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Effect of the ultraviolet light treatment and storage methods on the biological activity of a titanium implant surface

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ABSTRACT

Objective. We evaluated whether the biological activity of the surface of titanium, when stored in an aqueous solution, in low vacuum, and under ambient conditions after ultraviolet light (UV) treatment is comparable to that of the surface immediately after UV treatment for 15 min and that after dielectric barrier discharge (DBD) plasma treatment for 15 min.

Methods. Grade IV titanium discs with machined surfaces were irradiated with UV and their surface properties were evaluated immediately and after storage for 28 days in distilled H_2O (dH_2O), a vacuum desiccator (31.325 kPa), and a sealed container under air. Their surface characteristics were evaluated by atomic force microscopy, X-ray diffraction, contact angle analysis, and X-ray photoelectron spectroscopy. Biological activities were determined by analyzing the albumin adsorption, MC3T3-E1 cell adhesion, and cytoskeleton development.

Results. Hydrophilicity of titanium surfaces stored in dH_2O was comparable to that immediately after UV treatment and higher than that immediately after DBD plasma treatment ($P < 0.001$). Storage in dH_2O and in low vacuum immediately after UV treatment prevented hydrocarbon contamination and maintained elevated amounts of titanium and oxygen. After 28 days, protein adsorption, cellular adhesion, and cytoskeletal development of MC3T3-E1 cells on the titanium surfaces stored in dH_2O were significantly enhanced compared to those stored in low vacuum and under ambient conditions while being comparable to those immediately after UV and DBD plasma treatments.

Abbreviations: DBD, dielectric barrier discharge; NTAPP, nonthermal atmospheric-pressure plasma; dH_2O, distilled water; EO, ethylene oxide; AFM, atomic force microscopy; XRD, X-ray diffraction; XPS, X-ray photoelectron spectroscopy; PBS, phosphate-buffered saline; OD, optical density; BSA, bovine serum albumin.

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Significance. UV treatment of the titanium implants followed by wet storage is useful for maintaining enhanced biological activity and overcoming biological aging during shelf storage.

1. Introduction

Titanium is mainly used as a material for prosthetic dental implants. It is used for the restoration of missing teeth, as an orthodontic miniscrew or miniplate to provide absolute anchorage for tooth movement in the dental area. Titanium dioxide exhibits favorable biocompatibility and corrosion resistance, and can achieve osseointegration by directly interacting with bones without forming fibrous tissues around the implant [1]. To improve the quality of osseointegration and primary stability for immediate or early loading toward reducing patient discomfort, much effort has been devoted to modifying the surface physicochemical properties of the implants mimicking the innate characteristics of bones. As a result, treatments such as surface micro/nanotopographical modification, sandblasting, acid-etching, anodic oxidizing, or a combination of these are commercially available of late [2–5].

However, many studies have indicated that the time-dependent uncontrolled biological aging of the titanium implant surface during the inevitable storage period of commercial distribution under sterile gas-permeable packaging can lead to decreased protein adsorption and osteogenic cell adhesion capacity, regardless of the surface topography. This is because the titanium implant surface can get progressively contaminated by organic impurities such as hydrocarbons and polycarbonyls from the atmosphere, during shelf storage [6,7]. As the percentage of hydrocarbons on a titanium surface increases, the surface changes from electropositive to electronegative, and this phenomenon prevents negatively charged plasma protein and the extracellular matrix of the osteogenic cells from adhering to the titanium implant surface, and subsequent cell attachment could decrease in correlation with decreased cell-protein interaction *via* Arg-Gly-Asp-binding integrins [8–10].

Recently, it has been reported that ultraviolet light (UV) and nonthermal atmospheric-pressure plasma (NTAPP) can eliminate the surface organic impurities and change the surface wettability from hydrophobic to hydrophilic, without altering the surface topography [11–13]. These titanium surface treatments remarkably increased their osteoconductivity, and at the same time enhanced new bone formation, the bone-implant contact, and the bacterial resistance relative to untreated or even freshly prepared titanium [14,15].

However, according to the report by Choi et al. [9], when titanium discs were stored in a sealed container in dark under air for 4 weeks after the UV or NTAPP treatment, osteoblastic cell adhesion capacity and cytoskeletal development significantly decreased compared to the discs immediately after these treatments, even with a slight hydrocarbon contamination. To maintain the residual biological effect for a long period after the UV treatment, without the need of this treatment for ~15 min immediately before the implant surgery, development of alternative packaging methods is required to enable commercially available UV-pretreated titanium implants, through the interception of hydrocarbon contamination.

Recently, storage of titanium implants in isotonic saline, distilled water (dH_2O), or gas-barrier (vacuum) packaging immediately after their manufacture has been shown to preserve their hydrophilicity which is an important property of osteoblast lineage cells [16–18].

Therefore, to commercialize UV-pretreated titanium implants that do not require special efforts before implantation as well as to maintain the surface hydrophilicity of the titanium implants during shelf storage by preventing contamination by hydrocarbons, we evaluated the biological activities of the titanium surfaces after storage in an aqueous solution, in low vacuum, and under ambient condition after an UV treatment to determine if they are comparable to that of the surface immediately after the UV treatment. As a positive control, a dielectric barrier discharge (DBD) plasma treatment, one of the most popular methods to produce NTAPP was chosen [19,20]. We hypothesized that there would be no differences in the biological activities of the titanium surfaces immediately after the UV and DBD plasma treatments and those stored in an aqueous solution or in low vacuum for 28 days after the UV treatment.

2. Materials and methods

2.1. *Preparation of titanium samples*

Disc-shaped titanium samples (12.0 mm diameter, 1.0 mm thickness) were prepared by machining the commercially available pure titanium (grade IV; Osstem Implant Co., Seoul, Korea). The titanium discs were ultrasonically cleaned with acetone, alcohol, and dH_2O for 15 min each. They were then sterilized using ethylene oxide (EO) gas at a temperature of 55 °C for 1 h [9,13]. The prepared titanium discs were fully aged by storing in sealed 12-well cell culture plates in dark under air at room temperature for at least 8 weeks [14,21]. At the end of the aging period, the samples were divided into 6 groups including the untreated control group ($n = 52$ per group in case of samples immediately after UV and DBD plasma treatment and the untreated ones; $n = 49$ per group for the three storage methods).

2.1.1. *Immediately after UV and DBD plasma treatment*

The titanium specimens were treated by UV or DBD plasma under ambient conditions. The samples were irradiated with UV light for 15 min using a photo device (TheraBeam Affiny; Ushio Inc., Tokyo, Japan) [9,22,23]. The UV light was deliv-

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