A B S T R A C T

Effect of ozone nano-bubble water on
dental implant storage method

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【Introduction】

Ozone (O_3) is attracting attention as a possible cleansing agent in the medical field as well as food and semiconductor industries. Further, ozone has strong antimicrobial activity against bacteria, fungi, protozoa, and viruses.

Mostly commercial dental implants are exposed to the air during storage and delivery which may contaminate with the hydrocarbon of the air. Titanium is considered one of the best currently known biocompatible biomaterials. However, biological aging of titanium is important factor to be considered. Thus, the degradation of bioactivity and osteoconductivity of titanium over time may affect the implant surfaces leading initial adhesion of cells.

Therefore, the aim of this study was to evaluate the new storage method of dental implant that can maintain the biocompatibility of the titanium by focusing on the role of ozone nano-bubble water.

【Materials and methods】

1. Specimen preparation and evaluation

1). Titanium samples and surface characterization

JIS grade 2 titanium disk (Φ13.0 × 3.0 ㎜) material was used, and colloidal silica was treated with argon plasma glow discharge as a surface to improve surface wettability.

2). Measurement of the contact angle

Hydrophilic status of the titanium surfaces was examined by the contact angle. After discharge treatment, samples were stored in the air, water, acetone and ozone nano-bubble water, respectively. The contact angle of each samples was measured at day 28.

3). X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to investigate the

surface chemical composition of hydrocarbon.

4). Cell attachment and density assays

Initial attachment of human mesenchymal stem cells (MSCs) was evaluated by measuring the amount of cells attached to titanium substrates after 4 h of incubation. The number of attached cells was counted with a hemocytometer.

5). Scanning electron microscopy.

Surface attached cell morphology was examined by Scanning electron microscopy (SEM) after 4 h of incubation.

6). Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was used to examine cell morphology and cytoskeletal arrangement. Actin filament was stained with fluorescent dye conjugated with rhodamine-phalloidin.

7). Alkaline phosphatase (ALP) activity

Alkaline phosphatase (ALP) was used as enzyme marker. The ALP activity of cultured osteoblasts was examined by culture area- and colorimetry-based assays. The ALP positive area on the stained images was calculated using an image analyzing software. The ALP activity was evaluated as the amount of nitrophenol released through the enzymatic reaction and measured at 405 nm wavelength using micro-plate reader.

2. Evaluation of implant placement.

1). Implant

Titanium implant (JIS Class2, Φ 1 mm × 2 mm) was hydrophilized by argon glow discharge. Titanium implant was stored in the ozone nano-bubble water for 7 days.

2) Operation

8 weeks old SD rats were used in this study. They were anesthesized by isoflurane and local anesthesia. Implants were placed into the rat femur. At 14 and 28 days of post-surgery, the strength of osseointegration was

evaluated.

(1) Removal torque test

Removal torque values of implant were evaluated by bone-implant integration. Removed implants were observed using SEM.

(2) Soft X-ray images and basic fuchsine-methylene blue stain

Embedded thigh bone was adjusted to 120 μm and images were taken using X-ray generator. Furthermore, samples were adjusted to 50 μm and stained with Basic–Fuchsine methylene blue. Histomorphometric evaluation of Bone-to-Implant Contact (BIC) on titanium implant was observed with microscope.

【Results and discussion】

The contact angle of water on titanium disk dramatically decreased with 4 degrees after glow-discharge plasma treatment.

Acetone-~~s~~torage titanium surface was changed immediately from hydrophilic to hydrophobic. After that the contact angle was increased to 40°in 24 h. Air-storage surface was gently changed to hydrophobic when compared to acetone-storage surface in 7 days. On the other hand, high hydrophilicity was maintained on the ozone nano-bubble water-storage surface at the same level of glow discharged surface for 28 days. Furthermore, XPS was used to analyze the surface of adventitious carbon contamination, and the level of carbon contamination on ozone nano-bubble water-storage surface was lower than glow discharged surface.

In addition, enhancement of cell attachment was observed on the ozone nano-bubble water-storage titanium surface. After 4 h incubation, the number of human MSCs attached to ozone nano-bubble water-treated surfaces (8.7×10^3 cells) was at the same level of glow discharged surface (8.0×10^3 cells), and 1.6 fold greater than to air-storage surfaces (5.4×10^3 cells).

Furthermore, while observing through scanning electron microscope

(SEM) and confocal laser scanning microscope (CLSM), pseudopodium of the adherent cells were connected and fixed on the surface of sample stored in the nano-bubble.

At day 7, 1.6 fold areas of cultured osteoblast were ALP positive on ozone nano-bubble water storage surfaces compared with air-storage surfaces. In addition, the ALP activity was higher on ozone nano-bubble water storage surfaces.

Titanium implants were placed into the rat femur. At day 14, the removal torque value of air storage titanium implant was 1.4 N・cm, whereas ozone nano-bubble storage implant was 2.1 N・cm. Ozone nano-bubble stored implants torque value was significantly higher compared to air storage titanium implant i.e , 1.6 times.

Ozone nano-bubble storage implant significantly induces new bone formation. On examination, extensive bone formation was observed around ozone nano-bubble-storage implant observed by Basic fuchsine-methylene blue stain and CMR image.

Ogawa et al., had reported changes in bioactivity of titanium surfaces, water contact angle, and contamination of hydrocarbon. Ozone nano-bubble water-storage titanium substantially enhanced its osteoconductive capacity by removal of hydrocarbons from the TiO_2 surface, and established bone–titanium integration.

Various kinds of surface processing and chemical modification are done to the storage method to influence the performance of the implant. This may ultimately improve the clinical results.

From this study, we concluded that the titanium implant stored in ozone nano-bubble water could maintain the bioactivity of titanium surface. Furthermore, it can prevent the aging of implant and contamination by the hydrocarbon.