Supporting Information

Titanium Substrate Preparation

The starting substrate were 1 x 1 inch cover glasses. These were cleaned with acetone (acetone, certified ACS, Thermo Fisher Scientific, Pittsburgh, PA), isopropanol (isopropanol IPA, Thermo Fisher Scientific, Pittsburgh, PA) and dilute hydrochloric acid (HCl certified ACS Plus, Thermo Fisher Scientific, Pittsburgh, PA) (10% in deionized (DI) water). To simulate the Ti implant, 100 nm of high purity Ti (0.9999) was deposited on the cover glass surface rf-magnetron sputtering (CMS-18, Kurt J. Lesker Company, Pittsburgh, PA) at a deposition pressure of 5 mTorr in Ar ambient. Prior to biofilm growth the Ti samples were rinsed with the following solvent sequence: acetone, IPA, and DI water. Upon exposure to ambient, low quality native oxides are present on all Ti surface. The native oxides were removed by sonicating the samples in 1:10 HCl : DI water, then treating with hydrogen peroxide at 60°C to grow a high quality oxide layer. Contact angle was used to verify consistent sample surfaces for each batch.

Titanium Nitride Substrate Preparation

1 x 1 inch cover glasses were also used for preparing TiN substrates, which were cleaned with acetone, IPA and dilute HCl. 100 nm of titanium was first deposited by rf-magnetron sputtering (CMS-18, Kurt J. Lesker Company, Pittsburgh, PA) at a deposition pressure of 5 mTorr in Ar ambient on cover glasses. The Ti base layer was employed to simulate a Ti implant being plasma spray coated with titanium nitride. Without removing the sample from the chamber, 50 nm of TiN was subsequently deposited by rf-magnetron sputtering with an alloyed TiN target at room temperature in a pure Ar ambient. The platen for holding the samples was biased at 30-40 V to improve uniformity and stoichiometry of the deposited TiN layer. Prior to biofilm growth the TiN samples were rinsed with the following solvent sequence: acetone, IPA, and DI water. Then, the

sample was sonicated in 1:10 of HCl: DI water. Contact angle was used to verify consistent samples surfaces for each batch.

Quaternized Titanium Nitride Substrate Preparation

Quaternized TiN substrates were prepared by converting nitrogen atoms on the TiN surface into quaternary nitrogen by submerging the TiN substrates in an acetonitrile (25 mL) and allyl bromide (100µL) solution for 1 hour to quaternize the surface. After quaternization, an isopropranol and deionized water (Fisher Scientific, Pittsburgh, PA) rinse were used to remove any excess solvent and reagent. Contact angle measurements were used to verify consistent samples surfaces for each batch.

Sessile Contact Angle Measurement

Contact angles were performed with a cold back lit source to prevent heating of the droplet or sample. A 5 µL droplet of DI water was placed on the surface and the contact angle, Θ, measured using a microscopic lens equipped with a goniometer. For each indvidiual sample the contact angle is the average of at least 5 or more values. For each material type (Ti, TiN, quaternized TiN), 50 samples were employed to determine an average value to check for non-uniformity of samples batch to batch. The individual sample measurement was required to be within two standard deviations of the average. The small variance in measurement demonstrates the high reproducibility of the surface following proper cleaning procedure prior to measurement (Table 1).

X-Ray Photoelectron Spectroscopy

The chemical composition of the deposited films was studied using X-ray Photoelectron Spectroscopy (XPS) with an ESCALAB 250Xi instrument (Thermo Fisher Scientific, Pittsburgh, PA) equipped with a monochromatic aluminum anode as the x-ray source. Wide range analyses (survey scans) were initially acquired from the surface of the deposited film with an electron pass

energy of 100 eV and step size of 1 eV. High resolution scans for detailed peak analysis were performed at an electron pass energy of 40 eV and an energy step size of 0.1 eV. To better understand the chemical bonding of atoms on the surface region, scans were acquired at 0º, 25º and 50º tilting with respect to the normal. New spectra were also acquired after a gentle 30 s sputtering with 500 eV Ar ions at 50º tilting to eliminate the effect of surface contaminants.

Biofilm Incubation and Growth

On the day of bio-film growth, a paper point was used to extract anaerobic subgingival bacteria between the lower last premolar and first molar of a subject volunteer. Institutional Review Board Approval was obtained and an informed consent was signed. The paper point was placed in ringer solution to suspend and preserve the bacteria. To remove the bacteria from the paper point, the sample was vortexed for 10 seconds and sonicated for 10 seconds, then vortexed again briefly prior to any removal of bacteria containing solution from the container. All bacteria work used sterilized materials and was performed under flame to prevent any cross contamination from outside sources.

Ti, TiN and quaternized TiN substrates were decontaminated by rinsing and submersion in an ethanol bath for 20 minutes immediately prior to growth and were kept in sealed containers until application of live bacteria. Each substrate for bacteria growth was placed in an individual sterile container. Bacteria solution was micro-pipetted onto the Ti, TiN or quaternized TiN substrate surface to form bacteria solution film thicknesses (z-height) of 75, 100 and 125 μm by

film thickness was controlled by the volume (21.5 μL, 28.6 μL, 35.8 μL) of the bacteria solution and the area of the top cover glass slip. A small gravitiational force on the top cover glass alowed the bacteria solution to spread evenly exactly to the edge of top cover slip. The bacteria solution was expected to not extend beyond the top cover glass slip because of the solution surface tension. These thicknesses were chosen as the expected immediate interaction length of the

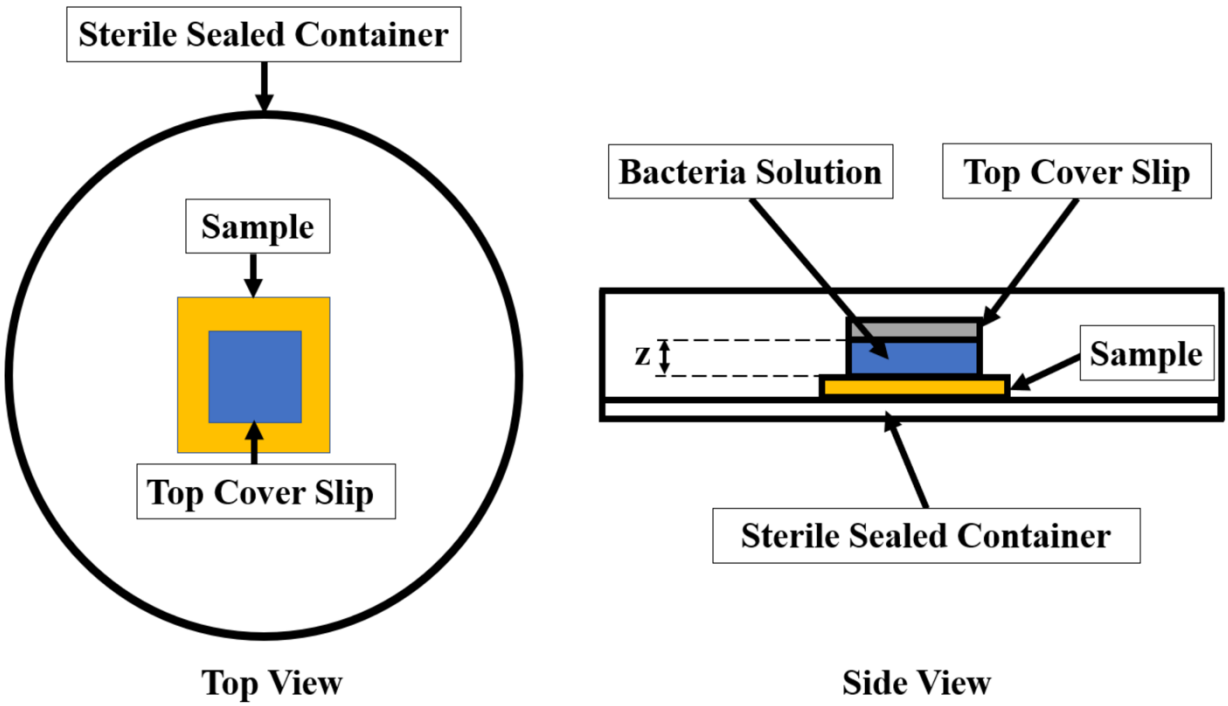

Figure 1. Top and side view of bacterial incubation setup to define the bacterial film thickness.

sample surface to the bacteria will only be within one monolayer of bacteria such that the surface is completely covered. Employing thicker films would not inform on the surfaces effect as the bulk would dominate which is non-interacting, evident through reduction in antibacterial efficiency at thicker film thicknesses.

The entire setup was incubated in a Bactron 300 anaerobic chamber (85% N_2, 10% CO_2, 5% H_2) at 37°C in a dark box for 4h. 4h was chosen because significant quantifiable differences in bacteria growth between the samples were detected for this time frame and incubating for longer periods such as 24hrs until the antibacterial surface has entirely removed all microbial presence is not realistic of end application. Previous groups Post incubation, the bacteria solution on Ti, TiN or quaternized TiN substrate and their respective cover glass slips were sonicated and vortexed in 5 ml fresh Ringer solution for 10 seconds. The resulting Ringer solution was further diluted and plated on Tryptic Soy Agar and allowed to grow under anaerobic conditions for 48h. Subsequently, the plates were removed and colony forming units (CFUs) counted to evaluate

antibacterial efficiency of the quaternized sample as compared to reference Titanium and Titanium Nitride samples. For every data point, three identical samples were used in order to provide statistical validity. The experiment was repeated four times to produce 108 data points. A primary concern as the bacteria solution collected from the patient varied on every trial is to ensure a similar bacterial concentration was used for plating and quantification of bacterial efficiency. To do so, 5 logs of diluted bacteria solution were used for all trials. The appropriate log dilution for data analysis was selected such that the CFU counts from each trial matched the Titanium reference states.

Antibacterial activity

The antibacterial activity of the Ti, TiN or quaternized TiN samples was evaluated using a live/dead staining kit (LIVE/DEAD® BacLight, Thermo Fisher) against *Streptococcus mutans* (ATCC 35668). Stocks of *S.mutans*, previous prepared in a glycerol solution and stocked at−80 °C, were thawed at room temperature and centrifuged at 4700 rpm for 10 min. The glycerol supernatant was discarded, and the pellet was resuspended in 5 mL of Brain Heart Infusion media (BHI) and allowed to grow for 24 h at 37 °C. The bacteria solution was adjusted to an OD600 nm (10^7 CFU/mL) and an aliquot of 500 µL of this solution was added over the Ti, TiN or quaternized TiN samples in a 24 well plate for 4 h. After this period the samples were gently rinsed with the phosphate buffer solution (PBS) and fixed with formaldehyde for 15 min, rinsed with PBS and incubated for 30 min at 37 °C in the staining solution prepared following the manufacturer's instructions. The staining solution contains SYTO® 9 to stain the living bacteria, which allows determination of the proliferation of live bacteria. Fluorescence images of the live bacteria were recorded in a fluorescence microscope (Zeiss) and analyzed by Image J software to determine the percentage of live bacteria over the samples.

Statistical analysis

ANOVA was used to test the effects of material and thickness on bacteria level. Bacteria level was used as the outcome and fixed factors were material, thickness and experimental run.