Characterization and Evaluation of Titanium Surfaces Functionalized with Gold Nanorods against *Staphylococcus aureus* Biofilms Using Photothermal Laser Ablation

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Abstract

Medical implant-associated biofilms can cause a significant increase in mortality and morbidity worldwide. Biofilms are communities of microbes encased within a self-produced extracellular polymeric substance attached to a surface and are highly resistant to antibiotics and conventional disinfection strategies. To improve patient health, novel biofilm elimination strategies are needed for medical devices. To address this knowledge gap, we evaluated Gold Nanorod (GNR) functionalized titanium surfaces via different immobilization techniques for biocompatibility and anti-biofilm effectiveness. We used thiol-terminated 3-mercaptopropyl-trimethoxysilane (MPTMS) or amine-terminated 3-aminopropyl-triethoxysilane (APTES) to silanize the Ti surface and deposit citrate-stabilized GNR (Ti-MPTMS-GNR or Ti-APTES-GNR, respectively) on titanium discs. Scanning Electron Microscopy and X-ray Spectroscopy data confirmed that Ti-MPTMS-GNR-functionalized surfaces achieved a higher amount of deposited nanorods compared to Ti-APTES-GNR-functionalized surfaces. Biocompatibility testing was conducted in vitro on various mammalian cells associated with host response and tissue healing utilizing ISO-10993 Part 5. Titanium discs with GNR functionalization using either method did not show significant cytotoxicity and showed favorable cell attachment and proliferation compared to Ti controls. Near-infrared (NIR) laser irradiation at a wavelength of 808 nm for 20 min (0.5 W/cm2) of GNR coated samples showed a significant temperature increase compared to uncoated titanium. Furthermore, Ti-MPTMS-GNR-functionalized surfaces functionalized discs showed higher photothermal activity than Ti-APTES-GNR-functionalized discs. The ability of NIR irradiation to eliminate early and late *S. aureus*, ATCC 25923 biofilms grown (2h or 24h at 37°C, respectively) on Ti samples coated with or without GNRs in the presence or absence of NIR irradiation (808nm for 20min at 0.5 W/cm2) was quantified by bacterial enumeration. As compared to Ti-APTES-GNR functionalized discs, the Ti-MPTMS-GNR-functionalized surfaces discs showed greater anti-biofilm effectiveness against both early and late-stage *S. aureus* biofilms with significant reduction in bacterial viability compared to the untreated control. The result of this research expands our understanding on the safety and efficacy of GNR-functionalized surfaces that are fabricated by different chemical methods with anti-biofilm claims.

Introduction

The implant-associated biofilms are a serious health care challenge causing persistent infections that are resistant to conventional antibiotics, complicating patient care. Bacterial biofilms are three-dimensionally structured communities of microbes encased within a self-produced extracellular polymeric substance (EPS) attached to a surface. Compared to their planktonic counterparts, biofilm bacteria embedded in EPS are highly resistant to antibiotics and conventional disinfection strategies. Surfaces functionalized with gold nanorods (GNRs) have been demonstrated to be promising as they can induce bacterial killing effects upon exposure to near-infrared (NIR) radiation. NIR light can penetrate as deep as 10 mm through tissue. When exposed to NIR, GNRs can generate plasmonic photothermal heat to eliminate microbial biofilms and reduce implant failures (Fig. A). However, the performances and biocompatibility of these surfaces may differ as the fabrication methods vary. To address these knowledge gaps in evaluating GNR-incorporated medical devices and design innovative strategies for eradicating persistent biofilms on implant surfaces, here we have assessed different immobilization techniques via thiol- or amine-terminated silane to deposit citrate-stabilized gold nanorods on titanium discs (Fig. B). Next, surface characterization, biocompatibility assessment on mammalian cells followed by photothermal anti-biofilm effects of these gold functionalized titanium surfaces were evaluated as part of this study.

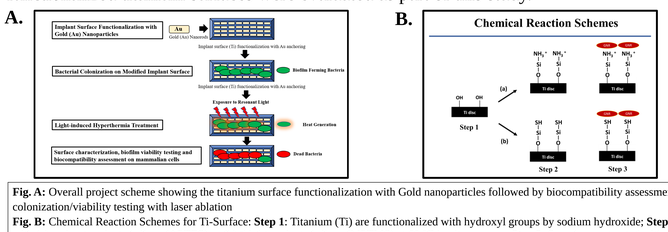

Fig. A: Overall project scheme showing the titanium surface functionalization with Gold nanoparticles followed by biocompatibility assessment and biofilm colonization/viability testing with laser ablation
Fig. B: Chemical Reaction Schemes for Ti-Surface: **Step 1**: Titanium (Ti) are functionalized with hydroxyl groups by sodium hydroxide; **Step 2**: Samples are pre-treated with (a) amine-terminated silane (APTES) or (b) thiol-terminated silane (MPTMS) at 60 ℃ in nitrogen gas ;**Step 3**: Samples pre-treated with different silanes are then deposited with citrate-stabilized GNR suspension

Results

Material Characterization

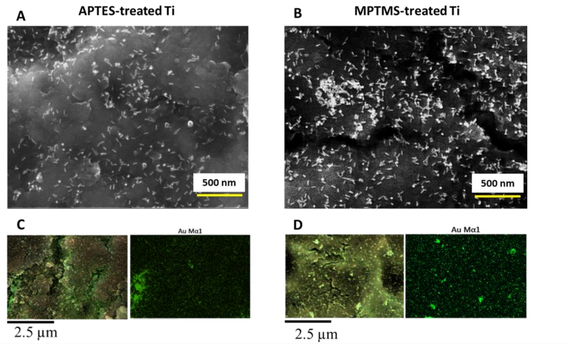

Figure 1. Scanning Electron Microscopy (SEM) images of GNR-functionalized **(A)** APTES-treated Ti and **(B)** MPTMS-treated Ti showed uniform coverage of GNRs. The presence of gold element was confirmed by EDS scanning analysis on **(C)** APTES-treated Ti and **(D)** MPTMS-treated Ti

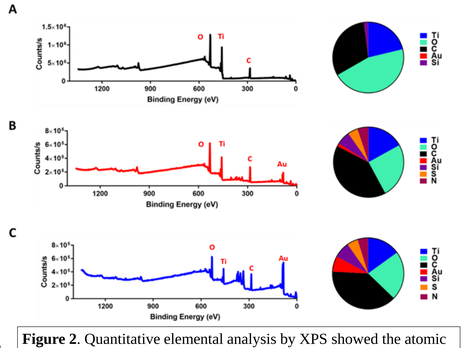

Figure 2. Quantitative elemental analysis by XPS showed the atomic percentage of different elements on **(A)** untreated control Ti, **(B)** GNR-coated APTES-treated Ti, and **(C)** GNR-coated MPTMS-treated Ti. After functionalized with GNRs, greater amount of GNRs was detected on MPTMS-treated Ti than on APTES-treated Ti.

Photothermal Anti-biofilm Effectiveness

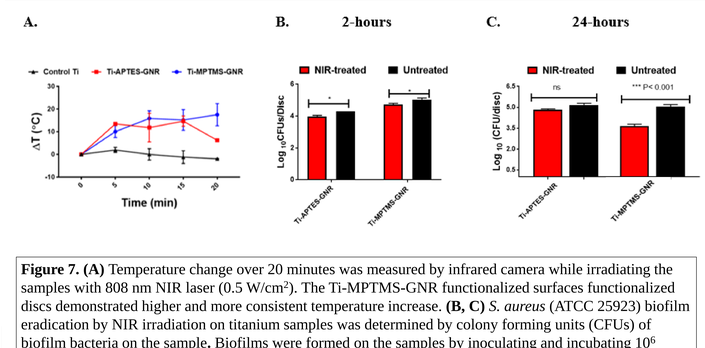

Figure 7. (A) Temperature change over 20 minutes was measured by infrared camera while irradiating the samples with 808 nm NIR laser (0.5 W/cm^2). The Ti-MPTMS-GNR functionalized surfaces functionalized discs demonstrated higher and more consistent temperature increase. **(B, C)** *S. aureus* (ATCC 25923) biofilm eradication by NIR irradiation on titanium samples was determined by colony forming units (CFUs) of biofilm bacteria on the sample**.** Biofilms were formed on the samples by inoculating and incubating 10^6 CFU/mL of *S. aureus* for 2h or 24 h. Samples with biofilms were exposed to 808 nm laser for 20 min in 0.5 mL PBS for 20 min at 37 °C. The Ti-MPTMS-GNR functionalized surfaces functionalized discs demonstrated more than 1 log-magnitude reduction of biofilm bacteria (***P<0.001 by ANOVA) for 24-h biofilm.

Biocompatibility Assessments on Mammalian Cells

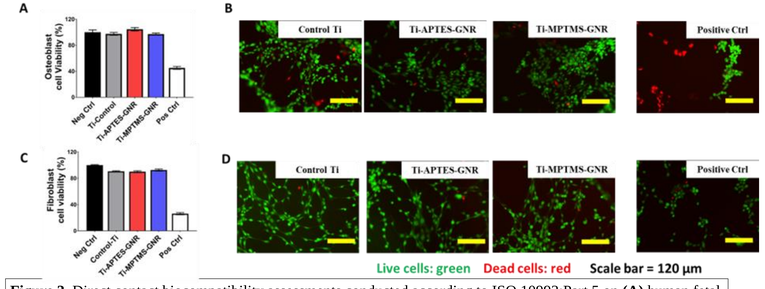

Figure 3. Direct contact biocompatibility assessments conducted according to ISO 10993:Part 5 on **(A)** human fetal osteoblast and **(C)** CCL-1 fibroblast and the cell viability was quantified by colorimetric MTS assay. Viable and dead cells were also stained by live/dead fluorescence assay **(B&D)**. Polyurethane film with 0.1% zinc diethyldithiocarbamate (PUA-ZDEC) was used as a positive control Fluorescence viability imaging showed high proportion of live cells with GNR-coated titanium similar to untreated control Ti.

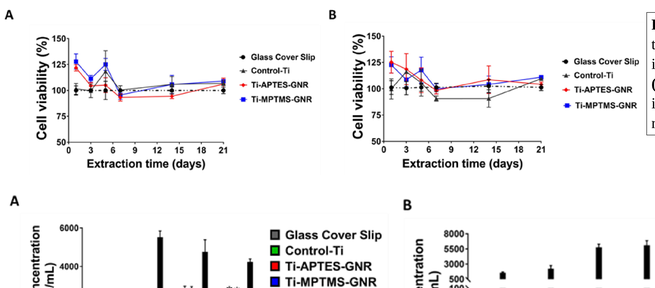

Figure 4. The extract test was performed according to ISO 10993:Part 5 to evaluate toxicity of leachables in sample extract over a 21-day extraction period on **(A)** osteoblast and **(B)** fibroblast cells. Results indicated that sample extracts induced no significant reduction in cell viability.

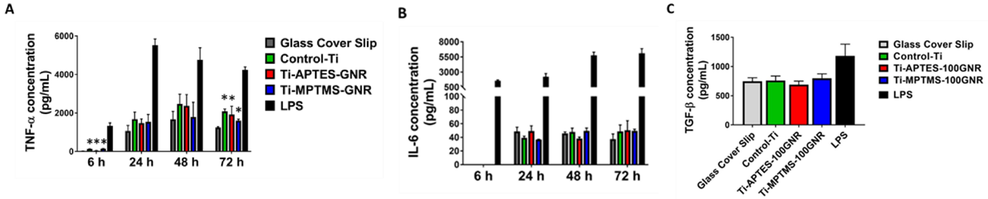

Figure 6. Cytokine secretion by macrophage cells in response to Ti samples was measured by ELISA. Macrophage cells incubated with Ti samples showed greater secretion of pro-inflammatory cytokine **(A)** TNF-α, but displayed no variation in **(B)** IL-6 secretion and **(C)** TGF-β (p<0.05 as determined by t-test).

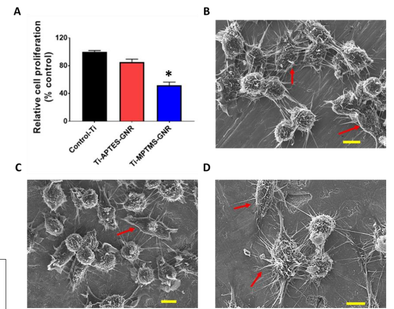

Figure 5. Study on RAW264.7 macrophage attachment on titanium samples after 3 days of incubation was conducted. Proliferating cells was then **(A)** quantified by MTS cell proliferation assay. Morphologies of macrophage cells on **(B)** control Ti, **(C)** Ti-APTES-GNR, and **(D)** Ti-MPTMS-GNR functionalized surfaces were also observed by SEM. The Ti-MPTMS-GNR functionalized surfaces contributed to less cell proliferation (*P<0.05 by two-way t-test compared to control-Ti) and spreading (red arrows) on the surface.

Conclusion

Two different chemical synthesis schemes were used to deposit GNRs on titanium surface. It is found that the thiol-terminated MPTMS induced greater GNR deposition compared to amine-terminated APTES. The functionalized titanium from both methods displayed good biocompatibility on mammalian cells associated with host response and tissue healing. As compared to Ti-APTES-GNR-functionalized discs, the Ti-MPTMS-GNR-functionalized surfaces discs showed greater photothermal anti-biofilm effectiveness against both early and late-stage *S. aureus* biofilms with significant reduction in bacterial viability compared to the untreated control. The result of this research expands our understanding on the safety and efficacy of GNR-functionalized surfaces that are fabricated by different chemical methods with anti-biofilm claims.

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