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The effect of surface microgrooves and anodic oxidation on the surface characteristics of titanium and the osteogenic activity of human periodontal ligament cells

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ABSTRACT

Objective: The purpose of this study was to investigate the effect of titanium (Ti) surface microgrooves and anodic oxidation on the surface characteristics of titanium and the osteogenic activity of human periodontal ligament cells (PLCs) cultured on theses surfaces.
Design: Mechanically ground Ti was used as the control substratum (NE0). Truncated V-shaped microgrooves, 60 μm-wide and 10 μm-deep in cross-sections, were created on the Ti substrata by photolithography (NE60/10). Anodically oxidized Ti (NE0AO) and anodically oxidized microgrooved Ti (NE60/10AO) were also prepared. Scanning electron microscopy, X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) were performed for surface characterization. Cell proliferation assay, osteoblast differentiation assay, and quantitative real-time PCR analysis were performed to compare the osteogenic activity of PLCs on NE0, NE60/10, NEAO, and NE60/10AO.
Results: A decrease in the microgroove-width of NE60/10AO compared to NE60/10 due to Ti oxide layer generation by anodic oxidation was detected with XRD and XPS. Cell proliferation, osteoblast differentiation, and osteo-related gene expression were enhanced on the NE60/10AO substrata compared with NE0, NE60/10, and NE0AO.
Conclusions: The combination of Ti surface microgrooves and subsequent anodic oxidation treatment synergistically upregulated osteo-related gene expression, despite showing limited ability to increase cell proliferation and osteoblast differentiation levels in PLCs.

1. Introduction

Microgrooves on the surface of titanium-coated epoxy resin can enhance *in vitro* osteogenic activity.[1] This finding has prompted investigation into the effect of introducing nanoscale or submicroscale topography secondary to the microgrooves. Both microscale and submicroscale topographies are essential for enhancing osteogenic activity on Ti surfaces,[2] and attempts to use Ti microgrooves with submicroscale acid-etched roughness as an osteogenic activator have been successful.[3] Acid etching reduces hydrocarbon contaminants on Ti surfaces[4]; however, it can also remove the existing oxide layer.[3] Thus, there is a need to develop alternative surface treatments that retain and/or augment the Ti oxide layer in order to increase the biocompatibility and will positively enhance cell response to the Ti surface.

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Nanoscale topography created by anodic oxidation in combination with microgrooves should elicit a favourable cell response and may be an alternative surface treatment that retains the Ti oxide layer. Indeed, previous animal studies have confirmed that anodic oxidation of the Ti oral implant surface in combination with 110- or 200 μm-wide and 70 μm-deep macroscopic grooves stimulates bone formation along the grooves and increases the resistance to shear load at the bone-implant interface.[5,6] However, results from an organized comparison of various Ti surface modifications such as smooth, grooved, smooth-anodized, and grooved-anodized surfaces have not been reported. In this study, we hypothesized that Ti surface microgrooves and subsequent anodic oxidation would alter the surface characteristics of Ti, resulting in enhanced osteogenic activity of cells on the Ti substrata. Since microstructures such as truncated V-shaped microgrooves were verified to enhance osteobalst differentiation,[7] combination of microstructural and chemical modification was applied in this study in order to obtain a synergistic effect on increasing osteogenic differentiation by microgrooves and anodic oxidation.

Previous studies suggest that human periodontal ligament cells (PLCs) can be used as an alternative to human bone marrow mesenchymal stem cells for determining the osteogenic potential of Ti and for assessing osseointegration in Ti oral implants. PLCs, similar to the human bone marrow mesenchymal stem cells (MSCs), can differentiate into several types of specialized cells.[8] PLCs proliferate rapidly and show highly expressed osteocalcin gene on rough Ti surfaces.[9] PLCs also showed comparable utility to MSCs for alveolar bone regeneration in a canine peri-implant defect model,[10] suggesting the efficacy of using PLCs in place of MSCs for various types of cell research in Oral Implantology.[8]

The Purpose of this study was to determine the effect of Ti surface microgrooves and anodic oxidation on the surface characteristics of Ti and on the osteogenic activity of PLCs.

2. Materials and methods

2.1. Fabrication of titanium substrata and anodic oxidation

Commercially pure titanium (cp-Ti) sheets of ASTM grade 2 (TSM tech Co. Ltd., Seoul, Korea) were buffered with emery powder compounds and mechanically ground using wool wheel to obtain Ra $\leq$0.1 μm. The Ti sheets were ultrasonically cleaned with pure isopropyl alcohol, acetone, and distilled water, respectively for 10 min. Cleaned sheets were cut into round discs of various diameter used as the control Ti substratum, NE0. Using photolithography, surface microgrooves and ridges having uniform widths, in parallel direction to the previously created texture, were created on Ti as previously described.[11] Microgrooves with 60 μm width, 10 μm depth, and 40 μm bottom width were created on Ti substrata (NE60/10) (Fig. 1). The anodically oxidized substrata were fabricated by using a platinum plate as the cathodic electrode and the NE0 and NE60/10 Ti substrata as the anode electrode. The anodically oxidized Ti (NE0AO) and anodically oxidized microgrooved Ti (NE60/10AO) were fabricated in a

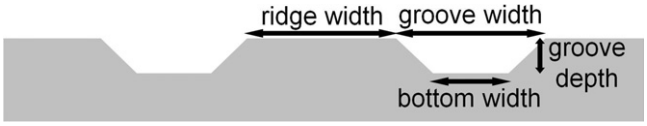

Fig. 1 – A schematic cross-sectional image and the structural nomenclature of the fabricated microgrooved Ti substrata using photolithography. Note that the ridge width and groove width were designed to be uniform in dimension, 60 μm. The groove depth is 10 μm and, according to the isotropic principle, the bottom width inside the microgrooves is 40 μm.

0.5 M H_2SO_4 electrolyte solution by supplying a constant voltage of 160 V. Anodic oxidation was carried out at room temperature with a direct power supply system for 180 s. During the anodic oxidation process, a magnetic stirrer achieved a homogeneous electrolyte solution. After anodic oxidation, the Ti substrata were rinsed with distilled water and dried in an oven. For all experiments, fabricated Ti substrata were cleaned three times in an ultrasonic device with sterile distilled water for 30 min. After being washed another three times with distilled water, Ti substrata were dried at room temperature overnight prior to use.

2.2. Scanning electron microscopy

The surface morphology of NE0, NE60/10, NEAO, and NE60/10AO was visualized using field emission scanning electron microscopy (FE-SEM; JSM-6700F, JEOL, Tokyo, Japan).

2.3. X-ray diffraction

The crystalline structure of NE0, NE60/10, NEAO, and NE60/10AO was evaluated using X-ray diffraction (XRD; D/max2200, Rigaku, Tokyo, Japan) with Cu Kα radiation at 40 kV and 30 mA, and at angles from 10° to 80° with a scanning rate of 1.2°/min.

2.4. X-ray photoelectron spectroscopy

The chemical composition and binding state of NE0, NE60/10, NEAO, and NE60/10AO were analysed using X-ray photoelectron spectroscopy (XPS; PHI5000, Physical Electronics Inc., Chanhassen, MN, USA). XPS experiments were performed in an ultra-high vacuum using Al Kα (1486.6 eV) radiation. XPS spectra were acquired from the specimen surface areas with diameter of approximately 0.3 mm. Only one spot on each specimen was analysed and total scan were performed in the binding energy range of 0–1200 eV with 1 eV step. High-resolution XPS analysis of the O1s and Ti2p peaks was performed in the range of 542–522 eV and 474–449 eV, respectively, with a step of 0.05 eV. The scan spectra were profile fitted with skewed Gauss–Lorenz line shapes using the XPS peak 4.0 software attached to the XPS analyzer.

2.5. Cell culture

PLCs were acquired from periodontal ligament tissues of freshly extracted bicuspid root surfaces in patients undergoing