Variation of Cell Spreading on Titanium Dioxide Film by Periodic Nanostructures Formation with Femtosecond Laser†

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Abstract

Coating of titanium dioxides (TiO_2) film on Ti plate has been conducted to improve biocompatibility of Ti. We have developed coating techniques of the film on Ti plate with an aerosol beam. Controlling cell spreading by periodic structure formation on biomaterials was also a useful method for improving the biocompatibility. In our previous study, the periodic nanostructures were formed on the film by femtosecond laser irradiation at a fundamental wave length (775 nm). Period of the nanostructures was about 230 nm. Cell tests shows cell spreading along the grooves of the nanostructures although it was not done for the film without the nanostructures. Then, influence of the periods of the nanostructures on cell spreading has not been investigated yet. The periods can be varied by changing the laser wavelength. In this study, the nanostructures with the periods of 130 and 230 nm were produced on the film by using the femtosecond laser at the wavelengths of 388 and 775 nm. After a cell test, cell spreading along the grooves of the nanostructures with the periods of 130 amd 230 nm was observed. These results suggested that cell spreading was controlled by using the nanostructures with the periods of 130 and 230 nm.

KEY WORDS: (Femtosecond Laser), (Titanium dioxide), (Aerosol beam), (Periodic nanostructures), (Cell test)

1. Introduction

Titanium (Ti) is suitable for biomaterials, because of its excellent anti-corrosion and mechanical properties. However, it has problems for long-term use[1]. Thus, adding new function to Ti is required. Recently, coating of the titanium dioxides (TiO_2) film on Ti plate has been proposed to improve biocompatibility of Ti[2]. We have developed coating techniques of the film on Ti plate with an aerosol beam[3]. It is also known that control of cell spreading by periodic structure formation on biomaterials is one of the useful methods to improve biocompatibility[4-7]. If direction of cell spreading of osteoblast was controlled, bone formation might be more enhanced[5]. Thus, new functional biomaterials can be created by control of cell spreading.

Femtosecond laser is one of the useful tools for creating periodic nanostructures on metals[8-11], semiconductors[12-17] and, in transparent materials[18]. Periodic nanostructures are self-organized on the laser focusing spot. The directional grooves of the nanostructures lie perpendicular to the laser electric polarization vector. We have already reported that periodic nanostructures were also formed on TiO_2 films with femtosecond laser whose wavelength is 775 nm [16]. In this case, period of the nanostructures was about 230 nm. Cell tests show that cell spreading was observed along the grooves of the nanostructures[16]. However, cell spreading did not show a definite direction on the bare film surface. The influence of the period on cell spreading has not been investigated yet. We showed that mechanism of the nanostructures formation on the film can be attributed to excitation of the surface plasmon polaritons (SPPs) [17]. Thus, the periods can be varied according to the laser wavelength.

In this study, the periodic nanostructures on the film were produced with the femtosecond lasers at wavelengths of 388 and 775 nm, respectively. After femtosecond laser irradiation, the laser irradiated area

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was observed with a scanning electron microscope (SEM). Periods of the nanostructures on the film were examined. Cell tests were conducted to examine the influence of the period on cell spreading. After a cell test, the direction of cell spreading on the film was observed with a fluorescence microscope.

2. Experimental

TiO_2 film was coated on Ti plate by aerosol beam irradiation. An aerosol beam is produced by mixing the TiO_2 particles and Helium (He) gas. The particles are accelerated by the flow of He gas and carried to the processing chamber. The films are deposited on the substrate when the particles impact with the substrate. Pure Ti plate was used as substrate in this experiment.

Schematic diagrams of femtosecond laser irradiation are shown in **Figs. 1 (a)**. A commercial femtosecond Ti: sapphire laser system was employed in our experiments, which were based on the chirped pulse amplification technique. The wavelength, pulse duration and repetition rate of the femtosecond laser were 775 nm, 150 fs and 1 kHz, respectively. The laser wavelength of 388 nm was obtained with a harmonic generator. The laser beam was focused on the film surface by using a lens and the Gaussian laser beam had a diameter of 60 μm (at the $1/e^2$ intensity points) on the film. The femtosecond laser focusing spot was scanned over the film surface using an XY stage, as shown in **Fig. 1 (b)**. Laser fluences with the wavelengths of 388 and 775 nm were 0.15 and 0.35 J/cm^2, respectively. Scanning speed with the wavelengths of 388 and 775 nm were 1.0 and 2.0 J/cm^2, respectively. Periods of the nanostructures on the film were observed with a SEM.

Cell tests were conducted using osteoblasts on films with and without the periodic nanostructures. The cells and samples were incubated in a humidified atmosphere of 5% CO_2 at 37°C. After incubation for 3 hours, the samples were rinsed with phosphate-buffered saline (PBS) and fixed in 8% paraformaldehyde (PFA). The cells were immunostained to investigate biocompatibility and cell spreading. The nucleus and actin of the cells on the film were observed with a fluorescence microscope and appeared blue and red, respectively. Then, direction of cell spreading was observed with the fluorescence microscope.

3. Results and Discussion

SEM images of TiO_2 film surface after scanning of femtosecond laser focusing spot at wavelengths of 388 and 775 nm are shown in **Figs. 2 (a) and (b)**, respectively. **Fig. 2 (c)** shows SEM image of bare film surface (no laser irradiated area). Periodic nanostructures lying perpendicular to the laser electric polarization vector *E* were formed on the laser irradiated area at the

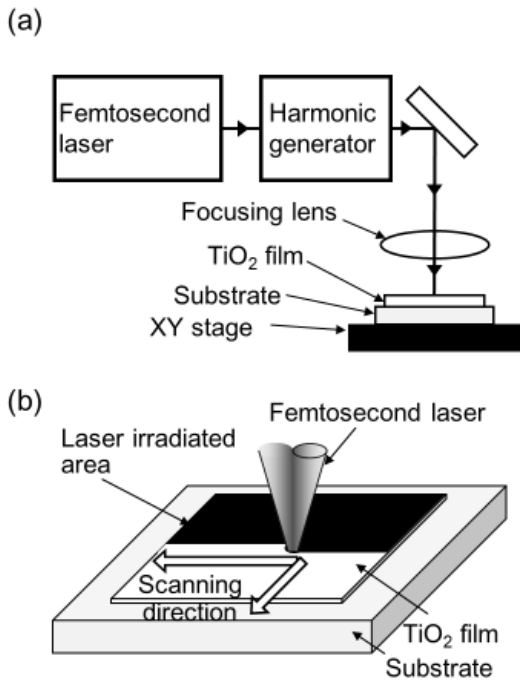

Fig. 1 (a) Schematic diagram of experimental setup for femtosecond laser irradiation. (b) Scanning of the laser focusing spot to produce the periodic nanostructures on the film.

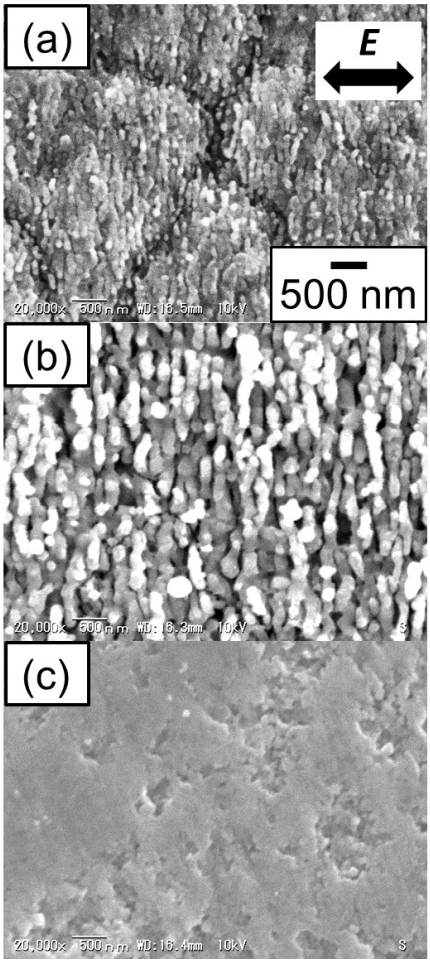

Fig. 2 SEM images of the film surface: (a) laser irradiated area for the wavelength of 388 nm and (b) 775 nm. (c) bare film surface (no laser irradiated area).

wavelengths of 388 and 775 nm although the nanostructures were not formed on the bare film surface as shown in Fig. 2(a), (b) and (c), respectively.

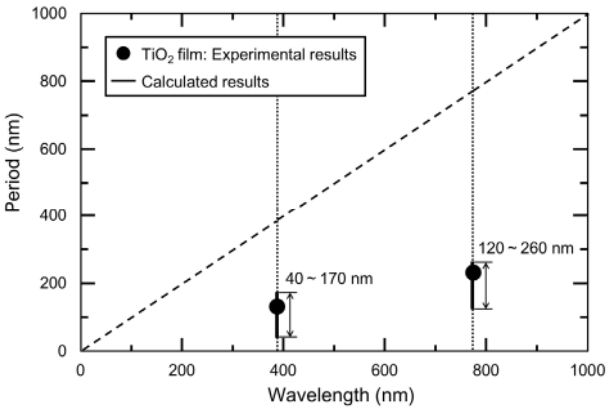

Fig. 3 Variation of periods on the film as a functional of the laser wavelengths.

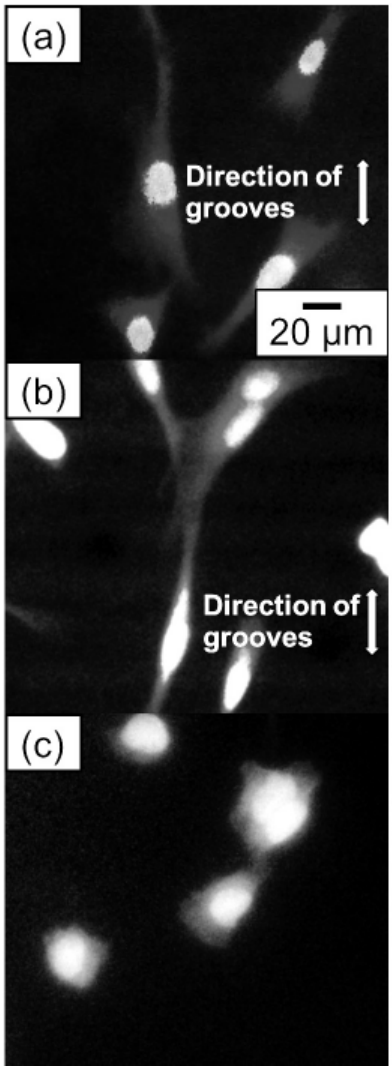

Fig. 4 Fluorescence microscope images of the film surface after cell test: (a) laser irradiated area for the wavelength of 388 nm and (b) 775 nm. (c) bare film surface (no laser irradiated area).

The periodicity of the nanostructures shown in Figs. 2(a) and (b) were examined as a function of the laser wavelengths and the results are shown in **Fig. 3**. Calculated periods by using our SPP model[17] are also shown in Fig. 3. Details of the SPP model are shown in Ref. [17]. The periods of the nanostructures increased from 130 to 230 nm as the laser wavelengths increased from 388 to 775 nm. Calculated periods for the wavelengths of 130 and 230 nm were in the range from 40 to 170 nm and in the range from 120 to 260 nm. These results show that experimental results were in the range of the calculated periods.

After the laser irradiation, a cell test was conducted to investigate the direction of cell spreading on the film with and without the nanostructures. Fluorescence microscope images of the laser irradiated area with the periods of 130 and 230 nm are shown in **Figs. 4 (a) and (b)**, respectively. Fluorescence microscope image of the bare film surface is also shown in **Fig. 4 (c)**. As Figs. 4 (a) and (b) show, cell spreading along the grooves of the periodic nanostructures was observed on the laser irradiated area with the periods of 130 and 230 nm, respectively. However, cell spreading did not show a definite direction on the film without periodic nanostructures as shown in Fig. 4 (c). These results suggested that the nanostructures with the periods of 130 and 230 nm were useful for control of cell spreading.

4. Summary

The periodic nanostructures were formed on TiO_2 film surfaces by scanning of the femtosecond laser focusing spot at 388 and 775 nm. The periods of the nanostructures for the wavelengths of 388 and 775 nm were about 130 and 230 nm, respectively. Cell tests show that cell spreading along the grooves of the nanostructures is observed on laser irradiated areas with the periods of 130 and 230 nm although it is not observed on the bare film surface. These results suggested that cell spreading was controlled by using the nanostructures with the periods of 130 and 230 nm.

Reference

1) T. Hanawa: Biofunctionalization of titanium for dental implant, Jpn. Dent. Sci. Rev. **46**, 2, (2010) 93–101.
2) X. Liu, P. K. Chub and C. Ding: Surface modification of titanium, titanium alloy sand related materials for biomedical applications, Mater. Sci. Eng. Rep., **47**, (2004) 49–121.
3) M. Tsukamoto, T. Fujihara, N. Abe, S. Miyake, M. Katto, T. Nakayama and J. Akedo: Hydroxyapatite coating on titanium plate with an ultrafine particle beam, Jpn. J. Appl. Phys., **42**, 2A, (2003) L120–L122.
4) J. Lu, M. P. Rao, N. C. MacDonald, D. Khang and T. J. Webster: Improved endothelial cell adhesion and proliferation on patterned titanium surfaces with rationally designed, micrometer to nanometer features, Acta Biomater., **4**, 1, (2008) 192–201.a
5) A. Matsugaki, G. Aramoto, and T. Nakano: The alignment

of MC3T3-E1 osteoblasts on steps of slip traces introduced by dislocation motion, Biomaterials, **33**, 30, (2012) 7327–7335.

6) S. Fujita, M. Ohshima, and H. Iwata: Time-lapse observation of cell alignment on nanogrooved patterns, J. R. Soc. Interface, **6**, (2009) S269–S277.
7) K. Matsuzaka, X. F. Walboomers, J. E. de Ruijter and J. A. Jansen: The effect of poly-L-lactic acid with parallel surface micro groove on osteoblast-like cells in vitro, Biomaterials, **20**, 14, (1999) 1293–1301.
8) M. Tsukamoto, K. Asuka, H. Nakano, M. Hashida, M. Katto, N. Abe and M. Fujita: Periodic microstructures produced by femtosecond laser irradiation on titanium plate, Vacuum, **80**, (2006) 1346–1350.
9) M. Tsukamoto, T. Kayahara, H. Nakano, M. Hashida, M. Katto, M. Fujita, M. Tanaka and N. Abe: Microstructures formation on titanium plate by femtosecond laser ablation, J. Phys. Conf. Ser., **59**, (2007) 666–669.
10) K. Okamuro, M. Hashida, Y. Miyasaka, Y. Ikuta, S. Tokita and S. Sakabe: Laser fluence dependence of periodic grating structures formed on metal surfaces under femtosecond laser pulse irradiation, Phys. Rev. B, **82**, 16, (2010) 165417.
11) S. Sakabe, M. Hashida, S. Tokita, S. Namba and K. Okamuro: Mechanism for self-formation of periodic grating structures on a metal surface by a femtosecond laser pulse, Phys. Rev. B, **79**, 3, (2009) 033409.
12) S. K. Das, D. Dufft, A. Rosenfeld, J. Bonse, M. Bock and R. Grunwald: Femtosecond laser induced quasiperiodic nanostructures on TiO_2 surfaces, J. Appl. Phys., **105**, 8, (2009) 084912.
13) D. Dufft, A. Rosenfeld, S. K. Das, R. Grunwald and J. Bonse: Femtosecond laser-induced periodic surface structures revisited: a comparative study on ZnO, J. Appl. Phys., **105**, 3, (2009) 034908.
14) G. Miyaji, K. Miyazaki, K. Zhang, T. Yoshifuji and J. Fujita: Mechanism of femtosecond-laser-induced periodic nanostructure formation on crystalline silicon surface immersed in water, Opt. Express, **20**, 14, (2012)14848–14856.
15) K. Miyazaki and G. Miyaji: Nanograting formation through surface plasmon fields induced by femtosecond laser pulses, J. Appl. Phys., **114**, 15, (2013) 153108.
16) T. Shinonaga, M. Tsukamoto, A. Nagai, K. Yamashita, T. Hanawa, N. Matsushita, G. Xie and N. Abe: Cell spreading on titanium dioxide film formed and modified with aerosol beam and femtosecond laser, Appl. Surf. Sci., **288**, (2014) 649–653.
17) T. Shinonaga, M. Tsukamoto and G. Miyaji: Periodic nanostructures on titanium dioxide film produced using femtosecond laser with wavelengths of 388 nm and 775 nm, Opt. Express., **22**, 12, (2014) 14696–14704.
18) Y. Shimotsuma, P. G. Kazansky, J. Qiu and K. Hirao: Self-organized nanogratings in glass irradiated by ultrashort light pulses, Phys. Rev. Lett., **91**, 24, (2003) 247405.