INFLUENCE OF SUPERHYDROPHILIC TREATMENT TO TITANIUM ON ADSORPTION OF BONE-ASSOCIATED CYTOKINE AND BEHAVIOR OF OSTEOBLAST-LIKE CELLS

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Abstract - The purpose of this study was to investigate the influence of superhydrophilic treatments to titanium on adsorption of bone-associated cytokine and the behavior of osteoblast-like cells. Superhydrophilic titanium specimens were prepared with atmospheric-pressure plasma treatment (Ti-Plasma) and ultraviolet treatment (Ti-UV) and were stored in distilled water for 3 days immediately after these treatments. Specimens stored in air for 3 weeks were used as a control Air group. Adsorption properties of CXCL12 were assessed using a QCM method. Subsequently, initial cell attachment and proliferation of mouse osteoblast-like cells MC3T3-E1 were evaluated. The specific binding of CXCL12 adsorption was increased on the superhydrophilic groups compared with Air (control). Possible mechanisms of increasing the CXCL12 adsorption on the superhydrophilic surfaces were consider due to the increasing the surface energy caused by removal of hydrocarbon and increasing hydrogen bond by relating the hydroxide. Initial cell attachment and proliferation of MC3T3-E1 were enhanced more on superhydrophilic groups than were Air specimens. These results suggested that the superhydrophilic modifications may enhance the adsorption of bone-associated cytokine to titanium implants, leading to initial attachment and proliferation of osteoblast-like cells.

Key words - Dental implants, Titanium, Superhydrophilic modification

I. INTRODUCTION

Surface wettability is an important physicochemical property such as protein adsorption and subsequent cell behavior [1). High surface wettability, which shows high surface energy, is generally reported to promote greater cell adhesion than is low surface energy [2]. Cold plasma and ultraviolet light treatment are commonly suggested methods for leading the high surface wettability, that is, superhydrophilicity.

It is recently reported that CXCL12 (SDF-1: Stromal cell–derived factor 1) - CXCR4 cytokine signaling is expressed during bone healing and osseointegration of implants, and these chemokines play a critical role in homing and migration of mesenchymal stem cells. Superhydrophilic surface modification is expected to enhance the adsorption of the proteins to titanium. Accordingly, the promoting the adsorption of these cytokines against titanium implants is getting important. On one hand, earlier studies confirmed that the greater the hydrophilicity of the surface, the greater the level of early-stage cell adherence, especially on superhydrophilic surfaces [3]. Cells spread much more widely on hydrophilic surfaces than on hydrophobic surfaces.This study aimed to investigate the influence of various superhydrophilic treatments of titanium on the binding behavior of cytokine CXCL12 as well as the behavior of osteoblast-like cells.

II. MATERIALS AND METHODS

A.Sample preparation and surface treatment

Titanium sensors for QCM-D instrument (Q-Sense AB, Göteborg, Sweden) and pure titaniumdisks (Ti, Grade 2, Kobe Seiko, Tokyo, Japan) were used in this study. The specimens were subjected to two types of super hydrophilic treatment. Air specimens (Air, control) were stored in air for 3 weeks. The plasma treatment (Ti-Plasma) was carried out using an atmospheric-pressure plasma treatment (NJZ-2820 , Nagano Japan Radio , Nagano, Japan) onto specimens with 10mm distance between plasma head and surface of specimen at room temperature for 10s. The ultraviolet treatment (Ti-UV) was performed using a UV ozone cleaner (PC440, BioforceNanosciences, Sweden) for 1h. This equipment emits UV radiation with excitation wavelengths of 185 and 254nm corresponding to ultraviolet C (UV-C) and 365nm ultraviolet A (UV-A), respectively. Plasma and UV specimens were stored in distilled water for 3 days immediately after the plasma and ultraviolet treatment.

B.Contact angle measurement

The specimens were measured against double-distilled water using a contact angle meter (Phoenix α, Meiwaforces, Tokyo, Japan).

C.Adsorption property of CXCL12

The 1 ppm solution of CXCL12 (SDF-1α, R&D Systems, MN, USA) was used as bone-associated cytokines diluted by PBS(-). For the adsorption assay of the CXCL12, a QCM-D instrument (QCM-D300, Q-Sense AB, Göteborg, Sweden) with AT-cut single-crystal quartz sensors, onto which Ti was coated, with 14 mm in diameter. Measurements were carried out at 298 K ± 0.05 K, and data were collected at 14.8 MHz for analysis. Monitoring the resonance behavior of piezoelectric oscillation enables

measurement of mass adsorption at the surface in real time, usually as a function of the decrease in resonance frequency (f). Frequency shift (Δ f) is related to adsorbed mass (Δ m). The amount of adhered protein was estimated according to the Sauerbrey equation [4]:
$\Delta f = \Delta m (-n/C)$,
where n indicates number of overtones, and C is the constant for a given kind of sensor. According to this equation, a 1 Hz decrease in frequency at an overtone of 14.8MHz corresponds to approximately 5.98 ng/cm^2 sensor mass gain.
In the experiment, nonspecific binding (w/o blocking) and specific binding (w/ blocking) behavior were evaluated. On the nonspecific binding, the sequence of injections into the QCM cell for an experimental run was as follows: 0.5 mL PBS (-) for 5 minutes, 0.5 mL each CXCL12 solution in PBS (100 μg mL^{-1}, pH = 7.4) for 20 minutes, and 0.5 mL PBS for 5 minutes. On the specific binding, surfactant (Tween 20: polyoxyetylenesorbitanmonolaurate, Polysorbate 20, Wako Pure Chemical, Japan) was used as a blocking agent to block nonspecific protein binding to the sensors. The sequence of injections into the QCM cell for an experimental run was as follows: 0.5 mL PBS (-) for 5 minutes, 0.5 mL PBS-T (PBS plus 0.1% -Tween 20) for 5 minutes, 0.5 mL each CXCL12 solution in PBS-T (100 μg mL-1, pH = 7.4) for 20 minutes, and 0.5 mL PBS-T for 5 minutes.

D.Cell culture

Mouse osteoblast-like MC3T3-E1 cells were purchased from RIKEN BioResource Center (Wako City, Japan). Cells were cultured at 310 K in an atmosphere of 5% CO^2 and 100% humidity in an alpha modification of Eagle's minimal essential medium (α-MEM, Life Technologies, MD, USA) containing 10% fetal bovine serum (FBS, Life Technologies, MD, USA). After 5 passages and reaching 80% confluence, the cells were trypsinized and seeded onto the test substrates. The medium was changed every 3 days.
For cell attachment assay, cells were seeded onto all Ti disks (13mm in diameter) placed in 24-well tissue culture plates at a density of 5×10^4 cells/cm^2 (in 1mL). Initial attachment of cells was determined using WST-1 based colorimetric assay (WST-1, Roche Applied Science, Mannheim, Germany) after remaining in the culture for 3, 6, and 12h. After each time period, the discs were washed 3 times with phosphate-buffered saline (PBS, pH7.4, Life Technologies, MD, USA) to remove unattached cells and transferred to new culture plates. The culture wells were incubated at 310 K for 1h with 50μL reagent containing tetrazolium salt (WST-1) and 500μL culture medium, after which 110μL reaction solution was moved to 96-well plates to measure absorbance. Formazan production was measured using a microplate reader (SpectraMax M5, Molecular Devices, Tokyo, Japan) at 450nm.
For cell proliferation assay, cells were seeded onto all Ti disks placed in 24-well tissue culture plates at a density of 1×10^4 cells/cm^2 (in 1mL). Cell proliferation was determined using WST-1 based colorimetric assay after remaining in the culture for 1, 3, 5, and 7 days in the same manner as the cell attachment ability assay.

E.Statistical analysis

Data were analyzed for statistical significance using an analysis of variance (ANOVA) followed by the Scheffe's test for multiple comparisons.

III. RESULTS

A. Contact angle

The contact angles on the Air specimen were 100 (±7) degree, whereas, the other specimens (Ti-Plasma, and Ti-UV) showed superhydrophilicity with a water contact angle of almost zero degree.

B. Adsorption property of CXCL12

The adsorbed mass per unit area of CXCL12 was demonstrated in Fig. 1. In w/o blocking, no significant differences in adsorbed mass of CXCL12 were recognized among the specimens, while on w/ blocking, adsorbed mass of CXCL12 in the superhydrophilic surface groups (Ti-Plasma and Ti-UV) were increased 2 times greater than that in Air (control).

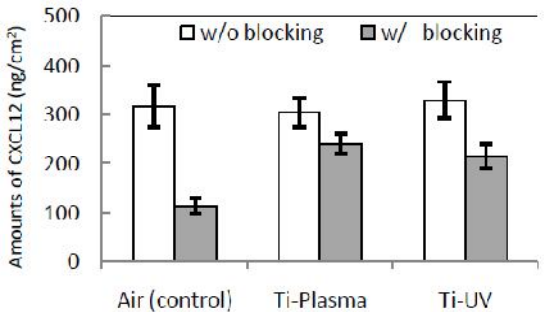


Fig. 1 Adsorbed mass of CXCL12

C. Cell attachment and cell proliferation

Initial cell attachment capability after 3, 6, and 12h of cultivation is shown in Fig. 2. In the superhydrophilic surface groups (Ti-Plasma and Ti-UV), cell attachment was greater than that in the Air (control) after all culture times.

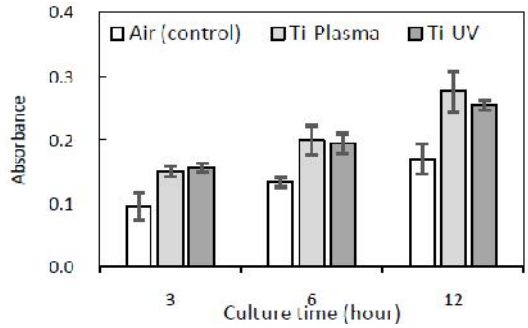


Fig. 2 Initial cell attachment

Cell proliferation capability after 1, 3, 5, and 7 days of cultivation is shown in Fig.3. Cell proliferation in the superhydrophilic surface groups (Ti-Plasma and Ti-UV) was greater than that in the Air (control) after after all culture times.

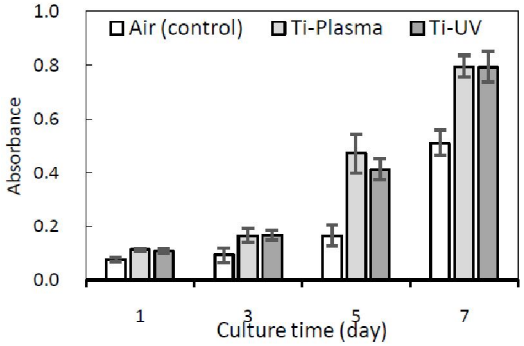


Fig. 3 Cell proliferation

DISCUSSION

It is generally recognized that superhydrophilicity was obtained with Plasma and UV treatments, and superhydrophilicity was maintained with immediate storage in distilled water after treatment [5]. The specific binding of CXCL12 adsorption was increased on the superhydrophilic treatment groups compared with Air (control) in this study. Specific binding on metal surfaces, where irreversible nonspecific binding can be avoided, may play an important role in achieving efficacious bio-fictionalization in metal surface modification. Possible mechanisms of increasing the CXCL12 adsorption on the superhydrophilic surfaces were consider due to the increasing the surface energy caused by removal of hydrocarbon and increasing hydrogen bond by introducing the hydroxide onto Ti surface.

Cell response was also enhanced on the superhydrophilic groups in this study. One possible reason was believe to introduction of the basic hydroxyl groups on the Ti surface. There are two kinds of hydroxyl groups on the outermost surfaces of Ti, negatively charged acidic hydroxyl groups and positively charged basic hydroxyl groups, which play important roles in protein adsorption[6]. Feng et al. [7] showed that at the initial stage of cell culture, the basic hydroxyl groups promote chemical interaction between osteoblasts and the Ti surface, indicating that the basic hydroxyl groups probably play a more important role in the bioactivity of Ti than do the acidic hydroxyl groups. In general, most proteins are negatively charged in a neutral environment. Accordingly, it is believed that protein adsorption related to cell attachment is promoted on superhydrophilic groups with increasing in the basic hydroxyl groups that were positively charged. As discussed above, the factors responsible for the remarkable cell response in the superhydrophilic groups over the Air specimens were the result of the synergistic effects of the following: elimination of hydrophobic hydrocarbons, introduction of basic hydroxyl groups, and an increase in the polar component.

In conclusion, the present study revealed that superhydrophilicmodifications are effective for enhancing the adsorption of bone-associated cytokine as well as the initial attachment and proliferation of osteoblast-like cells, indicating that superhydrophilic treatments of titanium implants may play important roles for enhancing the bone formation during the early stages of osseointegration.

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