

Antimicrobial Activity of Bioactive Glass-Ceramic Powder for Coating Titanium

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INTRODUCTION: Conventional calcium-phosphate and hydroxyapatite (HA)-based coatings for orthopedic and dental implants often lack the combined osteostimulative and antimicrobial properties needed for optimal bone healing and implant success. Silicated Ceramic (SiC), a DSM-Firmenich proprietary bioactive glass-ceramic, was developed to overcome these limitations. Partial or complete substitution of hydroxyapatite with SiC is hypothesized to improve antimicrobial activity compared to HA alone. To determine the surface antimicrobial performance of SiC-substituted HA coatings, titanium (Ti) discs were plasma spray-coated with one of three blends, containing either 15, 50, or 100 wt% SiC, these were compared to HA-coated and uncoated Ti discs.

METHODS: Plasma spray coating of the titanium discs was performed by Himed via an atmospheric spray system. All discs were blasted with Himed's MCD Apatitic Abrasive prior to coating, wherein one side of the disc was coated with either 100% HA, 85/15 HA/SiC, 50/50 HA/SiC, or 100% SiC, and, subsequently, gamma sterilized (25-40 kGy). Uncoated, blast-only Ti discs served as controls. Antimicrobial activity of the discs was assessed using three assays in triplicate (n=3). (1) The surface contact assay (ASTM E2180) involved applying a thin agar slurry containing *S. aureus MRSA (ATCC 15442)*, *P. aeruginosa (EDCC 5247)*, *E. coli (ATCC 10536)*, or *C. albicans (ATCC 10259)* to the coated surface. CFU counts were measured at 0- (Ti only) and 24-hours post-incubation, with a $\geq 99.9\%$ reduction relative to uncoated Ti after 24 hours considered indicative of antimicrobial activity. (2) The proliferation assay measured the growth of planktonic *S. aureus MRSA (ATCC 15442)* over 48 hours following removal of the disc via an Optical Density (OD) measurement, with $\geq 99.9\%$ inhibition at 6 hours relative to uncoated Ti indicating antimicrobial efficacy. (3) The bacterial cell viability (Resazurin) assay was used to assess the metabolic activity of *S. aureus MRSA (ATCC 15442)* adhered to the discs following incubation and subsequent washing of the samples. Fluorescence is measured over 4 hours; decreased fluorescence relative to uncoated Ti indicating antimicrobial activity, while absence of fluorescence indicating complete inhibition. A one-way ANOVA with post-hoc Tukey test ($\alpha = 0.05$) was used to determine statistical significance between groups.

RESULTS: In the ASTM E2180 surface contact assay, only plasma-sprayed coatings with 50 wt% or higher SiC achieved $\geq 99.9\%$ reduction of CFU numbers of *S. aureus* and *P. aeruginosa* relative to the growth of control Ti samples. Moreover, these groups demonstrated bactericidal activity – with the 100 wt% SiC coated disc yielding an average of 99.2% reduction in *S. Aureus* CFU and 98.7% reduction in *P. Aeruginosa* CFU compared to the inoculum after 24 hours (see Image 1). Additionally, the proliferation and viability assays confirmed surface antimicrobial activity of the 100% SiC coating against *S. aureus* as evidenced by a delayed onset of optical density beyond 6 hours and an average fluorescence reduction of 63.8%, despite only having one side of the disc coated. None of the coatings demonstrated sufficient antimicrobial activity against *E. Coli* or *C. albicans* per the surface contact assay. However, inhibition and/or reduction of *E. Coli* CFU by all coated disc groups was significant ($\alpha < 0.05$), demonstrating moderate antimicrobial activity compared to uncoated Ti.

DISCUSSION: Compared to HA alone, SiC-substituted HA coatings resulted in improved or equivalent antimicrobial activity. In particular, coatings containing higher SiC content ($\geq 50\%$) exhibited significant antibacterial activity against *S. aureus* and *P. aeruginosa* compared to both HA-coated and uncoated Ti discs. Activity against *E. coli* was moderate, likely due to its faster replication rate, which therefore requires further optimization. All groups were ineffective against *C. albicans*, however, fungal infection is considered rare, representing $\sim 2\%$ of periprosthetic joint infections [1]. Furthermore, optimization of coating application is still required, as evidenced by the high variability across replicates in all three assays. Moreover, as the discs were coated only on one side, outcomes of the proliferation and viability assay, which fully submerged the discs, represent antimicrobial performance in a worst-case scenario where an orthopedic or dental implant is only partially coated as is typical. Ongoing *in vitro* studies aim to screen for broad-spectrum activity and persistence of antimicrobial activity over time while planned *in vivo* studies will further evaluate preclinical performance.

SIGNIFICANCE/CLINICAL RELEVANCE: The increasing prevalence of implant surgeries and associated revisions, driven by an aging population, highlights the need for a next generation coating for orthopedic and dental implants to address failures associated with both aseptic and septic loosening [1]. Partial or complete substitution of hydroxyapatite with silicated ceramic may present a facile route to reduce biofilm formation and periprosthetic infection in medical implants.

REFERENECES:

- [1] F. S. Fröschen, T. M. Randau, A. Franz, E. Molitor, and G. T. R. Hischebeth, "Microbiological Profiles of Patients with Periprosthetic Joint Infection of the Hip or Knee," *Diagnostics*, vol. 12, no. 7, p. 1654, Jul. 2022, doi: 10.3390/diagnostics12071654.

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IMAGES AND TABLES:

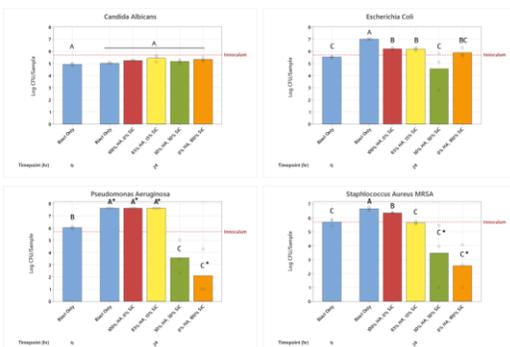


Image 1: Antimicrobial activity of plasma spray-coated titanium (Ti) discs evaluated using ASTM E2180 against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus MRSA*. Bars represent Log CFU/Sample at time 0 for uncoated Ti (inoculum control) and after 24 hours of incubation for all test groups.

Means that do not share a letter are significantly different using the post-hoc Tukey Method and 95% Confidence.

*One or more samples measured outside of the limit of detection. Detection limit was used to calculate the average.