Improvement of Biocompatibility of Silicone Elastomer by Surface Modification

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γ-Methacryloxypropyltrimethoxysilane (γ-MPS) was grafted to silicone due to emulsion polymerization to induce Si-OH groups, in order to provide silicone with bioactivity spontaneous deposition of apatite in body fluid and to improve cytocompatibility. Apatite deposited on the grafted silicone within 7 days of soaking in 1.5 times as concentrated as the Kokubo solution. Osteoblastic cells (MC3T3-E1) were cultured on the specimens up to 7 days. After 5 days of culture, the number of MC3T3-E1 cells on the grafted specimen was much greater than that on the original specimen. These results indicated that the biocompatibility of silicone elastomer was improved by the grafting γ-MPS.

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. 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. Kokubo 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 were embedded in human body. Kubo et al. have already reported that emulsion-polymerization-grafted γ-MPS molecules provided polymer substrates such as PE and PVC with bioactivity. This paper will report a successful attempt to graft γ-MPS on the silicone elastomer surfaces due to this chemical modification and improve biocompatibility of their surface.

2. EXPERIMENTAL

2.1. Sample preparation

Commercially available silicone rubber (KE106, Shinetsu Co., Japan) was used as the

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specimen. A room temperature vulcanizing (RTV) type liquid and a solidifying agent were mixed for 30 min. The mixture was cast into a mold, and heated for 30 minutes at 150 °C. Rectangular specimens of 20 X 20 X 1 mm³ were cut from a sheet of silicone rubber. The specimens were washed three times with ethanol for 3 minutes in an ultrasonic cleaner. Then, they were dried at 60 °C for 24 hours.

2.2. Emulsion polymerization

The specimens were held with aluminium wire placed in the midst of a three-necked separable flask. Distilled water (100 ml) was poured into flask so that the specimens were located at the half-height depth. Sodium lauryl sulfate (1.5 g) as the emulsifier was added. The solution was stirred at 60 °C for 30 minutes under an N₂ flow. γ-MPS was then added drop wisely into the solution with a syringe through one of the necks. Aqueous solutions (50 ml) of potassium peroxodisulfate (1.5 g) and sodium hydrogen sulfite (0.15 g) were prepared separately, and were added to the flask through one of the neck. The resultant solution was stirred at 60 °C in the N₂ flow for 120 minutes. The obtained specimens were washed with ethanol for 10 minutes in an ultrasonic cleaning and dried at 60 °C for 24 hours. The amount of grafted γ-MPS was calculated by the following equation:

\[
\text{Amount of } \gamma\text{-MPS grafted (µg/cm}^2\text{)} = \frac{(W_1-W_0)}{A}
\]

where \(W_1\) is the dry weight of grafted specimens, \(W_0\) is the dry weight of the original specimens, and \(A\) is area of the specimens.

2.3. Surface Analysis

The contact angle toward distilled water was measured using an automatic contact angle meter (Kyowa interface science, model CA-V) at room temperature with the sessile drop method. The surface structure was examined with Fourier-transform infrared (FT-IR) reflection spectroscopy. An infrared spectrometer (FT-IR 300, Jasco Co., Japan) was used, and the reflection angle to the normal was set at 75°. This technique enabled to detect a layer about 1 mm thick at the surface of the specimen. Some of the specimens were observed under a scanning electron microscope (SEM).

3.4. Soaking in 1.5 SBF

The obtained specimens were cut (10 X 10 X 1 mm³) and soaked in 24 ml of a 1.5 SBF (1.5 times as concentrated as a simulated body fluid; SBF, Kokubo solution⁵) kept at 36.5 °C which had inorganic species similar in concentration to those of human blood plasma. SBF is already
confirmed to well reproduce in vivo apatite formation on bioactive materials under in vitro conditions. After soaking in 1.5 SBF for various periods, the specimens were gently washed with ion-exchanged distilled water. Apatite formation was examined with a thin-film X-ray diffractometer and a scanning electron microscope (SEM). Phase identification was conducted with thin film X-ray diffractometer.

3.5. Cytocompatibility

Osteoblastic cells (MC3T3-E1) derived from mouse were seeded on the specimens. Initial density of the cell was $1.0 \times 10^4$ cells/ml. The specimens were incubated at 36.5°C under atmosphere of 5% CO$_2$ and 95% humidity. After incubation up to 7 days, the cells were removed from the substrates and the number of the cells was measured with hemocytometer. Some of the specimens were also observed under SEM.

4. RESULTS AND DISCUSSION

4.1. Surface microstructure

Fig. 2 shows the contact angle (CA) toward distilled water and amount (GA) of γ-MPS grafted. As γ-MPS was grafted, CA decreased while GA increased. When 3 ml γ-MPS were applied, CA was about 90°, 25° smaller than the original specimen. Fig. 3 shows FT-IR transmission spectra of the original specimen, grafted specimen and γ-MPS monomer. A new peak appeared at 1700 cm$^{-1}$ assignable to $\nu$ (C=O) for the grafted silicone. This result indicates the presence of γ-MPS on the silicone surface. The 2800 cm$^{-1}$ peak for –OCH$_3$ of γ-MPS was absent from the spectrum for the grafted specimen. This is interpreted as showing that the –OCH$_3$ groups were hydrolyzed to the –OH groups. SEM observation indicated that the original specimen had a smooth surface, but the grafted specimens had a Si rich layer. Thus it was concluded that emulsion polymerization grafted γ-MPS molecules onto the silicone surface and improved in wettability.

4.2. In vitro bioactivity

Fig. 4 shows thin-film X-ray diffraction patterns of the specimens after soaking up to 14 days in 1.5 SBF. Original specimen gave no peaks, whereas the grafted specimens (γ-MPS 3 ml) deposited apatite within 7 days in 1.5 SBF. When the volume of γ-MPS employed was less than 3
ml, i.e., 1 or 2 ml, no apatite deposited. Fig. 5 (a) and (b) respectively show SEM photographs for the grafted specimen surface after soaking in 1.5 SBF for 7 and 14 days. Apatite particles deposited on the grafted specimen surface within 7 days, then the apatite layer formed on the surface in 14 days. The results indicate that when adequate volume γ-MPS molecules grafted, silicone could form.

Fig. 3 FT-IR transmission spectra of the specimen.

\[ \text{γ-MPS monomer} \]

\[ \text{grafted specimen} \]

\[ \text{original specimen} \]

Fig. 3 FT-IR transmission spectra of the specimen.

\[ \square : \nu \text{ (C-H: -OCH\textsubscript{3})} \]

\[ \diamond : \nu \text{ (C=O)} \]

Fig. 4 TF-XRD patterns for the original and grafted specimens before and after soaking in 1.5 SBF up to 14 days.
a) original specimens
b) grafted specimens (γ-MPS 3 ml)

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4.3. Cytocompatibility

Fig. 6 shows cell proliferation curves of MC3T3-E1 cells cultured on original and grafted specimens. After 5 days cultured, the number of MC3T3-E1 cells on the grafted specimen surface was much greater than those on original specimen. SEM observation showed MC3T3-E1 cells on the grafted specimen surface had fibroblast-like shapes after 3 days culture. After 7 days, the cells attained a confluent state, their shapes changed from fibroblast-like to polygonal. This result
indicates that the grafted silicone surfaces have good osteoconduction and improve cell attachment. It is considered that improvement of cell attachment was contributed to decrease of wettability on the silicone surface. 5, 6

5. CONCLUSION

Emulsion polymerization grafted γ-MPS molecules on silicone elastomer. They were hydrolyzed to yield silanol (Si-OH) groups in the course of the reactions. Wettability of their surfaces was improved. The grafted surfaces deposited apatite in 1.5 SBF. Moreover, They promoted MC3T3-E1 cells attachment and proliferation. Therefore, it was concluded that the γ-MPS grafting is useful to improve biocompatibility of silicone elastomer.

6. REFERENCES