Effect of Ultraviolet-Mediated Photofunctionalization for Bone Formation Around Medical Titanium Mesh

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Purpose: The new technology of photofunctionalization with ultraviolet (UV) light for titanium implants has earned considerable attention. We hypothesized that UV light treatment would enhance bone formation on titanium mesh.

Materials and Methods: We implemented in vitro and in vivo experiments to examine the effectiveness of UV treatment for bone formation on titanium mesh surfaces. Titanium mesh for medical use was prepared as samples, which were autoclaved and stored under dark ambient conditions for 4 weeks. UV treatment was performed for 12 minutes. Carbon contamination, hydrophilicity, and protein adhesion of the titanium mesh surface were examined in an in vitro model. Bone tissue formation around the titanium mesh was observed in a rat femur bone model. The Mann-Whitney *U* test was used to examine differences between the untreated and UV-treated groups. *P* values of $< .05$ were considered significant.

Results: UV-mediated photofunctionalization reduced carbon contamination rates on the untreated titanium mesh surfaces. The hydrophobic surface of the untreated titanium mesh became superhydrophilic after UV-mediated photofunctionalization ($P < .01$). The amount of protein adsorbed onto the titanium was 1.5 to 3 times greater on the photofunctionalized titanium mesh surfaces than on the untreated titanium mesh surfaces ($P < .01$). In the animal experiment, the newly formed bone on the UV-treated titanium mesh was approximately 2.5 times greater than that on the untreated mesh ($P < .05$).

Conclusions: UV-mediated photofunctionalization is effective, as demonstrated by the enhanced bone tissue formation on the titanium mesh. Future studies will focus on bone augmentation using an UV-mediated photofunctionalized titanium implant and mesh.

J Oral Maxillofac Surg 72:1691-1702, 2014

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This study was supported by a research gift from Ushio and a Grant-in-Aid for Scientific Research (C) (grant 25463141) from the Japan Society for the Promotion of Science.

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Received January 8 2014

Accepted May 8 2014

0278-2391/14/00557-6$36.00/0

http://dx.doi.org/10.1016/j.joms.2014.05.012

The new technology of ultraviolet (UV)-mediated photofunctionalization for titanium implants has earned considerable attention. Titanium is an important biomaterial widely used in oral and maxillofacial surgery. Recently, studies have revealed that the titanium surface is covered with hydrocarbon after some production processes, such as a machine cut-and-etch treatment.[1-3] Hydrocarbon contamination on titanium surfaces causes biologic aging of titanium and reduces osteoblast activity on the surface.[1-3] Four weeks are required for aging after the production or processing of titanium surfaces, and 4-week-old titanium surfaces appear hydrophobic, with less attachment by proteins and osteoblasts.[1-5] To improve the aging of titanium surfaces, a photofunctionalization technique with UV light has been developed.[1-3] The UV treatment, by a combination of UVA and UVC for the aging titanium surface, dramatically changes the surface properties, expressed by superhydrophilicity, decontamination of accumulated carbon, and positively converted electrostatic charges. These factors act synergistically to increase the recruitment, attachment, and retention of osteogenic cells.[1-3]

Since Boyne et al[6] first reported the osseous restoration of deficient alveolar ridges using titanium mesh, bone augmentation surgery with the titanium mesh applied to the atrophied alveolar bone before implant therapy has been developed.[7] The alveolar ridge bone augmentation technique using autogenous bone and titanium mesh is well established. It is a reliable procedure for the restoration of approximately 5 mm to the height of a bone defect around an implant,[7-9] indicating that the titanium mesh ensures a bone-regenerative environment.

A UV-mediated photofunctionalized titanium surface enhanced bone generation in a bone-healing environment,[10] suggesting that photofunctionalized titanium can be used as a material for bone regeneration. Thus, UV treatment could be effective for the additional enhancement of osteoconductivity of the titanium mesh.

The osteoconductivity of the titanium mesh and its possible enhancement have not previously been viewed as important biologic requirements. The present study describes the effectiveness of UV treatment of titanium mesh as an osteoconductive material by analyzing the osteoblast activity in vitro and establishing 3-dimensional in vivo morphogenetic profiles of the newly formed bone tissues on the surface.

Materials and Methods

TITANIUM MESH SURFACE CHARACTERIZATION AND PHOTOFUNCTIONALIZATION

Titanium mesh (0.2 mm thick), made of commercially pure grade 2 titanium with anodizing treatment (Synthes K.K., Tokyo, Japan), was trimmed to fit 12 well culture plates for in vitro studies or cut to 3 × 10 mm for in vivo studies. All meshes were autoclaved and stored in a dark room for 4 weeks. The titanium mesh contained a round hole (1.8 mm diameter) within a titanium frame (0.3 to 0.4 mm wide), with a calculated hole ratio of 51.0%. The surface morphology and chemistry were examined using a scanning electron microscope (SEM; XL30, Philips, Eindhoven, The Netherlands) and energy-dispersive x-ray spectroscopy (EDS), respectively. The hydrophilic and hydrophobic properties of the titanium mesh surfaces were evaluated by measuring the contact angle with 5 μL of water. For evaluation of the hydrophilicity of the mesh surfaces, the trimmed mesh was placed on a clean, flat table. A 5-μL droplet of water was placed on the mesh surface. Photofunctionalization was performed by treating the titanium mesh with UV light for 12 minutes using a photo device (Ushio, Tokyo, Japan) immediately before the in vitro or in vivo experiment. The machine was optimized for UV light efficacy to the titanium surface.

PROTEIN ADSORPTION

Bovine serum albumin (Pierce Biotechnology, Rockford, IL) and bovine plasma fibronectin (Sigma-Aldrich, St Louis, MO) were used as model proteins. A 300-μL quantity of protein solution (1 mg/mL protein/saline) was pipetted onto and spread over the trimmed titanium mesh that had been placed into each well of a 12-well plate. The trimmed mesh was not fixed to the well, but remained stationary during the incubation. After either 6 or 24 hours of incubation in sterile humidified conditions at 37°C, the solution containing nonadherent proteins was removed and mixed with micro-bicinchoninic acid (Pierce Biotechnology) at 37°C for 60 minutes. The amount of protein was quantified using a microplate reader at 562 nm.

ANIMAL EXPERIMENT

Eight-week-old male rats (Charles River, San Diego, CA) were anesthetized by inhalation with 1 to 2% isoflurane. After their legs had been shaved and scrubbed with 10% povidone-iodine solution, the distal aspects of the femurs were carefully exposed by a skin incision and muscle separation. The corner angle of the femur was used for titanium mesh placement. The bone defect for implantation of the titanium mesh into the bone marrow cavity was created by drilling a rectangular osteotomy (0.25 mm × 10 mm) along the longitudinal axis of the femur using a burr and scalpel. An untreated or photofunctionalized titanium mesh was placed in the bone with passive retention (Fig 1). The surgeon