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Cytocompatibility of Silicone Elastomer Treated with Hydrogenperoxide Containing Tantalum Chloride

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Silicone elastomer was chemically treated at 60°C for 7 days with 30 wt% H_2O_2 solutions with or without $TaCl_5$ and soaked for various periods in a simulated body fluid(Kokubo solution) up to 21 days. Apatite formation ability of the surface of the silicone elastomer specimens was investigated with thin-film X-ray diffraction and FT-IR reflection spectroscopy. These silicone specimens did not deposit apatite or calcium phosphates, irrespective of chemical treatment. Osteoblast-like cells (MC3T3-E1) derived from mouse were cultured on the specimens at 36.5°C under 5%CO_2 and 95% humidity. Similar degree of proliferation of cells was observed at 7 days among three specimens, while the no treatment specimen after incubation for 5 days showed a lower degree of proliferation than the silicone treated with 30 wt% H_2O_2 solutions with or without $TaCl_5$. Alkaline phosphatase activity of the cells proliferated on the no treatment specimen was lower than those of the silicone treated with 30 wt% H_2O_2 solutions with or without $TaCl_5$. These results indicate that the cytotoxicity of the silicone could be improved by the chemical treatment with 30 wt% H_2O_2 solutions with or without $TaCl_5$.

1. INTRODUCTION

Silicone elastomer is one of the most important soft-tissue substitutes that are widely used in clinical applications such as finger joints, hydrocephalus shunts and breast implants[1]. However, it is only biocompatible and cannot directly bond to surrounding tissues because of the formation of a non-adherent fibrous capsule when embedded in the body. It is reported that the essential condition for glasses and glass-ceramics to bond to living bone was the formation of a biologically active bone-like apatite layer on their surfaces when they are embedded in human body[2]. Such apatite layer also showed high biocompatibility with soft-tissue[3]. Recently, we have confirmed that Ti metal treated with a $TaCl_5$-containing hydrogen peroxide solution was bioactive enough to deposit such apatite layer *in vitro*[4]. Thus one can expect that such treatment gives the silicone elastomer apatite-forming ability or bioactivity. In addition, to be noted are both biological activity and the chemical and physical stability of the silicone elastomer in the physiological environment. In the present study, we investigated fundamentally the effect of the chemical treatment with 30 wt% H_2O_2 solutions with or without $TaCl_5$ on the bioactivity of the silicone elastomer, where we examined not only the apatite formation on the surface of the silicone specimens when soaked in a simulated body fluid up to 21 days but we cultured osteoblast-like cells (MC3T3-E1) derived from mouse on the specimens at 36.5°C under 5%CO_2 and 95% humidity. The contact angle toward distilled water and the surface roughness were also examined and the effects of chemical treatment on the surface morphology of silicone substrate were discussed.

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2. EXPERIMENTAL PROCEDURE

2.1. Sample preparation

Commercially available silicone sheet was used as the specimens. Rectangular specimens of 10 x 10x 1 mm^3 were cut from the silicone sheet. The specimens were washed three times with ethanol for 3 min in an ultrasonic cleaner. Subsequently the surface was chemically treated with 24 ml(=10^3 dm^3) of 30 wt% H_2O_2 aqueous solution with or without $TaCl_5$ in concentration of 5 mM($mmol \cdot dm^{-3}$). After soaking at 60°C for 7 days, the specimens were removed from the solution, and then washed with ion-exchanged and distilled water.

2.2. Soaking in SBF

The obtained specimens were soaked in 24ml of a simulated body fluid(SBF; Kokubo solution) kept at 36.5°C, which had inorganic species similar in concentration to those of the human blood plasma. The fluid was prepared by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in distilled water as described elsewhere[5]. It was buffered at pH 7.4 with 50mM trishydroxymethyl-aminomethane ($(CH_2OH)_3CNH_2$) and 45mM HCl, and its temperature was kept at 36.5°C. SBF is already confirmed to be able to well reproduce the apatite formation on the surfaces of glasses and glass-ceramics in the body environment[6].

2.3. Surface Analysis

The contact angle toward distilled water was measured using an automatic contact angle meter(Kyowa interface science, CA-V) at room temperature with the sessile drop method. The surface roughness, Ra, was estimated by surface texture measuring instrument (Tokyo SEIMITSU surfcom 1500A). After soaking in the SBF for various periods up to 21 days, specimen was removed from the fluid and gently washed with ion-exchanged distilled water. The surface structure was examined with thin-film X-ray diffraction and Fourier-transform infrared(FT-IR) reflection spectroscopy. An X-ray diffraction attached with a thin-film attachment was used, and the glancing angle was fixed at 1°, while an infrared spectrometer (FT-IR 300, Jasco Co., Japan) was used, and the reflection angle to the normal was set at 75°. Both techniques enabled to detect a layer about 1 μm thick at the surface of the specimen.

2.4. Cell culture

Osteoblast-like cells (MC3T3-E1) derived from mouse were seeded on the specimens placed on the bottom of polystyrene petri dishes containing α-minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum. Initial density of the cell was 1.0 x 10^4 cells/ml. The specimens were incubated at 36.5°C under atmosphere of 5% CO_2 and 95% humidity. After incubation for 1, 3, 5 and 7 days, the specimens together with the cells were taken out. The cells were removed from the substrates by immersion in a 0.1M (=mol/dm^3) phosphate-buffered saline aqueous solution (pH 7.4) containing 0.01wt% actinase E and 0.02wt% EDTA-2Na at 36.5°C for 3 minutes. After centrifuged, the cells were suspended in α-MEM, and the number of the removed cells was measured with hemocytometer.

Some of the specimens were also observed under a scanning electron microscope (SEM). Cells on the specimen after culturing were fixed due to immersion in a 0.1 M phosphate-buffered solution containing 2% glutaraldehyde for 2 hours at 4°C. After washing with the 0.1M phosphate-buffer solution, the specimens were dehydrated using graded ethanol-water solutions of 50% to 100% concentration in 15 minutes at each step. Then, the specimens were immersed in 100% 3-butanol in 30 minutes. The specimens were freeze-dried at 13.3Pa (0.1Torr), -5°C with JFD-310(JEOL, Japan). After coated with thin gold film, specimens were observed with SEM at an accelerating voltage of 10kV.

Alkaline phosphatase (ALP) activity was measured for the cells incubated for 1, 3, 5 and 7 days, as described in the previous report[7]. After incubation, the specimens were rinsed with the 0.1M phosphate-buffer solution and add 500μl of 5mM Tris-HCl buffer solution (pH 7.45). Then, the specimens were incubated at 37°C with 100μl solution that contained 2mM Na_2-p-nitrophenylphosphate and 2mM $MgCl_2$ in a 0.1M 2-amino-2-methyl-1-propanol buffer (pH 10.5). After 24 hours, the reaction was stopped by adding 100μl of 1N NaOH. ALP activity was derived from the optical absorption at 415nm of the reacted solution.

3. RESULTS AND DISCUSSION

SEM observation of the surfaces of all the specimens treated with the H_2O_2 solutions with or without $TaCl_5$ shows no significant changes in their surface morphology even after soaking in SBF up to 21 days. Figure 1 shows that thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of the silicone specimens after soaking for 21 days in the SBF. No peaks were present assignable to apatite in TF-XRD patterns. The IR reflection peaks at 500, 800, 1100 and 1250 cm^{-1} were ascribed to Si-O bending vibration, Si-C stretching vibration, transverse optical mode of Si-O stretching vibration and longitudinal optical mode of Si-O stretching vibration, respectively. No changes were found either in IR spectra and TF-XRD analyses indicate that these silicone specimens do not deposit apatite or calcium phosphates, irrespective of chemical treatments.

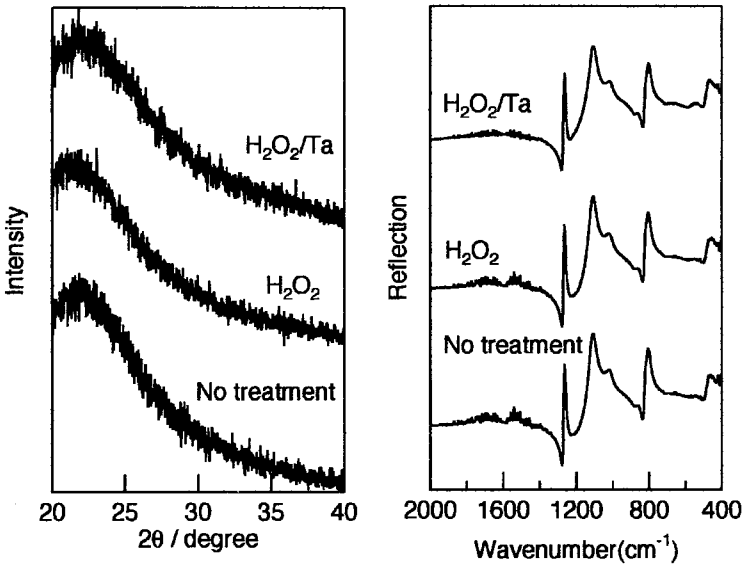

Figure 1 Thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of the silicone specimens after soaking for 21 days in the SBF.

Figure 2 shows changes in contact angle toward distilled water and roguhness, Ra, due to chemical treatment of silicone sheet as a function of treatment time. Note that in the silicone specimen treated with the H_2O_2 solutions with $TaCl_5$ the surface roughness increases with treatment time, while the silicone specimens treated with the H_2O_2 solutions without $TaCl_5$ showed the surface roughness to be almost constant irrespective to the treatment time. That is, the contact angle almost parallels to roughness. The decrease in the contact angle is accounted for the siloxane (Si-O-Si) bonds near the surface of silicone specimens are cleaved due to chemical treatment with the H_2O_2 solution with $TaCl_5$.

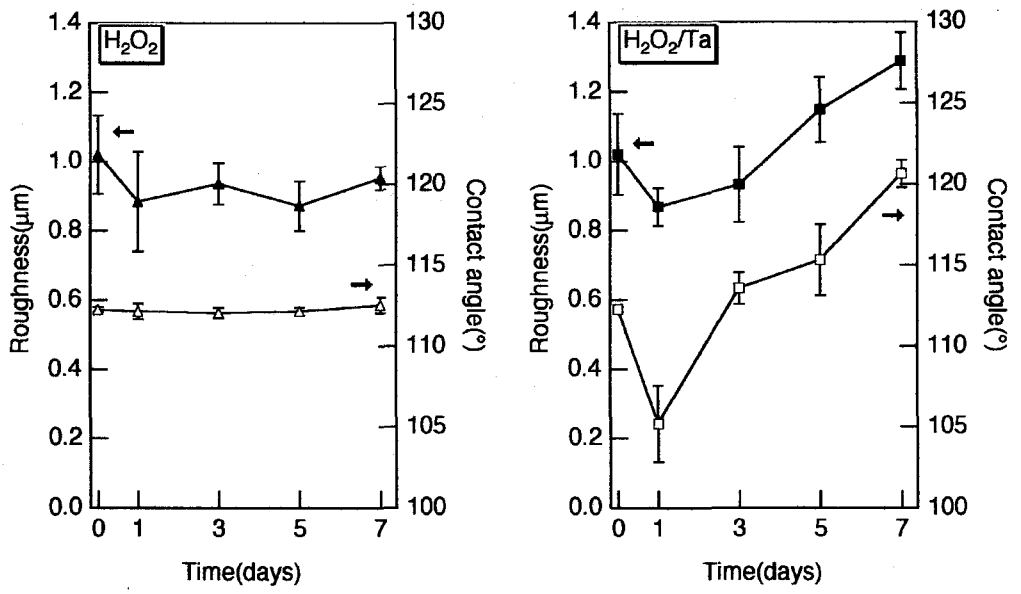

Figure 2 Changes in contact angle toward distilled water and roughness, Ra, due to chemical treatment of silicone sheet.

Figure 3 shows cell proliferation curves of MC3T3-E1 cultured on non-treated, silicone specimens chemically treated with the H_2O_2 solutions with or without $TaCl_5$. Cell numbers cultured without specimens are also shown on Fig. 3, denoted as "Reference". After 5days incubation the silicone specimen treated with the H_2O_2 solutions with or without $TaCl_5$ showed almost similar proliferation and the cell number on those two specimens were higher than non-treated silicone specimen, although the cell number on Reference was higher than others. After 7 days the cell number on the silicone specimens treated with the H_2O_2 solutions with or without $TaCl_5$ showed almost similar to that of Reference. These results indicate that the cytotoxicity of silicone substrate is remarkably improved by chemical treatment with the H_2O_2 solutions with or without $TaCl_5$.

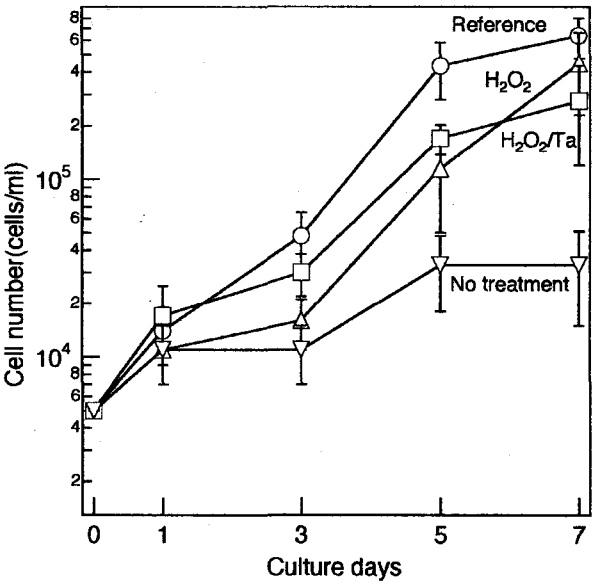

Figure 3 Cell proliferation curves of MC3T3-E1 cultured on non-treated, silicone specimens chemically treated with the H_2O_2 solutions with or without $TaCl_5$.

Figure 4 shows that ALP activity of cells cultured on the silicone specimens after incubation. The degree of ALP activity of the cells cultured on the silicone specimen treated with the H_2O_2 solutions with or without $TaCl_5$ was almost similar to non-treated silicone specimen and much lower than that of Reference. The degree of ALP activity of the cells cultured on the silicone specimen was in the order: Reference $>H_2O_2/Ta \sim H_2O_2 >$ No treatment. The cell number after incubation for 7 days on each specimen was in the order: Reference $> H_2O_2 \sim H_2O_2/Ta >>$ No treatment. Therefore, the cells cultured on H_2O_2 and H_2O_2/Ta have lower activity than that of Reference. These result suggest that the surface of silicone chemically treated with the H_2O_2

solutions with or without $TaCl_5$ has little negative effects on proliferation of the osteoblastic cells but their ALP activity is weakened. It is known that the low molecular weight silicon-containing compounds exhibit a wide variety of biological activities depending on their molecular size, configuration and the substituents on the silicon atoms[1]. One should take into consideration that such low molecular weight silicon-containing compounds present near the surface of the silicone substrates possess a little cytotoxicity.

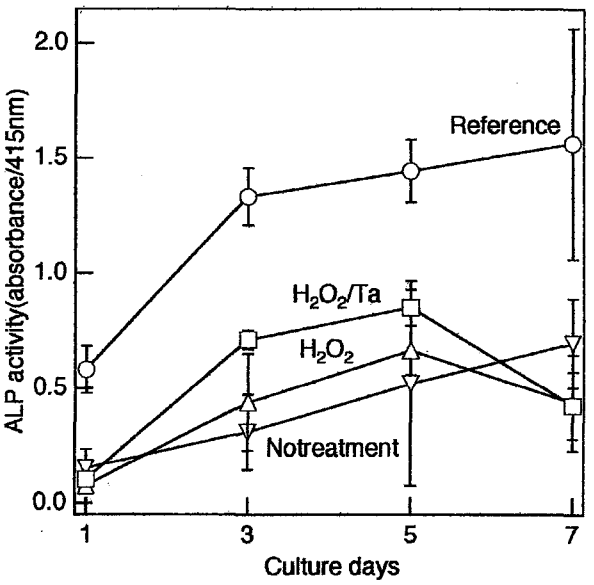

Figure 4 Alkaline phosphatase activity of cells cultured on the silicone specimens after incubation.

4. SUMMARY

We examined the apatite formation on the surface of the silicone specimens in the simulated body fluid up to 21 days and cultured osteoblast-like cells (MC3T3-E1) derived from mouse on the specimens at 36.5°C under 5%CO_2 and 95% humidity in order to investigate fundamentally the effect of the chemical treatment with 30 wt% H_2O_2 solutions with or without $TaCl_5$ on the bioactivity of the silicone elastomer. These silicone specimens did not deposit apatite or calcium phosphates, irrespective of chemical treatment. Silicone elastomer was chemically treated at 60°C for 7 days with 30 wt% H_2O_2 solutions with or without $TaCl_5$ and soaked for various periods in a simulated body fluid(Kokubo solution) up to 21 days. Similar degree of proliferation of cells was observed at 7 days among three specimens, while the no treatment specimen after incubation for 5 days showed a lower degree of proliferation than the silicone treated with 30 wt% H_2O_2 solutions with or without $TaCl_5$. Alkaline phosphatase activity of the cells proliferated on the no treatment specimen was lower than those of the silicone treated with 30 wt% H_2O_2 solutions with or without $TaCl_5$. These results indicate that the cytotoxicity of the silicone could be improved by the chemical treatment with 30 wt% H_2O_2 solutions with or without $TaCl_5$.

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