# Biofunctionalized Titanium with Anti-Fouling Resistance by Grafting of Thermo-Responsive Polymer Brushes for Prevention of Peri-implantitis

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# Electronic Supplementary Information (ESI)

## Experimental: materials and methods

1. Materials.

Titanium disks (diameter: 12 mm, thickness: 1 mm) were supplied by Biotem Co., Ltd. (Korea). The glycidyl methacrylate (GMA) monomer (97%), *tert*-butyl peroxide (TBPO) initiator (98%), and amine-terminated poly( N-isopropylacrylamide) (Mn = 2500, Sigma-Aldrich) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *Porphyromonas gingivalis* ATCC 33277 (*P. gingivalis*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*) were obtained from the American Type Culture Collection (Manassas, VA). MesenPRO RS™ medium (MPRO medium) and StemPro® Human ADSCs (adipose derived stem cells) were obtained from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS), streptomycin, and penicillin were purchased from GIBCO® (Gran Island, NY). All other reagents and solvents were of analytical grade and used without further purification.

2. Preparation of pGMA deposited Ti by iCVD.

The method for iCVD deposition of pGMA has been previously described.[1-4] Briefly, the pGMA polymeric films were deposited on the Ti substrates in a custom-built iCVD chamber (Daeki Hi-Tech Co. Ltd, Korea). The GMA monomer was vaporized and fed into a 35℃ heated chamber. An initiator (TBPO) was fed into the chamber without heating and mixed with the GMA monomer in the chamber. The flow rate of the initiator and the GMA monomer were kept at 1.0 sccm and 2.0 sccm, respectively. The deposition process was performed at a filament temperature of 200 ℃ and a subtracted temperature of 25 ℃. The filament temperature was continuously controlled by using a thermocouple directly attached to one of the filaments. The *in situ* growth rate of the pGMA films was monitored using a He-

Ne laser (JDS Uniphase, Milpitas, CA, USA) during the deposition process in order to achieve a predefined thickness of approximately 60 nm.

3. Preparation of pGMA deposited Ti surface grafted with PIPAAM.

After deposition of the pGMA films on Ti substrates, amine-terminated PIPAAM was grafted on the Ti surface by using a dip-coating method. Prior to dip-coating, amine-terminated PIPAAM was dissolved in distilled water at a concentration of 1g/30ml at 50 ℃. Subsequently, Ti substrates were placed in the PNIPAM solution for 24 hours with agitation. After dip-coating, the PIPAAM coated Ti-substrates were rinsed with distilled water and sterilized by UV light prior to *in vitro* testing.

4. Analytical equipment.

Surface observations were measured by a field emission-scanning electron microscope (FE-SEM) using a LEO supra 55, Genesis 2000 (Carl Zeiss, Germany) at an acceleration voltage of 10 kV. Additionally, the surface was observed using Atomic Force Microscopy (AFM, Park-System XE-100, Korea) at a scan area of 10 x 10 $um^2$ measurements at room temperature. X-ray photoelectron spectroscopy (XPS) was performed using a K-Alpha (Thermo Electron, UK) to confirm the treated surface chemistry. Water contact angle measurements were conducted on a contact angle meter (Phoenix 150, SEO, Korea) using an 8 μl distilled water droplet at room temperature and 50 ℃, respectively. Focused ion beam milling was performed using a Helios NanoLab 600 (FEI Company, Netherlands) with energy dispersive X-ray analyzer (EDX, EMAX-7000, Horiba Co. Ltd., Japan). Cellular adhesion on the Ti surface was observed using confocal laser scanning microscopy (CLSM, Eclipse E600W, Nikon, Japan). All analysis were performed in triplicate.

5. Observation of hADSC cell adhesion on treated Ti surfaces.

hADSCs were used to examine cellular adhesion on Ti surfaces. The cell culture procedure of hADSCs was same as described in our previous research publications.[4-6] hADSCs were cultured using MesenPRO RS™ medium (MPRO medium, Invitrogen) and the third passage of ADSCs (Invitrogen) was used. Cell culture was done in an incubator under a humidified atmosphere with 5% $CO_2$ at 37℃. MPRO medium was supplemented with MesenPRO RS™ Growth Supplement (2% FBS, 2 mM glutamine). The hADSCs were drop seeded onto treated Ti surfaces. Briefly, each Ti specimen was placed in a 48 well culture plate and then 60μL of hADSCs, at a density of 5 x $10^3$ cells/mL, were drop seeded on top of the Ti surface. After 2 hours, 940 μL of cell culture medium added to each well. After 1 day of incubation the cells were washed with 37℃ PBS and fixed with 3.9% formaldehyde for 12 hours. After this, the cells were washed with PBS at 37℃ again. The samples were stained with 1 ml of 0.5 wt% of Oregon green 514 phalloidin and 0.1 wt% of DAPI in order to fluorescently label the cells.

6. Bacterial binding assay.

Prior to bacterial testing, *P. gingivalis* were grown in half-strength brain heart infusion broth (Difco Laboratories, Detroit, MI) supplemented with 5 μg/ml of hemin and 1 μg/ml of vitamin $K_1$ at 37℃ under an atmosphere of 85% $N_2$, 10% $H_2$, and 5% $CO_2$. S. *aureus* was grown in tryptic soy broth (Difco) at 37 ℃ aerobically. Two identical 24-well plates containing Ti disks fixed to the bottom of the wells with silicone sealant (Silicone II; GE Sealants and Adhesives, Huntersville, NC) were prepared. The bacterial binding assay was performed as described previously with some modifications.[7] Briefly, bacterial cells were

grown to mid-log phase ($OD_{600}$ = 0.5) and were harvested by centrifugation. They were subsequently rinsed with 50 mM KCl solution supplemented with 1 mM $KH_2PO_4$, 1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ buffered at pH 6 (buffered KCl), and then resuspended in pre-warmed (37℃) buffered KCl. The volume of the fresh buffer was equivalent to the volume of the original culture. Aliquots (1 ml) of the bacterial cell suspensions were added to each well of the 24-well plates and then the assay plates were incubated at 37℃ for 1 hour. One assay plate was then allowed to stand for 15 min at room temperature, prior to rinsing the Ti disks with room temperature buffered KCl solution to remove non-adherent bacterial cells. The other assay plate was strictly maintained at 37 ℃ while the Ti disks were rinsed with pre-warmed buffered KCl. The number of bacterial cells adherent to Ti-disks was enumerated by ATP-bioluminescence quantification using a BacTiter-Glo™ Microbial Cell Viability Assay Kit (Promega, Madison, WI) according to the manufacturer's protocol. In order to characterize the adhesion of bacteria onto the Ti surface using FE-SEM, the bacterial cells adherent to Ti were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 1 h. Subsequently, they were post fixed with 1% $OsO_4$ in 0.1 M phosphate buffer for 1 h at 4℃. They were then dehydrated through a graded ethanol series. The samples were finally dried by critical point drying.

## References


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| | C1s (%) | O1s (%) | N1s (%) | Ti2p (%) |
|---|---|---|---|---|
| Ti | 44.57 | 42.14 | 0 | 13.29 |
| Ti + GMA | 71.26 | 28.74 | 0 | 0 |
| Ti + GMA + PIPAAM | 76.84 | 11.9 | 11.26 | 0 |

177 **Figure. S1.** Quantification of atomic chemical composition of bare Ti and surface treated Ti.

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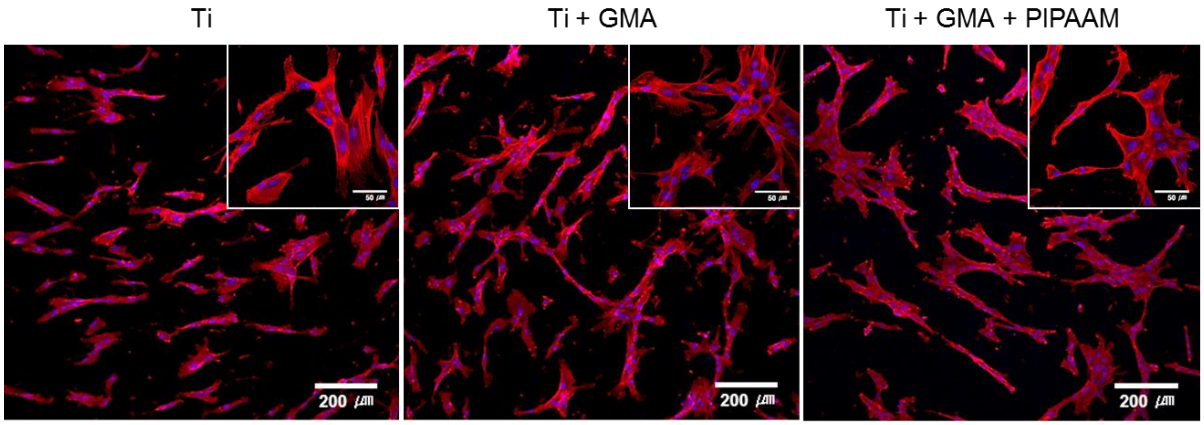
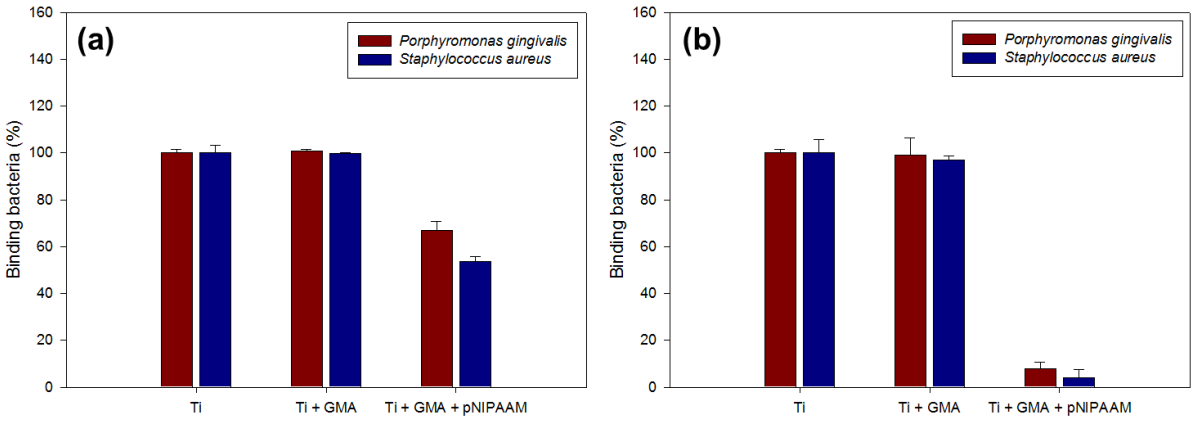



179 **Figure. S2.** Confocal laser scanning microscope images of bare Ti and surface treated Ti

180 showing adhesion of hADSC on each Ti specimen after 24 h.




181 **Figure. S3.** Quantification of bacteria on each specimen after rinsing at oral temperature (a),

182 and after rinsing at room temperature (b). All graphs are relative to pristine Ti.