Silanization of titanium and hydroxyapatite for loading and release of 2-heptylcyclopropane-1-carboxylic acid

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INTRODUCTION

Implanted biomaterials are often susceptible to bacterial contamination and provide a substrate for biofilm formation. Bacteria within a biofilm can evade immune cell clearance and withstand up to 1000 times the minimum inhibitory concentration of antibiotics, making them particularly difficult to treat[1]. Molecules termed diffusible signal factors have been shown to prevent bacterial attachment to surfaces and to eradicate pre-formed biofilms. However, many of these molecules are susceptible to isomerization and become less effective when exposed to light, radiation, and other common sterilization methods. A synthetic DSF developed in our lab, 2-heptylcyclopropane-1-carboxylic acid (2CP), is stable and resistant to isomerization, making it a potentially efficacious antibacterial coating for medical implants.[2]

This study sought to modify titanium and hydroxyapatite coupons with silanization using n- decyltrimeth-oxysilane (DTMS) and n-hexyltrimethoxysilane (HTMS) and to then load with 2CP (Figure 1). Efficacy of surface modification was investigated by measuring water contact angle before silanization, after silanization, and after loading with 2CP. Initial 2CP release was investigated in a 3-day elution study.

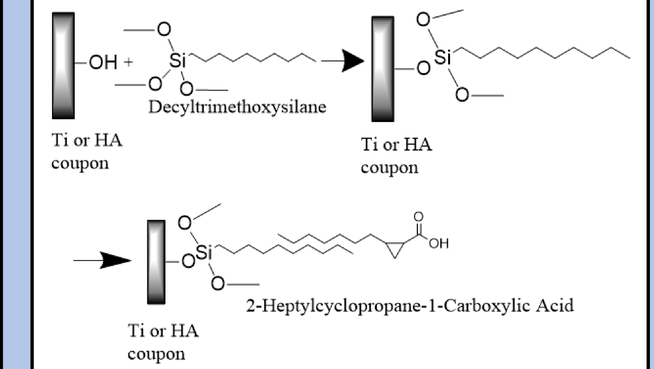

Figure 1 (A) Proposed mechanism for DTMS and 2CP interactions with biomaterial coupons. A similar mechanism is proposed with HTMS, which is similar in structure to DTMS but with a shorter carbon chain (not shown).

METHODS

Titanium coupons were sonicated in deionized water and dish soap for 5 min, followed by acetone for 10 min, and ethanol for 10 min. Coupons were then placed in a 5M sodium hydroxide solution an incubated at 60°C for 24 h to enhance surface hydroxyl formation. Because hydroxyapatite already has available surface hydroxyl groups, these steps were only performed on titanium coupons. Coupons were washed 2x in deionized water, then treated with 2% (v/v) of either DTMS or HTMS in ethanol. Silanated coupons were placed on a shaker for 10 min then rinsed in ethanol to remove non-adhered silane and dried in a 110°C oven overnight. Coupons were then either loaded with ~2035 µM of 2CP in 100% ethanol or left unloaded. After ethanol evaporation and drying, water contact angles of loaded and unloaded coupons were determined using a VCA optima measurement machine (AST products, INC, USA)3. Water droplets (5 µL) were placed carefully onto the coupon surfaces. A digital camera recorded the photographs of the droplets after approximately one minute. The goniometry software of VCA OptimaXE calculated the contact angles. Loaded coupons (n=3 for titanium, n=1 for hydroxyapatite) were placed in sterile phosphate buffered saline and eluates were collected by complete solution change at time points of 24, 48, and 72 h. Concentration of 2CP in the eluates was measured using a Free Fatty Acid Fluorometric Assay Kit (Cayman Chemical).

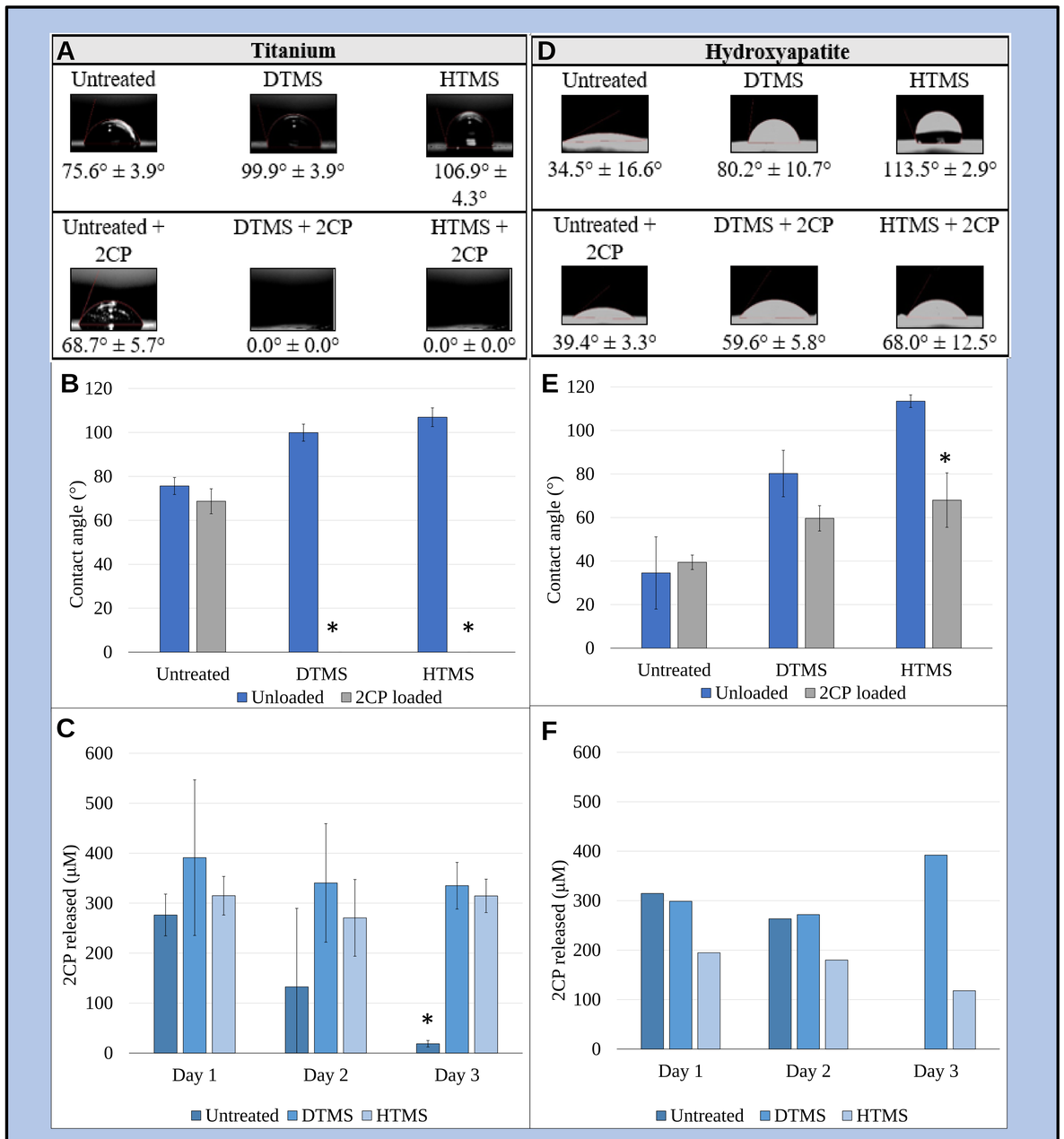

Figure 2 Titanium results for contact angle (A) images, (B) graphs, as well as (C) evaluation of 2CP release. Hydroxyapatite results for contact angle (D) images, (E) graphs, as well as (F) evaluation of 2CP release. Each bar represents mean and error bars indicate standard deviation.* indicates significant difference between groups detected using one-way ANOVA with Holm–Sidak post hoc tests ($p < 0.05$).

RESULTS

Contact angle results indicate that silanization of titanium and hydroxyapatite increases hydrophobicities of both materials Figure 2A/D). The addition of 2CP appeared to decrease contact angle (Figure 2B/E). Furthermore, silanization of both materials resulted in a sustained release of 2CP for 3 days compared to untreated controls, which only released 2CP through day 2 (Figure 2C/F).

CONCLUSIONS

The increased hydrophobicity after silanization then decrease after 2CP loading may confirm successful loading of 2CP and the protrusion of hydrophilic groups. Due to material limitations, only one hydroxyapatite coupon was investigated in the elution study, which is a significant limitation. Future studies will increase sample size and repeat this study, as well as perform FTIR to confirm successful modification. Finally, studies to determine antimicrobial efficacy of these materials will be performed.

Acknowledgments

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References

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