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学 位 論 文 題 名

Surface Control of Titanium by Electrochemical Modification for Biofunctional Improvement and its Application

（生体機能性向上のための電気化学的処理による
チタンの表面制御とその応用）

学位論文内容の要旨

Titanium and titanium alloy have the characteristic of good biocompatibility, and they have been used widely as dental implants, orthopedic surgical hip joints, etc.. Many studies examining various surface modification methods to improve the surface for dental and orthopaedic implants have been reported. Among the many surface modification methods, anodic spark oxidation can satisfy simultaneously the abovementioned advantages, therefore many researchers have been carrying out studies on the anodic oxidation. In particular, TiO_2 nanotube (TN) arrays produced by electrochemical anodization have been extensively explored in recent years as a new biomaterial for implants, drug delivery system, immunoisolations, biosensors, cell growth, bioartifical organs and tissue engineering. In this study, the electrochemical modification by anodic oxidation was performed to increase bioactivity and biocompatibility of titanium. In addition, we evaluated the characteristics of modified layer by changing the parameters of process. Moreover, we fabricated a novel platform on Ti implants by loading gelatin stabilized gold nanoparticles into anodized titania nanotube using simple lyophilization method.

In section 1, electrochemical treatments were performed while applying a direct current, a pulse current, and a reverse pulse current during anodic spark oxidation. A mixed solution of 0.015M DL-α-GP (DL-α-Glycerophosphate Disodium salt) and 0.2M CA (Calcium Acetate) was used as the electrolyte. The micropore size generated after anodic spark oxidation was smallest in the group exposed to the reverse pulse current followed in order by the pulse current and direct current. Anatase was the major crystal phase of the TiO_2 produced on the surfaces treated with 280 V, and the rutile phase was additionally detected in the group treated with 320 V. The crystals precipitated on the surface after the hydrothermal treatment were HA crystals, and the crystals had a polygonal bar shaped needle structure. Good activity was observed in the 320 V pulse treated group in which very thin needle shaped crystals being observed after immersing the samples in Hanks' solution for 4 weeks. The cell viability was increased greatly by anodic spark oxidation, and the surface roughness was also increased. It is believed that the excellent characteristics of the surface treated using a pulse current is suitable for applications to biomaterials.

In section 2, the bioactive and electrochemical properties of titanium implant materials with a nanotube surface treatment and various types of post-treatments were examined. Two types of amorphous TiO_2 nanotubes were grown homogeneously on the surface: one with a larger diameter (approximately 85 nm) and one with a smaller diameter (approximately 50 nm). Amorphous TiO_2 nanotubes were partially crystallized to anatase and rutile by heat treatment at 500℃ for two hours. The corrosion potential (E_{corr}) of the heat-treated sample (HT) had a novel value of 0.102 V due to the stable TiO_2 crystal phase compared to the -0.106 V observed in the anodic oxidation sample (AN). The corrosion current density (I_{corr}) ranged from 0.20 to 0.64 ㎂/㎠ according to the post-treatment conditions. After evaluating the hydroxyapatite-forming ability by immersion in a simulated body fluid (SBF) solution, the CP process induced the adsorption of Ca and P onto HT. A comparison of the time-dependent amount of Ca and P adsorption showed that Ca adsorption plays a role in determining the rate at which hydroxyapatite (HA) is formed. For the induction of HA formation, a level of Ca adsorption above a critical level is required.

In section 3, Gelatin-stabilized gold nanoparticles (AuNPs-gelatin) with controlled particle size were synthesized with simple variation of concentration of gelatin by reducing in situ tetrachloroauric acid with sodium citrate. The nanoparticles showed excellent colloidal stability. Transmission electron microscopy (TEM) revealed the formation of well-dispersed gold nanoparticles (AuNPs) with different sizes. The methodology produces particles 10–15 nm in size depending on the concentration of gelatin used. The measured AuNPs are 10, 11, 12, 13, 14, and 15 nm for AuNPs-gelatin 1, 0.5, 0.25, 0.1 and 0.05%, and pure AuNPs, respectively. The AuNPs-gelatin exhibit size-dependent localized surface plasmon resonance behavior as measured by UV–visible spectroscopy. UV–vis spectroscopy and TEM results suggest that higher concentration of gelatin favor smaller particle size and vice versa. FTIR spectroscopy analysis of AuNPs-gelatin revealed the amino bands and carboxyl peak of gelatin. The crystalline nature of AuNPs was investigated by X-ray diffraction.

In section 4, we fabricated highly ordered TiO_2 nanotube arrays via electrochemical anodization of high purity Ti plate in fluorine containing electrolytes with the addition of cationic and anionic surfactant, respectively. The effects of anodization parameters (both surfactant in electrolyte and applied voltage) on nanotube morphology were comprehensively investigated. It was observed that the nanotube topography, diameter, length and wall thickness were clearly influenced by the addition of surfactant in the electrolyte and applied voltage. As a consequence, TiO_2 nanotube arrays with average tube diameters ranging from 65 to 120 nm and wall thicknesses ranging from 20 to 28 nm were obtained. The average tube diameter was found to decrease with using surfactant in the electrolyte, while the length of the tube was found to increase. A linear increase in nanotube diameter and length with increasing applied potential is evident. At 30 V tube to tube spacing is increased using surfactant as a composition of electrolyte. At 40 V tubular surface morphology completely collapsed but no effect on tube length in electrolyte without surfactant. The TiO_2 structure depends on the heating condition, amorphous phase is found at room temperature, the anatase phase is the predominant phase at 500°C in the XRD pattern. The mean average roughness (R_a) value of nanotube surface fabricated with addition of surfactant in electrolyte is lower than without surfactant.

In section 5, we investigated in vitro cell-materials interactions using MC3T3-E1 mouse osteoblast cells on four different types of titanium surfaces: polished Ti surface, TiO_2 nanotube surfaces fabricated in fluorinated glycerol solution, fluorinated glycerol solution with 1wt% anionic surfactant sodium dodecyl sulphate, and fluorinated glycerol solution with 1wt% cationic surfactant cetyl trimethyl ammonium bromide, respectively. Four different types of surfaces show distinctive surface morphologies and geometrical features. Mouse osteoblast cell growth behavior was studied with four different surfaces using MC3T3-E1 cell line for 1, 3 and 5 days. When anodized surfaces were compared for cell-materials interaction, it was noticed that each of the surfaces has different properties, which led to variations in cell-materials interactions. Colonization of the cells was noticed with distinctive cell-to-cell attachment in the TN. Good cellular adherence with extracellular matrix extensions in between cells was noticed for samples TN. The TiO_2 nanotubes grown in surfactant assisted fluorinated electrolyte did not show significant cell growth on the surface and some cell death was noticed. Cell adhesions and differentiation and alkaline phosphatase results were more pronounced on TN surface. 3- (4, 5-Dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium assays also showed increase in living cell density and proliferation with TN surfaces. It was clear that rough surface morphology was important factor for better cell materials interaction.

In section 6, we developed a very simple method for surface modification of TiO_2 nanotube to tailor new interfacial properties important in many biomedical applications. TiO_2 nanotubes were fabricated by electrochemical anodization of titanium plate using 70wt% glycerol/30 wt% H_2O/1wt% NH_4F. Lyopholization method has been applied to impregnate gelatin stabilized gold nanoparticles into the TiO_2 nanotube followed by vacuum dry.

In conclusion, such a novel TiO_2 nanotube platform on Ti implant surface can be useful as an excellent bioactive surface for orthopedic and dental applications as well as the cell adhesion and bone growth on implant surface can be significantly accelerated.

学位論文審査の要旨

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Surface Control of Titanium by Electrochemical Modification for Biofunctional Improvement and its Application

(生体機能性向上のための電気化学的処理による チタンの表面制御とその応用)

審査は、審査員が一同に会し、申請者に対して提出論文とそれに関連した学科目について口答試問により行われた。以下に、提出論文の要旨と審査の内容を述べる。

［目的］　チタン(Ti)の生体活性と機能性を向上させるために、電気化学的表面処理を行い、処理条件に対する特性変化の評価、アパタイトやチタニアナノチューブ形成の最適条件を調べ、さらにナノチューブ内に金ナノ微粒子を担持し、人体埋入時の化学的安定性と薬剤除放性治療剤として応用するための基礎研究を行うことを目的とした。

［材料と方法］　Ti の表面形状と粗さを調整するために陽極酸化法を適用した。

(1)陽極酸化条件：-　多孔性酸化皮膜を形成するため、電解液として 0.015M DL-α-GP と 0.2M CA の混合溶液、電源として直流、パルス、逆パルスの各電源を適用し、280V 及び 320V の高電圧下で陽極酸化を行った。さらに熱水処理を行い、各条件で得られた表面特性を評価した。

(2)チタニアナノチューブの形成：20V 及び 30 mA/cm² の低電圧領域で、NH_4F : H_2O : Glycerol を 1 : 20 : 79wt%の比で調合した電解液内で陽極酸化を行い、さらに熱処理及び石灰化前処理を行い電気化学的特性と生体適合性を評価した。

(3)金ナノ微粒子の作製：クエン酸ナトリウムを添加した四塩化金酸をゼラチン濃度を変化させながら還元し、粒径を調節したゼラチン安定化金ナノ微粒子を作製しその特性を評価した。

(4)ナノチューブの調整：ナノチューブの直径、長さを調整するために、界面活性剤を添加して 20V, 30V, 40V で表面処理し、電圧及び界面活性剤の皮膜特性に及ぼす影響を評価した。

(5)ナノチューブ内部への金ナノ微粒子の担持：金ナノ微粒子を Ti 表面に形成されたチタニアナノチューブ内部に挿入し、その特性を評価した。

(6) 金ナノ微粒子担持チタニアナノチューブ表面被覆チタンの生体親和性評価：骨芽細胞を培養し、その形態観察と細胞活性、ALP 活性から生体適合性を評価した。

［結果と考察］　320V の表面処理で得られたチタニアの結晶構造はアナターゼとルチルであり、水熱処理群ではハイドロキシアパタイト相が検出された。ハンクス溶液に４週間浸漬することにより、高い生体活性が得られた。低電圧領域で形成された非晶質のチタニアナノチューブは熱処理によりアナターゼとルチルに結晶化した。SBF 溶液浸漬時のアパタイト形成では陽極酸化＋熱処理＋水熱処理群で最良の結果を示した。ナノチューブの直径と長さは付加電圧に比例して増加した。界面活性剤を電解液に添加し 20V で処理すると均一の直径のナノチューブが得られた。平均のナノチューブ外径、内径、長さは 30V と 40V で増加した。金ナノ微粒子担持チタニアナノチューブ表面上で骨芽細胞は大きく伸展して糸状仮足を多数発達させ高い親和性を示した。内径が小さく長さが長いナノチューブは体内での薬剤除放の調節がしやすく、ゼラチン安定化金ナノ微粒子担持チタニアナノチューブは、体内除放機能性インプラントのプロトタイプとして今後の臨床治療への応用の観点から画期的と考えられる。

これに対して審査委員から、

① チタニアナノチューブの長さと径の調整方法
② アモルファスと結晶相のチタニアナノチューブの強さ
③ チタニアナノチューブの長さの強度に及ぼす影響
④ 金ナノ粒子の作製方法は他の金属にも適用できるのか？
⑤ 金ナノ粒子の作製時のゼラチンの効果
⑥ 金ナノ粒子はチタニアナノチューブのチューブ内にどのくらい入るのか？
⑦ 細胞の形態と MTT アッセイの結果の関係
⑧ 金ナノ粒子を担持したチタニアナノチューブ上で細胞がよく伸展する理由
⑨ 長さが大きめのチタニアナノチューブコーティングを関節軟骨部に適用する可能性
⑩ 今後の応用として、歯科領域以外の可能性は考えられるか？

等の質問やコメントが出され、論文提出者はそれぞれに的確に回答し、考察や展望についても言及した。

論文提出者は、本研究を通して各種成長・抑制因子や薬剤除放性等の機能性を付与したインプラントの可能性を提起し、臨床応用につながる将来性の点においても評価できる。よって、学位申請者は博士（歯学）の学位授与に値するものと判断し、主査ならびに副査は合格と判定した。