Abstract: Titanium mesh plate (Ti mesh) used for bone augmentation inadvertently comes into contact with medical gloves during trimming and bending. We tested the hypotheses that glove contact degrades the biological capability of Ti mesh and that ultraviolet treatment (UV) can restore this capability. Three groups of Ti mesh specimens were prepared: as-received (AR), after glove contact (GC), and after glove contact followed by UV treatment. The AR and GC meshes were hydrophobic, but GC mesh was more hydrophobic. AR and GC meshes had significant amounts of surface carbon, and Si content was higher for GC mesh than for AR mesh. UV mesh was hydrophilic, and carbon and silicon content values were significantly lower in this group than in the AR and GC groups. The number, alkaline phosphatase activity, and mineralization ability of attached osteoblasts were significantly lower in the GC group than in the AR group and markedly higher in the UV group than in the AR group. In conclusion, glove contact caused chemical contamination of Ti mesh, which significantly reduced its bioactivity. UV treatment restored bioactivity in contaminated Ti mesh, which outperformed even the baseline Ti mesh.

Keywords: titanium mesh; photofunctionalization; osteoblasts; guided bone generation.

Introduction

Titanium (Ti) is a lightweight, non-magnetic, corrosion-resistant material with excellent biocompatibility and many applications in medicine. It is widely used as a substitute for hard tissues in areas that require high mechanical strength and durability and helps patients with diminished or lost biological function after disease or injury. In addition to its semi-permanent applications (eg, as implants) in orthopedic surgery and dentistry, Ti is also used as an immobilizing, supporting, and fixating material, eg, in bone plates and mini-screws, stents for circulatory organ surgery/medicine, and mesh plates used in pre-implant bone regeneration surgery (1-3). Ti plates with holes (Ti mesh plates or Ti meshes) are often used in dental applications such as bone augmentation in guided bone regeneration (GBR) (4,5).

Placement of dental implants requires a jaw bone with adequate width and height (5). The Ti mesh is used to hold bone graft materials and to support/maintain the three-dimensional structure of the bone to be regenerated (6,7). However, Ti mesh is usually sold as a non-sterile two-dimensional sheet of uniform thickness and must
be sterilized in an autoclave or with other sterilization equipment. During surgery, Ti mesh is cut to the required size and bent into the desired shape by the surgeon (8). In some cases, Ti mesh is trimmed and shaped on stone and polymer models created from patient X-ray data (9). During these procedures, Ti mesh is exposed to medical gloves, surgical instruments, and other materials, which leads to biological contamination. To remove these contaminants, the mesh surface must be re-sterilized. However, Ti mesh can also develop chemical contamination, which has not been previously studied. It is therefore unclear whether such chemical contamination has biological effects.

Photofunctionalization—the process of activating Ti surfaces with ultraviolet (UV) treatment immediately before use—promotes adhesion of osteoblasts and increases osseointegration capacity. In animal models, photofunctionalization increased the bone-to-implant contact ratio to nearly 100%, which approximately tripled the mechanical stability of a bone/implant. These effects are due to decomposition and removal of carbon compounds (such as hydrocarbons) from the Ti surface during photofunctionalization (10-12). UV treatment of Ti mesh eliminates carbon species from the Ti surface and makes it superhydrophilic, which may improve osteoconductivity (13-18). Furthermore, the clinical use of photofunctionalization has yielded positive results in dental implants (19-23).

We examined the effects of intraoperative contact between surgical gloves and Ti mesh. Our primary goals were to identify chemical contaminants on the Ti mesh surface caused by glove contact and determine the biological effects of such contaminants. The secondary goal was to determine whether UV treatment of contaminated Ti mesh altered the chemical and biological properties of the mesh.

Materials and Methods

Ti mesh samples and surface characterization
Ti mesh (thickness 0.2 mm) was fabricated from grade 2 commercial pure Ti (Micro Mesh, Karl Leibinger Medizintechnik, Mühlheim, Germany) and cut to fit 12-well culture plates for in vitro studies. The mesh had circular holes (diameter 1.8 mm).

Three groups of Ti mesh samples were prepared: as-received Ti mesh (AR), Ti mesh after glove contact (GC), and Ti mesh subjected to UV treatment after glove contact (UV). The GC group was exposed to sterilized non-powdered gloves for 3 min under pressure from a 100-g weight. UV treatment was performed with a TheraBeam SuperOsseo device (Ushio, Tokyo, Japan) for 12 min.

The surface morphology of the specimens was examined by scanning electron microscopy (SEM; Nova 230 Nano SEM, FEI, Hillsboro, OR, USA), and hydrophobicity was estimated by measuring the contact angle formed by 3 µL of sterile ultra-pure water (ddH2O).

The chemical properties of Ti mesh surfaces were evaluated by X-ray photoelectron spectroscopy (XPS; Axis Ultra DLD, Kratos Analytical Ltd, Manchester, UK) under high-vacuum conditions (corresponding to a background pressure of 6 × 10⁻⁷ Pa).

Osteoblast cell culture
Bone marrow-derived osteoblastic cells isolated from the femurs of 8-week-old male Sprague-Dawley rats were placed into alpha-modified Eagle’s medium (Minimum Essential Media, Thermo Fisher, Waltham, MA, USA) supplemented with 15% fetal bovine serum, 50 µg/mL ascorbic acid, 10 mM Na-β-glycerophosphate, 10⁻⁸ M dexamethasone, and an antibiotic/antimycotic solution containing 10,000 units/mL penicillin G sodium, 10,000 mg/mL streptomycin sulfate, and 25 mg/mL amphotericin B. Cells were incubated in a humidified atmosphere of 95% air and 5% CO2 at a temperature of 37°C. After reaching a confluence of 80%, the cells were detached with a 0.25% trypsin-1 mM EDTA-4Na solution and seeded onto Ti meshes placed on a 12-well culture dish at a density of 3 × 10⁴ cells/cm². The culture medium was renewed every 3 days.

Cell attachment and proliferation
Using a tetrazolium salt (WST-1)-based colorimetric assay (Roche Applied Science, Mannheim, Germany), we assessed initial attachment of cells to Ti meshes by measuring the number of cells on mesh surfaces after incubation for 6 and 24 h. A culture well was incubated with 100 µL of WST-1 reagent at a temperature of 37°C for 25 h. The amount of formazan produced was measured at a wavelength of 420 nm by using an enzyme-linked immunosorbent assay (ELISA) reader (Synergy HT, BioTek Instruments, Winooski, VT, USA).

Osteoblast morphology and spread
The spread and cytoskeletal arrangement of osteoblasts seeded onto Ti mesh surfaces were examined by confocal laser scanning microscopy (TCS SP5, Leica, Wetzlar, Germany). After seeding for 6 and 24 h, the cells were fixed in a 10% formalin solution and stained with fluorescent rhodamine phalloidin dye (actin filament, red color; R415, Thermo Fisher) and vinculin (green color; ab11194, Abcam, Cambridge, MA, USA). The areas,
perimeters, Feret diameters, and densities of rhodamine- and vinculin-positive regions were quantified with image analysis software (ImageJ, NIH, Bethesda, MD, USA).

**Alkaline phosphatase activity**

Image-based assays were used to determine alkaline phosphatase (ALP) activity of osteoblasts on days 5 and 10. Cultured cells were washed twice in Hanks’ solution and then incubated with 120 mM of Tris buffer (pH 8.4) containing 0.9 mM naphthol AS-MX phosphate and 1.8 mM Fast Red TR salt for 30 min at a temperature of 37°C. ImageJ software was used to determine the ALP-positive area on stained images, which was calculated as [stained area/total Ti mesh area] × 100 (%).

**Mineralization assay**

von Kossa stain was used to visualize mineralized nodules of osteoblastic cells at day 14. Cultured cells were fixed by applying a 50% ethanol + 18% formaldehyde solution for 30 min and then incubated in a 5% silver nitrate solution under UV irradiation for 30 min. After that, the cells were washed twice with ddH$_2$O and incubated in a 5% sodium thiosulfate solution for 2 to 5 min. The mineralized nodule area was defined as [stained area/total Ti mesh area] × 100 (%) and estimated with ImageJ software.

**Statistical analysis**

All *in vitro* culture assays were performed in triplicate (*n* = 3), except for cytomorphometry, which was performed in six cells (*n* = 6). Measurement of contact angle and XPS elemental quantification were performed on three samples (*n* = 3). Differences between experimental groups were analyzed with the Bonferroni-adjusted Mann-Whitney *U* test. A *P* value of <0.05 was considered to indicate statistical significance.

**Results**

**Surface morphology of Ti mesh plates**

High-magnification SEM images showed that AR Ti mesh fabricated by machine cutting or pressure forming had surfaces with irregular coarseness; no exogenous structure or attachment was observed at low- or high-magnification (Fig. 1). Low-magnification SEM images showed scattered discolored areas in GC and UV meshes. Higher magnification allows for clearer observation of Ti mesh structure and identified these areas as granular exogenous structures or structures of undefined shape (Fig. 1).

**Measurement of contact angle**

The surfaces of AR and GC Ti meshes were hydrophobic, but their hydrophobicity significantly differed. The contact angle of water was 60° for AR mesh and about 75° for GC Ti mesh. On the UV mesh surface, the water droplet spread widely, which indicated a contact angle of 0°. This mesh should therefore be regarded as superhydrophilic (Fig. 2).

**Surface properties of Ti mesh**

XPS spectra recorded for the AR and GC Ti meshes had strong carbon peaks, which was not true for the UV Ti mesh (Fig. 3). In addition, the silicon peak for the GC Ti mesh was greater than those for the AR and UV Ti meshes. The amount of carbon on the surface of UV Ti meshes was 35% lower than values measured for the surfaces of the AR and GC Ti meshes. The silicon content on the surface of GC Ti meshes was 1.66 times that on the surface of AR meshes and was 20% higher than that on the surface of the UV Ti mesh. The Ti content on the surface of GC Ti meshes was about 1/3 that on AR mesh surfaces and was lower than that on UV Ti mesh surfaces by a factor of four. The oxygen content on the surface of UV Ti meshes was roughly two times that on the surfaces of AR and GC Ti meshes (Fig. 3).
Osteoblast attachment to Ti mesh surfaces

We examined the numbers of osteoblasts that adhered to Ti meshes during the early stage of the experiment. For GC Ti mesh, the number of attached osteoblasts after 6 h of culture was about 20% that for the AR Ti mesh. The number of attached cells was significantly higher in the UV group than in the AR group. The results obtained after culture for 24 h were consistent with those after 6 h (the number of adhering cells was significantly lower for the GC Ti mesh, and the cell adhesion properties of GC Ti mesh recovered after UV treatment; Fig. 4A). These findings were confirmed by confocal laser fluorescence microscopy; UV Ti mesh had the highest numbers of cells after culture for 6 and 24 h, and GC Ti mesh had the lowest numbers (Fig. 4B).

Initial behavior of osteoblasts on Ti mesh

After culture for 6 and 24 h, osteoblasts were stained with actin and vinculin dyes and observed with confocal laser fluorescence microscopy. After 6 h of culture, cells attached to GC Ti mesh were significantly smaller than those on AR mesh, and their shape was less protruded and elongated. The cells that proliferated on UV Ti mesh were larger than those on GC Ti mesh and similar in size to those attached to AR Ti mesh. Cell elongation was advanced on UV Ti mesh, and this trend was more pronounced after culture for 24 h. Furthermore, actin expression was greatest for UV Ti mesh (Fig. 5A). Cytomorphology was examined by image analysis. After culture for 6 and 24 h, the areas, perimeters, and Feret diameters of osteoblasts on GC Ti mesh were significantly smaller than those of osteoblasts on AR Ti mesh. The values in the UV group were equivalent to or greater than those in the AR group (Fig. 5B).
Densitometric analysis after 6 and 24 h of culture revealed that osteoblasts on GC Ti mesh had significantly lower actin and vinculin expressions per cell than did those on AR Ti mesh. However, actin and vinculin expressions in the UV group were equal to or even greater than those in the AR group (Fig. 5B).

**Functional phenotypes of osteoblasts grown on Ti mesh**

AR Ti mesh was positively stained for ALP at day 5. In contrast, GC Ti mesh was faintly stained, while red staining of UV Ti mesh was similar to or more intense than that of AR mesh. The same trend was observed after 10 days of culture. The results of quantitative analysis of the obtained images showed that the degree of staining measured for GC Ti mesh at day 5 was 20% that obtained for AR Ti mesh. The magnitude measured for UV Ti mesh was 10 times that of GC Ti mesh and two times that of AR Ti mesh. After 10 days, the degree of staining for GC Ti mesh was 25% less than that for AR Ti mesh, and UV and AR Ti meshes exhibited similar osteoblast expression levels (Fig. 6).

After 14 days of culture, the AR Ti mesh became almost entirely black after von Kossa staining. In contrast, GC Ti mesh was only faintly stained. The staining intensity for UV Ti mesh was similar to that for AR Ti mesh. Qualitative image analysis showed that almost 100% of the AR and UV Ti meshes were stained, indicating the presence of mineralized nodule areas; however, only 1/8 of GC Ti mesh was stained (Fig. 7).

**Discussion**

This study investigated the effectiveness of a UV treatment, known as photofunctionalization, in eliminating carbon and other impurities caused by glove contact and restoring bioactivity in Ti mesh. We hypothesized that areas of Ti mesh affected by chemical contamination would adversely affect osteoblast attachment and subsequent function and that UV treatment would restore osteoblast activity. Although we used powder-free sterilized gloves, we noted areas of GC mesh with uneven, discolored contrasts 4.1) and the presence of small granular exogenous particles adsorbed on the mesh surface, which suggested contamination. Foreign debris were still present in the UV group, which indicates that UV treatment did not remove the presumed contamination. Silicon content was higher on the surface of GC Ti mesh than on the AR Ti mesh surface (Fig. 3), probably because silicon is a constituent element of the gloves. Carbon content was lower on the surface of UV Ti mesh than on the surfaces of AR and GC Ti meshes, and Si content was lower on the surface of UV Ti mesh than on the GC Ti mesh surface. These findings suggest that, although UV treatment did not remove the presumed contamination, it reduced chemical contamination.

The AR and GC Ti meshes were both hydrophobic, but the GC Ti surface exhibited greater hydrophobicity (Fig. 2). In contrast, UV Ti mesh was superhydrophilic. Surface adhesion of osteoblasts was weaker for GC Ti mesh than for AR Ti mesh but was strongest for UV Ti mesh (Fig. 4). The size and perimeter of osteoblasts were larger for those adhering to GC Ti mesh than for those adhering to AR Ti mesh; however, the values were largest for UV Ti mesh (Fig. 5). The expression of osteoblast functional phenotypes obtained for GC Ti mesh was much lower than that for AR Ti mesh; however, expression was greatest for cells on the surface of UV Ti mesh (Figs. 6 and 7).

During GBR, a membrane is positioned against the area of lost bone in order to cap off soft tissue, which allows osteoblasts to migrate to the inner surface and generate bone tissue. This treatment is usually required when bone height is relatively low, which creates problems during implantation. In addition, installation of implants in the...
resorbed alveolar ridge compromises the crown-to-root ratio, which degrades biomechanical conditions and results in poor cleanability. Hence, use of GBR restores esthetics and ensures long-term predictability of clinical outcomes. The height and width of the bone defect must be restored; however, it is difficult to grow tissues of ideal height and width in three dimensions. Ti mesh is expected to provide a three-dimensional structure that can be maintained for the required time, thus improving the degree of ossification and overall outcomes of GBR. However, the bone-healing period during GBR is relatively long, and predictions of the final bone structure and its preservation are not entirely reliable. Hence, enhancing the properties of Ti mesh could potentially improve overall outcomes.

Previous studies reported that UV treatment helped remove carbon species from the surface of Ti mesh plates and enhanced bone conductivity. UV radiation makes the hydrophobic Ti surface hydrophilic, thus changing the surface charge from negative to positive. UV-irradiated Ti surfaces exhibit stronger osteoblast adhesion and more active cell spreading and promote cell differentiation, which potentially increases bone conductivity in dental implants (13-18). Furthermore, photofunctionalization has yielded consistently positive results in clinical studies (19-23) and was effective for various types of Ti implants.

Carbon, silicon, Ti, and oxygen species were detected on the surface of Ti meshes (Fig. 3). The amount of carbon on GC Ti mesh was equal to about 75% of the surface of Ti meshes, and was higher than the carbon content for ordinary Ti disks. This finding is attributable to carbon transfer from the gloves. Interestingly, a carbon content of as much as 75% was also detected in the AR group. Hence, medical gloves may be contaminated during packing, and contact with sterile gloves may thus not have further increased carbon content. In addition, silicon species were detected on AR Ti mesh but not on ordinary Ti disks, perhaps because Ti mesh was handled and packed with gloves. Owing to excessive adsorption of carbon and silicon atoms, Ti content on the surfaces of AR and GC Ti meshes was 2% and 1%, respectively. UV irradiation of mesh decreased the amounts of carbon and silicon species, thereby significantly increasing Ti concentration on the surfaces.

The GBR Ti meshes examined in this work are usually exposed to many other instruments (including scissors and handling equipment). We are planning future studies of the biological effects of contact with these instruments and their mitigation by UV irradiation, which will be followed by animal and clinical studies.

Contact with medical gloves causes structural and chemical contamination of Ti mesh and severely diminishes its bioactivity. UV treatment of contaminated mesh increases bioactivity to a level similar to or greater than that before contamination.

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**Conflict of interest**

The authors declare no conflict of interest.

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