

行政院國家科學委員會專題研究計畫 成果報告

鈦金屬經水雷射處理後之生物相容性研究 研究成果報告(精簡版)

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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 our knowledge, a simple but efficient method for implant surface modification is not well developed, and currently still under investigation.

Recently, a new generation of Erbium, Chromium: Yttrium, Scandium, Gallium, Garnet (Er,Cr:YSGG) laser uses a combination of laser energy, water, and air to ablate enamel, dentin, bone, and soft tissue⁷⁻¹². The wavelength (2780 nm) of the Er,Cr:YSGG laser has an affinity for water. The ablation is accomplished by hydrokinetic energy that prevents the temperature from rising. Although the Er,Cr:YSGG hydrokinetic laser system is widely used for dental purposes, its potential application to implant biocompatibility modification is still not available in the literature.

2. Purpose

This study proposed a fast and useful surface modification method, i.e. 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.

3. Materials and methods

Commercially pure Ti disks were used as the test substrate (ϕ : 15 mm; thickness: 1 mm). The Ti substrate surface was ground with SiC paper to #1500 and had a surface roughness of around 0.15 μm . According to our previous study¹³, the ground #1500 Ti surface (R_a 0.15 μm) 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/cm^2 , 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-3500 N 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 phenotype¹⁴. Complete McCoy's 5A medium was supplied to culture the U-2 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 counter¹⁵ 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 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 U-2 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 onto 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 a thin platinum film onto the specimens.

4. 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 the specimen preparations. After laser treatment (specimens L-125 and L-190), 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 cavitations¹⁶. In this study, the laser-energized water with the energy densities used could locally melt the Ti surface.

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 our recent article¹³, 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 electrochemical¹⁹⁻²¹, 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. Similar phenomenon was also observed for the L-125 specimen. When the cell adhesion progresses on implant material surface, a thin membrane develops following the spread of filopodia on cell. The cell grows flattened onto the material surface, indicating high quality of cell growth and material biocompatibility²². 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 well 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.

5. 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.

6. References

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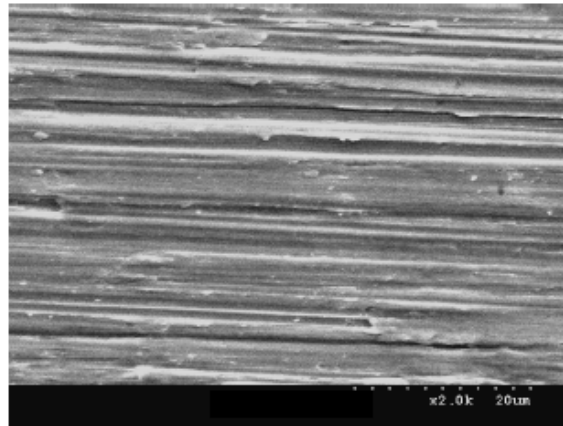
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7. 計劃成果自評

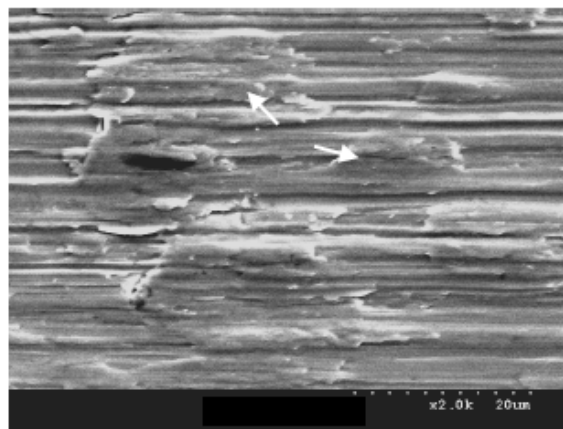
本研究計畫之成果已達預期目標，並有足夠之數據能發表於國際期刊，目前正在進行研究成果之彙整及撰寫論文。

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 letter (A and B) means significantly different; * standard deviations are given in parentheses.

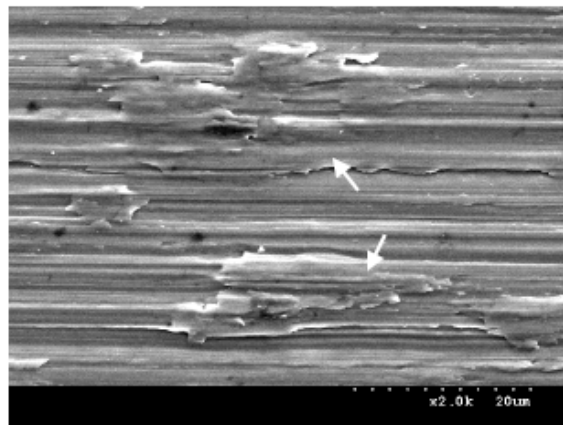
Materials	Cell Proliferation Index
L-0	$6.58 \times 10^4 (3.51 \times 10^3)^{A*}$
L-125	$7.94 \times 10^4 (3.65 \times 10^3)^B$
L-190	$8.51 \times 10^4 (3.09 \times 10^3)^B$



(a) L-0

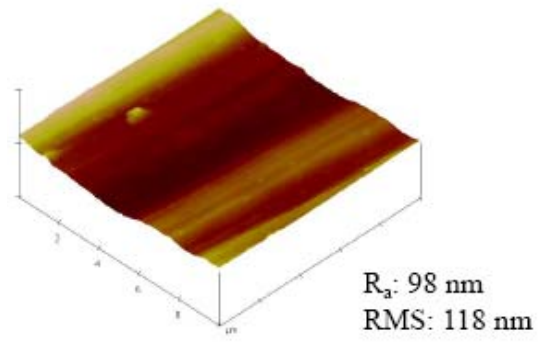


(b) L-125

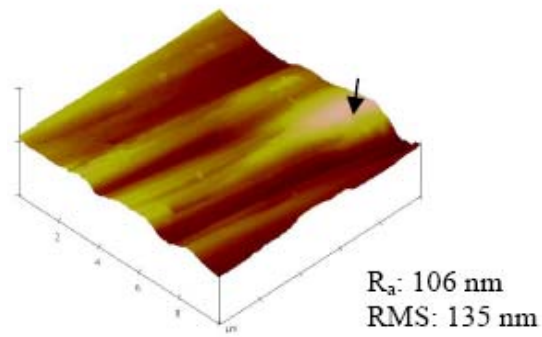


(c) L-190

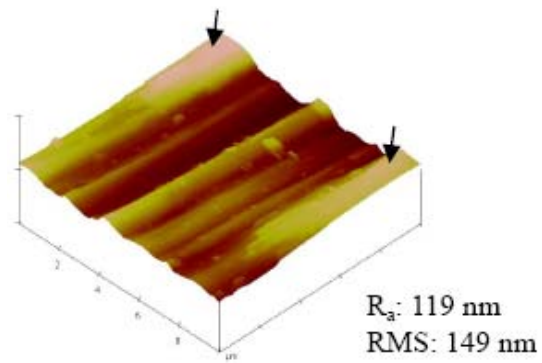
Fig. 1 SEM observations of the Ti specimens with and without Er,Cr:YSGG hydrokinetic laser treatment.



(a) L-0



(b) L-125



(c) L-190

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

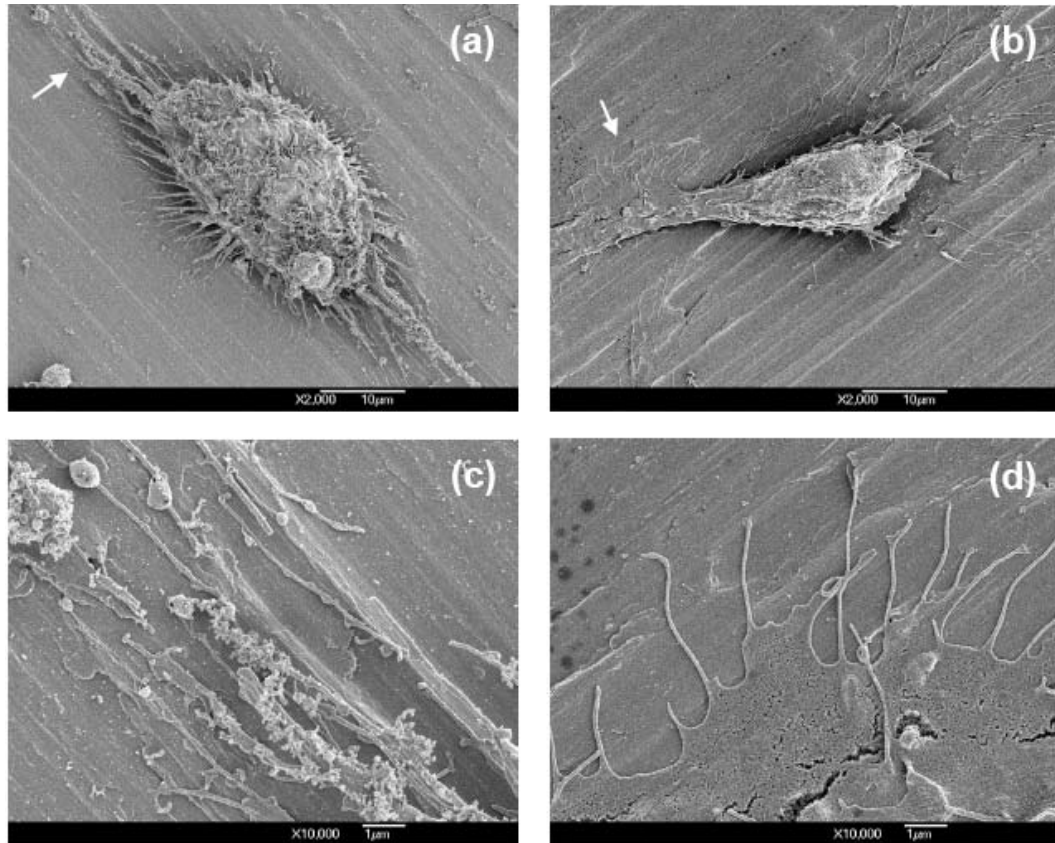


Fig. 3 FE-SEM observations of the Ti specimens, with and without Er,Cr:YSGG hydrokinetic laser treatment, after 1 day of cell culture: (a) L-0; (b) L-190; (c) and (d) higher magnifications of (a) and (b), respectively, as indicated by the arrow.