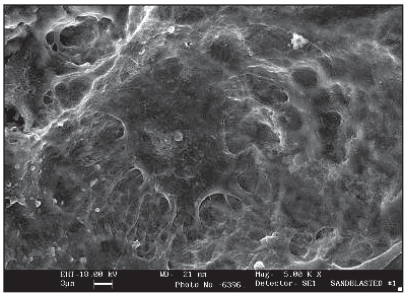

Fig 3c Sandblasted disks lased by Er,Cr:YSGG laser presented a spread of osteoblasts with good cell maturation (original magnification ×5,000).

Summary

In summary, all examined titanium surfaces were colonized well by osteoblasts. The cell morphology was similar for both the control and test groups. The cellular density of the test group was similar to that of the control group. The exact number of attached cells was not calculated.

However, in the machined group, the cellular density was higher in the laser-irradiated than in the nonirradiated specimens, probably because of the cleaner effect on superficial layers by the lasers. The lubricating fluids used in machined tools present on surfaces of this type of disk prevent cell adhesion and spreading on surfaces. It is possible that laser light eliminates these fluids and facilitates cell adhesion. Removal of organic contaminants from machined surfaces is very important for surface biocompatibility, since the organic spores may prevent cell spreading. The cell morphology presented on machined surfaces was typically flat. The other 3 disk surface types presented cells with pseudopodia, which is a feature of cell maturation.

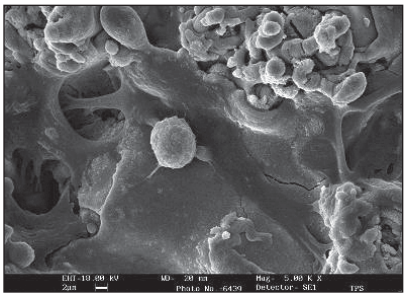

Fig 4a In the TPS nonirradiated surfaces (control group), the osteoblasts were spread over the surfaces (original magnification ×5,000).

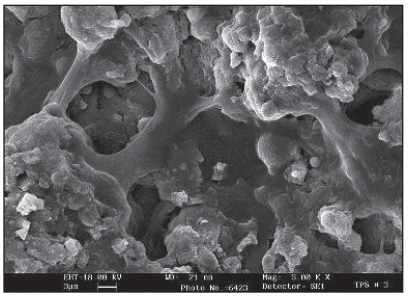

Fig 4b TPS-coated disks lased by CO_2 laser presented a spread of osteoblasts with good cell maturation (original magnification ×5,000).

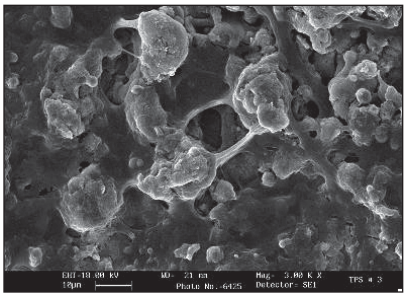

Fig 4c TPS-coated disks lased by Er,Cr:YSGG laser presented a spread of osteoblasts with good cell maturation (original magnification ×5,000).

DISCUSSION

Soft tissue cells, bacteria, and bacterial by-products may be attached in the micro-irregularities of the implant surfaces and can enhance bone resorption. Moreover, insufficient implant decontamination may compromise wound healing processes after treatment of peri-implantitis. Air-spray instrumentation alone or in combination with citric acid[21] may be effective for treatment of peri-implantitis. The risk of air emphysema[22,23] or insufficient reosseointegration with these techniques has been discussed.[21,24]

Laser application is not generally associated with such complications and may enhance reosseointegration according to histologic studies.[17] The present study showed that osteoblasts may grow on titanium surfaces with different patterns after CO_2 or Er,Cr:YSGG laser irradiation. SEM analysis demonstrated the formation of filipodia, representing cell maturation. Because of this cellular attachment on titanium disks, it may be possible to generate new bone formation, when implants are decontaminated in cases of peri-implant infection with additional bone loss. Because the laser wavelengths used in this study do not change the titanium surface but reduce significantly the pathogenic bacteria, they can be used to treat failing implants in association with augmentative procedures. Further studies should examine the activity of the osteoblasts dependent on the surface pattern and the laser wavelength to confirm the process of maturation and better explain the bone formation.

Based on SEM studies it has been demonstrated that the CO_2 laser does not change the implant surface, independent of the type of implant surface pattern (sandblasted, HA-, or TPS-coated).[13,25] Diode lasers with the specific wavelength of 980 nm appear to have no effect on lased implant surfaces, even if the power setting is high (10 W). In contrast to these effects, the Nd:YAG laser can be associated with dramatic changes of the implant surface, such as melting, crater formation, and cracks on different titanium surfaces.[5,12,13,18]

The physical properties of the CO_2 laser and the surgical effects of this wavelength allow soft tissue removal in peri-implant areas. Furthermore, the diode (980 nm) laser may be applied for such indications without concern for the implant surface. Moreover, many studies have shown temperature changes during laser implant irradiation.[26,27] The temperature change during laser irradiation does not seem to be significantly dependent on the laser mode used. In addition, the low-power CO_2 laser (2- to 4-W continuous-wave or 6-W pulse mode at a frequency of 20 Hz and width of 10 ms) may induce only small temperature changes.[28]

In vivo, corresponding histologic observations of 4-month sections showed evidence of new direct bone-to-implant contact after CO_2 laser-assisted therapy, especially when the implants with TPS coating were treated concomitantly with submerged membranes (guided bone regeneration technique). These results support previous findings that peri-implant defects can be treated successfully by CO_2 laser decontamination without damaging the surrounding tissues in the dog model.[17] Reosseointegration after treatment of peri-implantitis seems to be dependent on the surface pattern. However, Persson and colleagues[29] were able to treat peri-implant bony defects in dogs around implants with turned and sandblasted, large-grit, acid-etched surfaces after local surgical debridement therapy using cotton pellets soaked in saline solution and systemic antibiotic treatment with amoxicillin and metronidazole for 17 days. The bone fill was similar (72% versus 76%), but the amount of reosseointegration was 22% at the sites of the turned implants and 84% at the sites of the roughened implants.

The fact that possible laser effects of surface chemistry were not studied is a shortcoming of the present study. Also, the number of cells attached to each surface was not quantified, which suggests that the results were somewhat subjective.

CONCLUSIONS

The data of the present study showed that laser irradiation of titanium surfaces did not negatively influence osteoblast attachment. These findings may help to explain the effect of laser irradiation on implant surfaces and support the possibility of new bone formation after implant irradiation. More research is needed to see how this method of treating ailing implants affects wound healing and the potential for reosseointegration.

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