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Coating of Titanium Plate by Photocurable Azidophenyl Chitosan Derivative for Application to Implants

Si Yoong Seo,[1] Shin Hye Park,[1] Hyung Jae Lee,[1] Yun Heo,[1] Ha Na Na,[1] Kwang Il Kim,[1] Jae Hong Han,[1] Yoshihiro Ito,[2] Tae Il Son[1]

[1]Department of Biotechnology and Bio-Environmental Technology (BET) Research Institute, Chung-Ang University, Anseong, Gyeonggi-do 456-756, Korea

[2]Nano Medical Engineering Laboratory, Riken Advanced Science Institute, 2-1 Hirosawa, Wako-Shi, Saitama 351-0198, Japan

Correspondence to: T. I. Son (E-mail: tisohn@cau.ac.kr)

ABSTRACT: Titanium (Ti) is widely used as an implant material. There are various kinds of Ti surface modification methods to facilitate effective osseointegration. In this study, UV-curable azido-LMC (azidophenyl low molecular weight chitosan) was used to modify the Ti surface. Here we suggest a novel Ti coating material that confers complex, diverse improvements to the Ti surface through a simple process. First, a cytotoxicity test of azido-LMC against osteoblast MG-63 was performed. The curing ratio dependent on UV irradiation time and concentration was determined by a comparison of weight. A 5% azido-LMC solution, which showed a wide curing ratio range, was used to investigate the surface properties. The contact angle value was measured to compare hydrophilicity, and osteoblast MG-63 cells were cultured on the coating surface. The Bradford assay was used to assess the protein immobilization capability. Hydroxyapatite, which has a beneficial influence on osseointegration, was included on the coating surface and observed by scanning electron microscopy. Surface roughness was measured by atomic force microscopy. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 4322–4326, 2013

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INTRODUCTION

Titanium (Ti) has been used as an implant material in dental and orthopedic applications.[1] Many studies have shown that modified Ti implant surfaces have advantages in osseointegration compared to the typical Ti implant surface. Therefore, much effort has gone into the modification of Ti implant surfaces. According to these studies, the advantages are (i) hydrophilic implant surfaces allow for greater interaction with biological fluids, cells, and bone tissues leading to greater bone-to-implant contact versus hydrophobic implant surfaces.[2,3] (ii) Rough implant surfaces improve the early fixation and long-term mechanical stability of implants compared to smooth implant surfaces.[4,5] (iii) Since hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] has a similar chemical composition to the mineral aspect of bone, coating implant surfaces with HA allows for direct bonding between the implant and bone leading to better osseointegration and improved biocompatibility.[6–9] (iv) Implants coated with bone-stimulating agents such as growth factors, extracellular matrix (ECM), or antiresorptive drugs facilitate better osseointegration.[10–13] Since modified Ti implant surfaces have the advantages mentioned above, many surface modification methods have been reported and are used in the implant industry such as plasma-spraying, grit-blasting, acid-etching, electrophoretic deposition, sputter deposition, and sol–gel.[14–16] However, the issue of taking advantage of combinations through a single process still remains.

In this study, we attempted to coat Ti implant surfaces with photocurable natural polymers, especially chitosan derivatives. Photocurable natural polymers have received a considerable amount of attention in the field of tissue engineering, biomaterial coating, and drug delivery mainly due to their mild cross-linking ability compared to other methods.[17,18] Photocurable natural polymers can be used as matrices to immobilize various bioactive compounds such as HA, growth factors, drugs, proteins, and DNA.[19–21]

Chitosan was used as a base polymer in this study. A deacetylated form of chitin, chitosan is a natural polymer composed of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units.[22] Many studies have revealed its unique biological properties that include wound healing, stimulating effects on the secretion of fibroblast growth factor, in addition to antibacterial, hemostatic, fungistatic, antitumor, and anticholesteremic

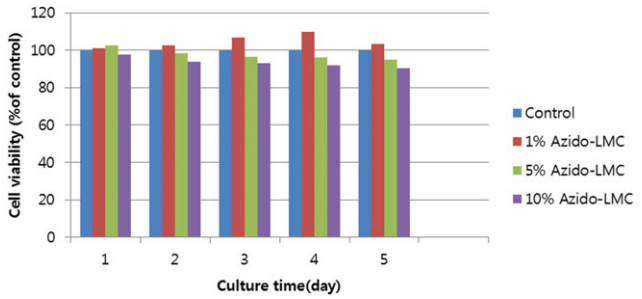

Figure 1. Viability of human MG-63 osteoblasts in DMEM containing 10% (vol/vol) FBS and 2% (vol/vol) penicillin-streptomycin after treatment with various concentrations of azido-LMC solution (1, 5, and 10% F-LMC) as determined by the WST assay (control: not treated with azido-LMC). Within 90.4%–109% of surviving cells number was observed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

activities.[23–26] In this study, we investigated the possibility of using Ti implant surfaces coated with photocurable chitosan derivatives (azidophenyl low molecular weight chitosan; azido-LMC)[21] as a novel coating material in order to take advantage of the combination of modified surfaces through a single process.

EXPERIMENTAL

Materials

Azido-LMC was synthesized according to a previously described method.[21] The Ti plate consisted of a glass plate coated with Ti (Imvac, pressure, 2×10^{-6} Pa). A UV irradiator, Spot cure-9, was purchased from Ushio, Japan. Human MG-63 osteoblasts were obtained from the Korean Cell Line Bank. Cell culture reagents, including Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco (Life technologies Korea, Seoul, Korea).

Trypsin-ethylenediaminetetraacetic acid (EDTA), BSA (bovine serum albumin), and HA (synthetic) were purchased from Sigma-Aldrich Korea (Yong-In, Korea). Cell counting kit-8 (CCK-8) for the water-soluble tetrazolium (WST) assay was purchased from Dojindo, Japan.

WST Assay Test for Cytotoxicity

Cytotoxicity was assessed using the WST salt assay. Osteosarcoma MG-63 fibroblast cells were cultured in DMEM containing 10% FBS and 2% penicillin-streptomycin. An 80 μL aliquot of MG-63 cells (3×10^4 cells/mL) was seeded in a 96-well plate (DK-4000 Roskilde, Kamstrup Vej 90, Nunc A/S, Denmark), and incubated at 37°C in a 5% CO_2 environment for 24 h. Ten microliters of various concentrations (1%, 5%, and 10%) of azido-LMC solution were added to the plate and incubated for the indicated length of time (24, 48, 72, 96, and 120 h). In addition, 10 μL of CCK-8 solution was added to each well of the plate and incubated for 2 h. The absorbance at 450 nm was measured using a microplate reader.

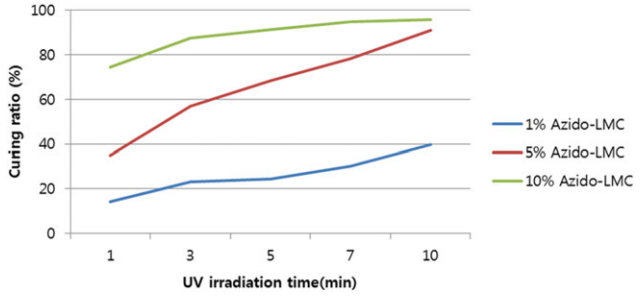

Figure 2. The curing ratio was influenced by UV irradiation time and the concentration of azido-LMC. The higher the concentration and the longer the UV irradiation time, the higher the curing ratio. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

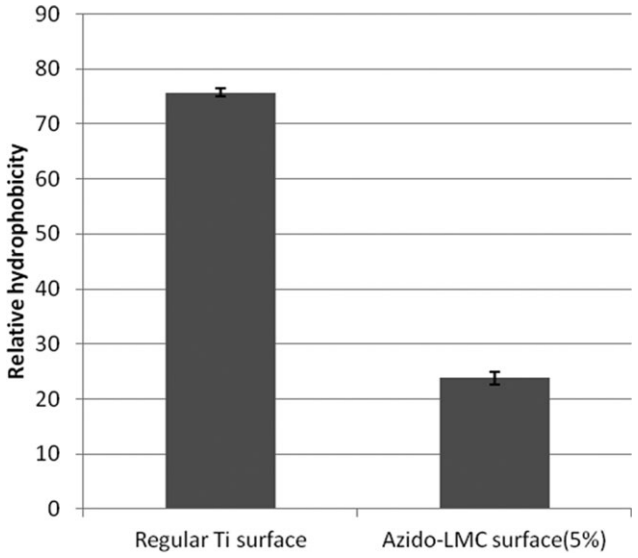

Figure 3. The contact angle of azido-LMC was significantly smaller than that of the regular Ti surface, indicating that the azido-LMC surface was considerably more hydrophilic than the Ti surface.

Curing Ratio

The curing ratio was determined by comparing the weight of azido-LMC on the Ti plate. Various azido-LMC solutions (1%, 5%, and 10%) were casted at 20 μL each on a Ti plate. Each casted Ti plate was exposed to a UV irradiator for different time periods (1, 3, 5, 7, and 10 min) and weighed. The Ti plates containing cured azido-LMC were immediately washed using distilled water for 5 min on a shaker with mild shaking, dried in an incubator at room temperature without any light, and weighed.

The curing ratio was calculated as follows:

$$\text{Curing (\%)} = (\text{washed weight (g)})/(\text{initial weight (g)}) \times 100$$

Initial weight: Cured azido-LMC weight; washed weight: Remaining weight of the cured azido-LMC after washing.

Contact Angle Measurement

Surface contact angle measurements were performed to compare the hydrophilicities between the regular Ti surface and the cured azido-LMC surface. Twenty microliters of azido-LMC solution (5%) were dropped on the Ti plate and spread laterally followed by exposure to UV light (48 mW/cm^2) for 5 min. The plates were then washed with dH_2O and dried. The contact angles

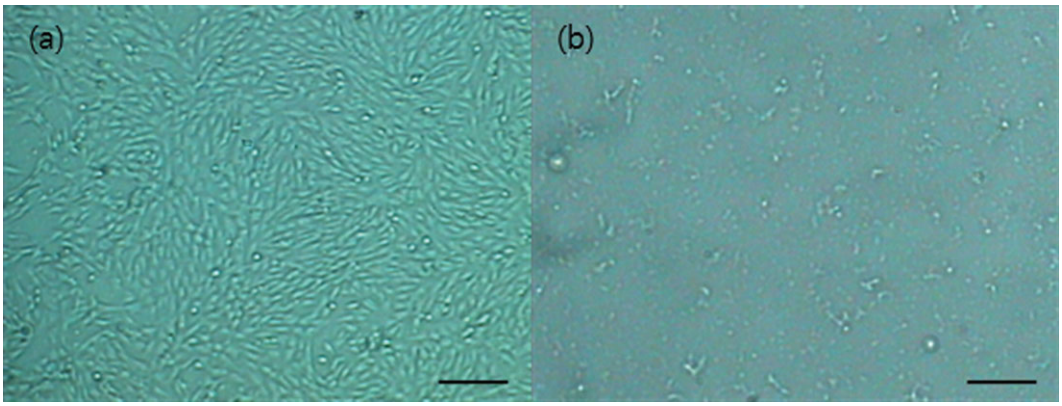

Figure 4. Proliferation of the MG-63 osteoblasts seeded on the azido-LMC-cured surface (a) and on the surface of regular Ti (b); scale bar = 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

values were measured using a CA-W Automatic Contact Angle Meter (Kyowa Interfaces, Co., Saitama, Japan).

Cell Morphology on the Cured Azido-LMC

Osteosarcoma MG-63 fibroblast cells were cultured on a Ti plate coated with azido-LMC (20 μL of 5% Azido-LMC, 5 min UV exposure) in DMEM containing 10% FBS and 2% penicillin-streptomycin. A 1 mL aliquot of MG-63 cells (3×10^4 cells/mL) was seeded in a 12-well plate (Falcon 3043, Becton Dickinson Labware, Franklin Lakes, NJ) containing Ti plates. After 18 h of incubation at 37°C in a 5% CO_2 environment, the cells on the Ti plate coated with azido-LMC were compared to the cells on the noncoated Ti plate through microscope images and a WST salt assay.

Protein Release Test

A 5% azido-LMC solution was the most sensitive to UV irradiation time and was thus used for the protein release test. BSA was immobilized with azido-LMC on the Ti plate, and the amount of released BSA was measured by Bradford's method. The 5% Azido-LMC solution was prepared and BSA was added (50 μg/μL). The solution containing azido-LMC and BSA was casted at a volume of 20 μL on the Ti plates and then exposed to UV light (48 mW/cm^2) for various time periods (1, 3, and 5 min). The coated Ti plates were washed with dH_2O to remove the nonimmobilized BSA and azido-LMC and then immersed in dH_2O. The absorbance of the dH_2O containing BSA was measured every 24 h by Bradford's method using a UV/vis spectrophotometer (Optizen 3220UV, Mecasys Co., Ltd. Daejeon, Korea).

Immobilization of HA and Surface Roughness Measurement

The azido-LMC (5%) was cured with HA powder (10 μg/μL, Ø < 50 μm) on the Ti plate by UV irradiation for 5 min and washed with dH_2O to remove the nonimmobilized materials. The surface was then analyzed by scanning electron microscopy (SEM; S-3400N, Hitachi, Kyoto, Japan) and atomic force microscopy (AFM; XE-100, PSIA, Korea).

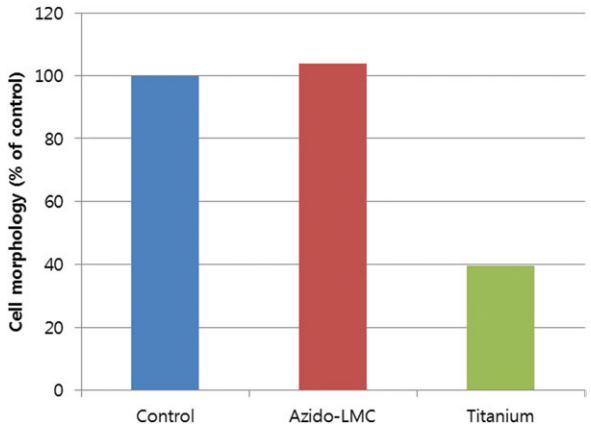

Figure 5. Proliferation of human MG-63 osteoblasts on the cured azido-LMC surface and regular Ti surface as determined by the WST assay (control: cell culture-treated plate). Cell number on azido-LMC surface was 103.9% that of the control. In contrast, cell number on the regular Ti surface was less than 40% that of the control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

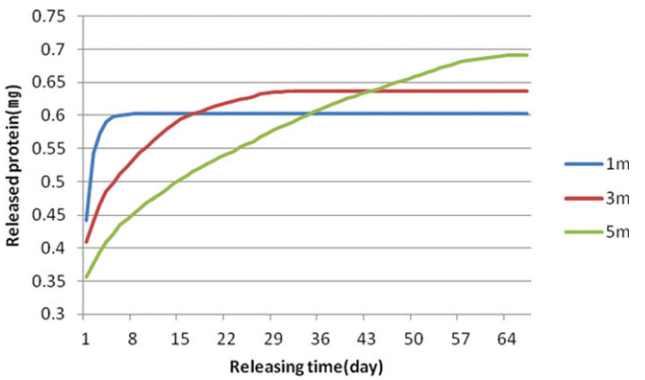

Figure 6. Twenty microliters of 5% azido-LMC solution was cured with protein BSA (50 μg/μL). The amount of immobilized BSA increased depending on the UV irradiation time [1 min: 60.3 ± 1.2%, 3 min: 63.7 ± 2.0%, 5 min: 69.1 ± 2.4%]. The BSA release time was longer depending on the UV irradiation time (1 min: 8 days, 3 min: 31 days, 5 min: 64 days). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Average Surface Roughness Determined by AFM

	Regular Ti	Azido-LMC	Azido-LMC + BSA	Azido-LMC + HA	Azido-LMC + BSA + HA
Average roughness	0.488 ± 0.008	12.008 ± 0.351	16.452 ± 1.358	67.610 ± 0.025	71.351 ± 4.667

AFM, atomic force microscopy; BSA: bovine serum albumin; HA: hydroxyapatite.
The regular Ti surface had the lowest roughness and the azido-LMC coating increased the surface roughness. Surfaces containing BSA (50 μg/μL) showed a slight increase in roughness, and surfaces containing HA (10 μg/μL) exhibited significantly increased roughness.

RESULTS AND DISCUSSION

Cytotoxicity Test of Azido-LMC

The lack of cell toxicity is an essential prerequisite for medical applications. Therefore, in this study, the WST assay was carried out to compare cell proliferation in media containing various concentrations of azido-LMC using regular media as a control. There was no significant difference in the number of surviving cells cultured in media treated with the different concentrations of azido-LMC (0%, 1%, 5%, and 10%) compared to the control (Figure 1). Within 90.4%∼109% of surviving cells number was observed.

Curing Ratio

The physical properties of the prepared azido-LMC were altered after UV irradiation. At first, the azido-LMC was water soluble, but it became water insoluble and formed a hard film after UV irradiation. The curing ratio was influenced by the azido-LMC concentration and UV irradiation time. The 1% azido-LMC solution exhibited a slight curing ratio (14–40%), and the 5% azido-LMC solution showed a wide curing ratio range (35–90%). The 10% azido-LMC solution exhibited the highest curing ratio (74–96%) (Figure 2).

Contact Angle Measurement

The contact angle of the cured azido-LMC surface was half of that measured for the regular Ti plate surface. A lower contact angle indicates greater hydrophilicity (Figure 3). The contact angle values measured in this study did not absolutely indicate hydrophilicity, but it was clear that the azido-LMC surface was more hydrophilic than the regular Ti surfaces of currently used implants. This indicates that coating Ti implant surfaces with azido-LMC can change the hydrophobic surface of regular Ti into a hydrophilic surface.

Cell Proliferation on the Cured Azido-LMC

MG63 osteoblast cells grew well on the cured azido-LMC surface in comparison to the regular Ti surface (Figure 4). Cell proliferation on the azido-LMC surface was compared to cell proliferation on the regular Ti surface using the WST assay. There was no remarkable difference in cell proliferation between the azido-LMC surface and the control, a cell-culture treated surface. Cell proliferation on azido-LMC surface was 103.9% that of the control. In contrast, cell proliferation on the regular Ti surface was less than 40% that of the control (Figure 5).

Protein Release Test

The longer UV irradiation time led to greater BSA immobilization and longer BSA release time. The sample irradiated for 1 min immobilized 60.3 ± 1.2% of the BSA, which was then completely released after eight days. The sample exposed to UV light for 3 min immobilized 63.7 ± 2.0% of the BSA, which was then completely released after 31 days. Finally, the sample exposed to UV light for 5 min immobilized 69.1 ± 2.4% of the BSA and took 64 days for complete release (Figure 6).

Immobilization of HA and Surface Roughness Measurement

The AFM data (Table I) indicates that the regular Ti surface had the lowest average roughness value of 0.488. The surface roughness increased with the azido-LMC coating. The surface containing BSA showed a slight increase in roughness, and that containing HA showed a significantly increased average

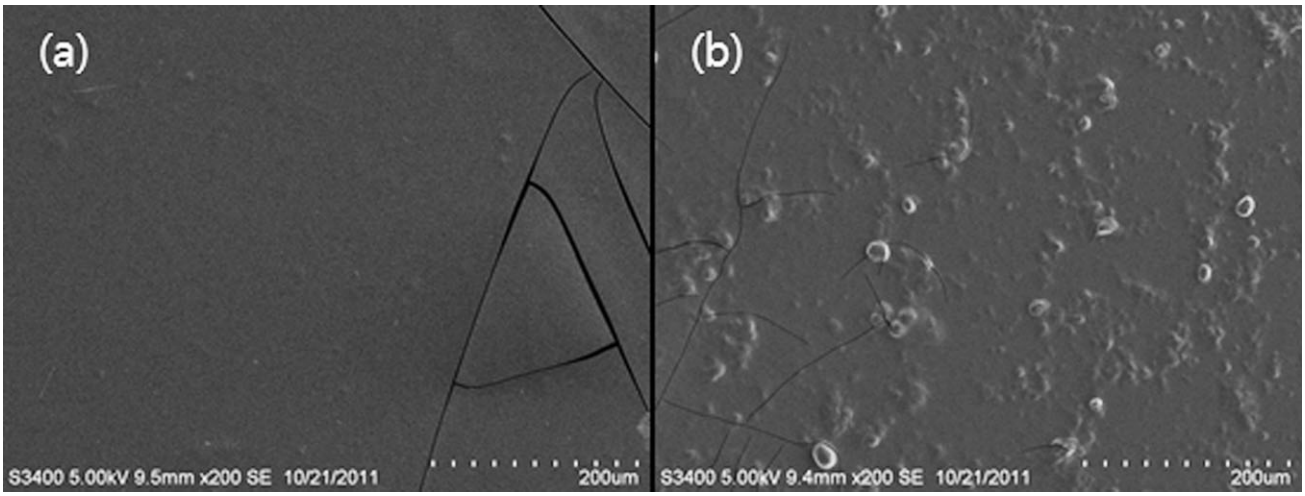

Figure 7. SEM images of the surfaces coated with azido-LMC (a) and with azido-LMC and HA particles. (b) HA particles, which have a beneficial influence on osseointegration, were buried under the cured azido-LMC surface.

roughness value greater than 67.610. The SEM images indicated that the azido-LMC immobilized HA internally (Figure 7).

CONCLUSIONS

In this study, azido-LMC was tested as a novel Ti coating material. According to the results, azido-LMC was not toxic to osteoblasts. The coating surface solidity value was altered depending on the concentration of the azido-LMC solution and UV irradiation time. Therefore, the biodegradation time of the coating surface and protein release time was adjustable. These findings indicate that azido-LMC can be used as a sustained release delivery system for Ti surfaces.

The azido-LMC coating surface was more hydrophilic than the regular Ti surface according to the contact angle value, allowing for greater interaction with biological fluids, cells, and bone tissues to facilitate better bone-to-implant contact than hydrophobic surfaces. The photo immobilization method using azido-LMC immobilized not only proteins but also huge particles such as hydroxyapatite, which enables effective osseointegration. Coating with hydroxyapatite considerably increased the surface roughness of the implant. The cured azido-LMC surface exhibited greater cell proliferation than the regular Ti surface. The cell proliferation on the azido-LMC surface was no different than that on the cell culture-treated plate. In this study, a single process was used to fabricate UV curable azido-LMC to create a novel surface that had various properties suitable for effective osseointegration. This study provides evidence for the potential of azido-LMC as a novel metal surface coating material.

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