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Improving the initial biocompatibility of a titanium surface using an Er,Cr:YSGG laser-powered hydrokinetic system

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ARTICLE INFO

Article history:

Received 26 May 2005

Accepted 8 February 2006

Keywords:

Er,Cr:YSGG laser

Titanium

Cell proliferation

Cell spreading

Biocompatibility

ABSTRACT

Objectives. This study was designed to improve the initial biocompatibility of titanium (Ti) using an Er,Cr:YSGG-powered hydrokinetic system.

Methods. The Er,Cr:YSGG-powered hydrokinetic system with different laser energy densities, 125 and 190 J/cm², were applied to the Ti substrate. Human osteosarcoma U2-OS cells were used. The difference in the attached cell number between 3- and 1-days cell culture was calculated and defined as the initial cell proliferation index (CPI). The initial CPI was statistically analyzed using one-way analysis of variance with the factor of applied laser energy density. The surface spreading morphology of the attached cells after 1 day incubation was observed using a field-emission scanning electron microscope.

Results. The Er,Cr:YSGG laser-treated Ti had a higher (1.2–1.3 times) initial CPI ($P < 0.001$) and better cell spreading morphology than the untreated Ti. Treating the Ti with higher Er,Cr:YSGG laser energy did not significantly improve the CPI and cell spreading morphology.

Significance. The initial biocompatibility of the Ti surface could be improved using an Er,Cr:YSGG laser-powered hydrokinetic system.

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1. Introduction

The biocompatibility of implant material in the human body is related to the interaction between the living cells and implant material surface. Not all implant material surfaces have truly long-term biocompatibility, even though many biomaterials have been used for clinical implantation. Therefore, various modification techniques have been considered to increase the surface biocompatibility of titanium (Ti) [1–6] which is one of the most popular implant materials for clinical applications. To the authors' knowl-

edge, a simple but efficient method for implant surface modification is not fully developed, and currently still under investigation.

Recently, a new generation of erbium, chromium: yttrium, scandium, gallium, garnet (Er,Cr:YSGG) lasers uses a combination of laser energy, water, and air to ablate enamel, dentin, bone, and soft tissue [7–12]. The wavelength (2780 nm) of the Er,Cr:YSGG laser has an affinity for water. Ablation is accomplished by hydrokinetic energy that prevents the temperature from rising. Although the Er,Cr:YSGG hydrokinetic laser system is widely used for

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doi:10.1016/j.dental.2006.02.012

dental purposes, its potential application for implant biocompatibility modification is still not available in the literature.

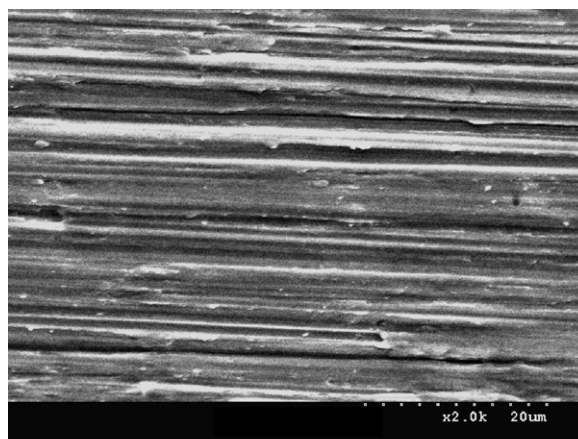
This study proposed a fast and useful surface modification method, i.e. an Er,Cr:YSGG laser-powered hydrokinetic system, for improving the biocompatibility of Ti metal. The initial biocompatibility on the laser-treated Ti surface was evaluated.

2. Materials and methods

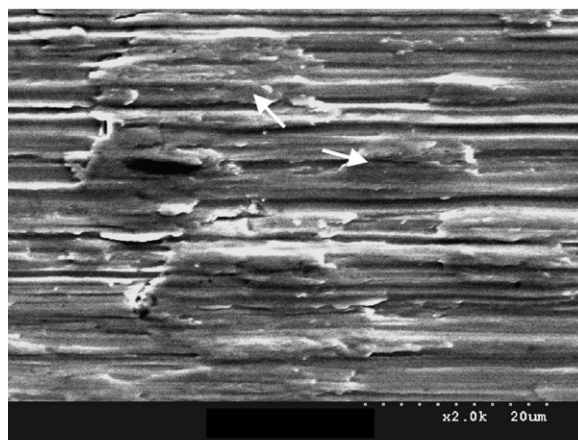
Commercially pure Ti disks were used as the test substrate (\varnothing : 15 mm; thickness: 1 mm). The Ti substrate surface was ground with SiC paper to #1500 and had a surface roughness R_a of around 0.15 μm . According to the authors' previous study [13], the ground #1500 Ti surface ($R_a = 0.15 \mu\text{m}$) exhibits the optimal initial cell adhesion condition. An Er,Cr:YSGG-powered hydrokinetic system was utilized to modify the Ti surface using Waterlase[®] (Biolase Technology, Inc., San Clemente, CA). The Er,Cr:YSGG hydrokinetic laser system was used with a 500- μm laser tip, with the air pressure setting at 45% and the water spray at 20% according to the manufacturer's advice. During the laser treatment, the distance between the laser tip and specimen surface was controlled at a value of about 2 mm. In this study, two different laser energy densities, 125 and 190 J/cm², were applied to the ground Ti substrate. The corresponding specimens were designated as L-125 and L-190. The specimen without Er,Cr:YSGG laser treatment was used as control group and designated as L-0. Surface topography of the tested Ti specimens was observed using an S-3500N scanning electron microscope (SEM) and a Nanoscope III atomic force microscope (AFM).

Human osteosarcoma U2-OS cells (American Type Culture Collection (ATCC) HTB-96) were used to imitate the behavior of osteoblasts because this cell line exhibits the osteoblast phenotype [14]. Complete McCoy's 5A medium was supplied to culture the U2-OS cells with a density of 5×10^4 cell no./cm² on the test specimens in an incubator with a content of 5% CO₂ + 95% air at 37 °C. The trypan blue exclusion method and cell counter [15] were used to calculate the number of attached cells on the specimens after 1 and 3 days of cell culture. The difference in the attached cell number between 3- and 1-days cell culture was defined as the initial cell proliferation index (CPI), statistically analyzed using one-way analysis of variance (ANOVA) for evaluating the applied laser energy density factor. Tukey's test ($\alpha = 0.05$) was chosen as the following multiple-comparison technique when necessary. The specimen number for each test group was five.

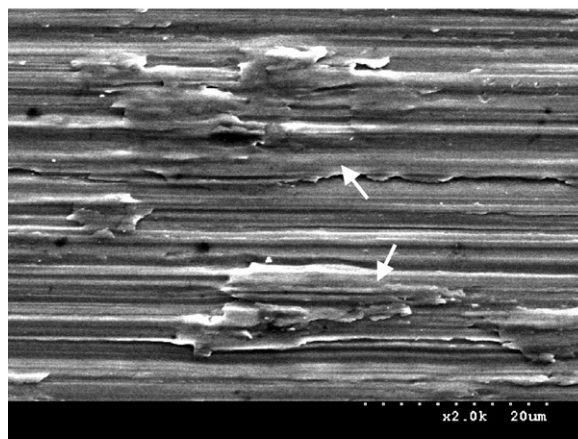
The U2-OS cells were cultured on the Ti test specimens (density: 5×10^4 cell no./cm²) for 1 day. The unattached cells and culture medium were then removed, followed by washing the specimens with phosphate-buffer saline. The cells attached to the Ti test specimens were sequentially fixed and dehydrated. The surface spreading morphology of the attached cells was observed using a JSM-6700F field-emission scanning electron microscope (FE-SEM) after coating the specimens with a thin platinum film.



(a) L-0



(b) L-125



(c) L-190

Fig. 1 – SEM observations of the Ti specimens with and without Er,Cr:YSGG hydrokinetic laser treatment.

3. Results and discussion

Figs. 1 and 2 show the SEM and AFM observations of the Ti specimens with and without Er,Cr:YSGG hydrokinetic laser treatment. The L-0 specimen (without laser treatment) revealed a surface morphology with parallel grooves ascribed to the mechanical grinding procedure during specimen preparation. After laser treatment (specimens L-125 and L-190),

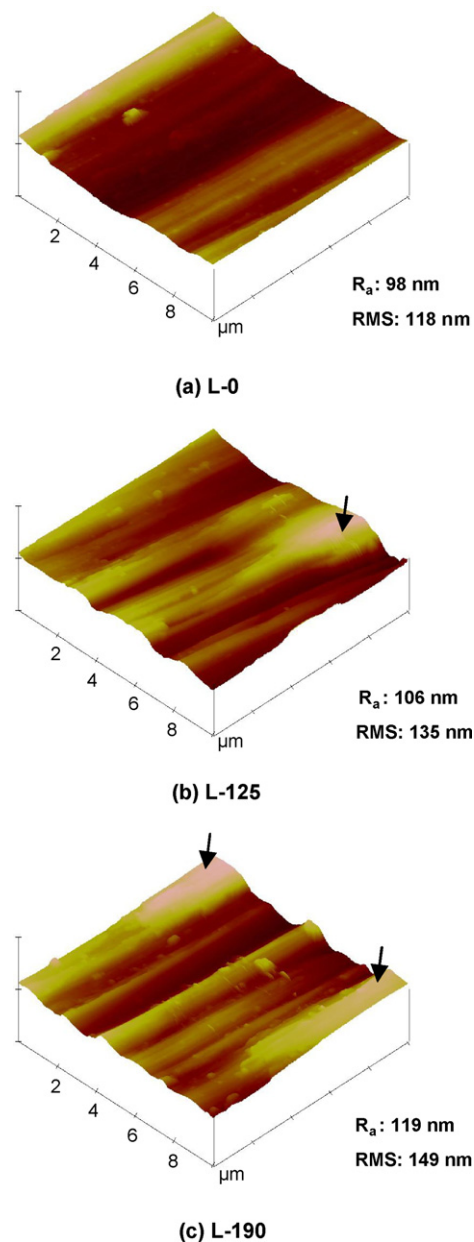


Fig. 2 – AFM observations of the Ti specimens with and without Er,Cr:YSGG hydrokinetic laser treatment.

locally melted morphology was observed on the specimen surface, as indicated by arrows in Figs. 1 and 2. By comparing specimens L-125 and L-190, it showed that increasing the applied energy density from 125 to 190 J/cm² led to a slight increase in both the melted area (Fig. 1) and surface roughness (Fig. 2). When using the Er,Cr:YSGG hydrokinetic laser, the water molecules become energized and propelled by the laser light. A related article reported that the hydrokinetic laser with lower energy density of 5.6 J/cm² can result in a macroscopic etched-like surface on enamel, whereas higher power energy results in visible cavitations [16]. In this study, the laser-energized water with the energy densities used could locally melt the Ti surface.

Table 1 – Initial cell proliferation index, difference in attached cell number between 3- and 1-days cell culture, of Ti specimens with and without Er,Cr:YSGG laser treatment: different letters (A and B) mean significantly different

Materials	Cell proliferation index
L-0	6.58×10^4 (3.51×10^3) A*
L-125	7.94×10^4 (3.65×10^3) B
L-190	8.51×10^4 (3.09×10^3) B

* Standard deviations are given in parentheses.

Table 1 shows the initial CPI, difference in the attached cell number between 3- and 1-days cell culture, for Ti specimens with and without Er,Cr:YSGG hydrokinetic laser treatment. One-way ANOVA results showed that the laser energy density had a statistically significant influence on the initial CPI for the Ti specimen ($P < 0.001$). Furthermore, Tukey's test results showed that there was no significant difference between the L-125 and L-190 specimens, although the L-190 specimen showed a slightly higher CPI than the L-125 specimen. It was obvious that the Er,Cr:YSGG hydrokinetic laser system was a potential candidate for improving the initial biocompatibility, in terms of CPI, of a titanium surface.

According to the authors' recent article [13], the ground #1500 Ti with a R_a of 0.15 μm shows the optimal cell adhesion behavior with respect to either the rougher or smoother specimens. In this study, the laser-treated specimens apparently increased (1.2–1.3 times) the initial CPI ($P < 0.001$) with respect to the ground #1500 Ti specimen. Furthermore, during the Ti surface treatment process via Er,Cr:YSGG hydrokinetic laser system, the total operating period for each specimen was around 90 ± 5 s. Compared with some recent Ti surface modification methods, chemical [17,18] or electrochemical [19–21], the laser-powered hydrokinetic system used in this study produced more rapid Ti surface biocompatibility modification.

Fig. 3 shows the FE-SEM observations of the Ti specimens, with and without Er,Cr:YSGG hydrokinetic laser treatment, after 1 day of cell culture: (a) L-0; (b) L-190; (c) and (d) higher magnifications of (a) and (b), respectively, as indicated by the arrow. As shown in Fig. 3, the attached cell with a flattened membrane was observed on the laser-treated L-190 specimen compared to the untreated L-0 specimen. A similar phenomenon was also observed for the L-125 specimen. When cell adhesion progresses on the implant material surface, a thin membrane develops following the spread of filopodia on the cell. The cell grows flattened onto the material surface, indicating a high quality of cell growth and material biocompatibility [22]. From the initial biocompatibility point of view, the attached cell morphology observation results (Fig. 3) are consistent with the CPI listed in Table 1.

In this study, the improved Ti surface biocompatibility mechanism after Er,Cr:YSGG hydrokinetic laser treatment is still not fully understood. However, the physical and/or chemical variations, including the three dimensional change in the surface topography, on the outermost surface of the laser-treated Ti substrate might play an important role in the initial biocompatibility, and needs further investigation.

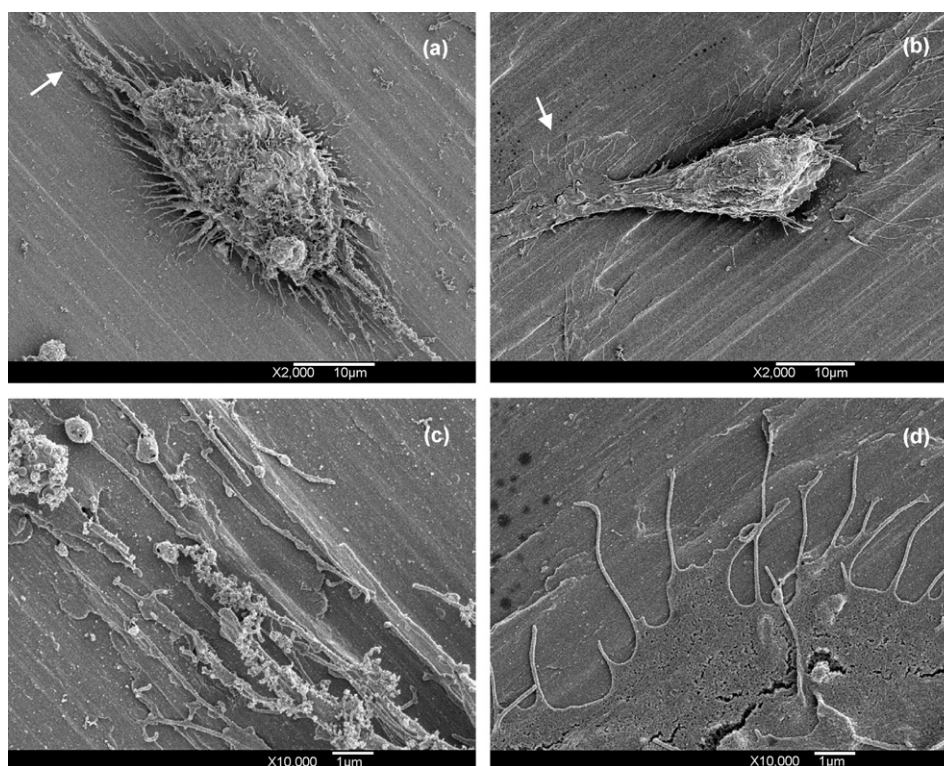


Fig. 3 – FE-SEM observations of the Ti specimens, with and without Er,Cr:YSGG hydrokinetic laser treatment, after 1 day of cell culture: (a) L-0; (b) L-190; (c) and (d) higher magnifications of (a) and (b), respectively, as indicated by the arrow.

4. Conclusions

The initial biocompatibility of a Ti surface, in terms of the CPI and cell adhesion morphology, was improved using a fast and efficient surface modification technique, Er,Cr:YSGG laser-powered hydrokinetic treatment. The laser-treated Ti specimen had a higher (1.2–1.3 times) initial CPI ($P < 0.001$) and better cell spreading morphology. The specimen with higher applied laser energy had somewhat better biocompatibility, but there was no statistical difference between the laser-treated specimens with dissimilar laser energy. For long-term clinical applications, further investigations on improving the cell differentiation behavior using Er,Cr:YSGG hydrokinetic laser treatment is suggested.

Acknowledgement

The authors would like to thank the financial support by National Science Council, Taiwan (grant no. 93-2216-E-040-001).

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