Hydroxyapatite coupons loaded with 2-heptylcyclopropane-1-carboxylic acid inhibit *P. gingivalis*

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INTRODUCTION: There are over 700 different bacterial species in the oral cavity, including opportunistic bacteria, such as *Porphyromonas gingivalis* [1,2]. Implanted biomaterials are highly susceptible to bacterial contamination, and once bacteria colonize the material surface and form biofilm, extracellular polymeric substances (EPS) are produced and increase the difficulty of removal [3]. Bacteria that invade the spaces between the tooth and gingiva can progress beyond the gingiva into the alveolar bone, limiting efficacy of antimicrobial components of saliva and common treatments [1]. In severe cases, loss of gum tissue and bone can occur, and bacteria can enter the blood, traveling throughout the body and potentially causing chronic diseases [4]. Chlorhexidine is used for skin and medical instrument disinfection, as well as for post-implantation oral rinses [5,6]. Hydroxyapatite (HAp) is a natural inorganic component of bone in mammals, which makes it highly biocompatible, and is the most widely used calcium phosphate bioceramic coating for metal implants [7-9]. Previous research has suggested that unsaturated fatty acids disperse and inhibit biofilm formation [10,11]. In this study, hydroxyapatite coupons were loaded with 2-heptylcyclopropane-1-carboxylic acid (2CP) to determine the interaction with *P. gingivalis*.

METHODS: Hydroxyapatite Coupon Discs (12.7 mm, >95%, BioSurface Technologies Corporation) were immersed in 2.5 mg/mL 2CP in EtOH for 3 hours at 40°C and 50 rpm and dried overnight in a fume hood. Oral rinse coupon groups were immersed in 0.12% chlorhexidine gluconate oral rinse commonly prescribed after dental implantation (Xttrium Labs). Biofilm prevention properties of 2CP-loaded, 2CP+oral rinse-loaded, oral rinse-loaded, and unloaded hydroxyapatite coupons (n=4; fully loaded and following 3-day elution) were tested by direct inoculation with approximately 10^5 CFUs of *P. gingivalis* by addition of bacteria solution in 100 µL increments to avoid significant loss of bacteria from surface of coupon. After 24 hours, coupons were removed from wells, rinsed, and sonicated to detach biofilm-associated bacteria. Wells from which coupons were removed were also washed with PBS and the attached biofilm quantified. Bacterial viability quantification (n=4 planktonic; n=3 biofilm) was determined using BacTiter-Glo® Microbial Cell Viability Assay (Promega). Select coupons from each group (n=1) were imaged with FilmTracer Live/Dead assay (Invitrogen). Statistical analysis was performed using GraphPad Prism 7.2 software to conduct one-way analysis of variance (ANOVA) followed by Holm-Sidak's post-hoc test.

RESULTS: 2CP and oral rinse groups (before and after elution) reduced planktonic growth, compared to the unloaded control; however, significance was not observed due to high standard deviation in the control group (Figure 1A). 2CP and oral rinse groups reduced biofilm directly on coupons, compared to the unloaded control; however, post-elution 2CP-loaded coupons did not reduce biofilm significantly (Figure 1B). Live/Dead staining produced similar results, with oral rinse-loaded HAp eliminating almost all biofilm growth on the coupon and slightly more growth for 2CP-loaded HAp groups.

DISCUSSION: Oral rinse-loaded and 2CP+oral rinse-loaded HAp coupons had the greatest inhibition of *P. gingivalis* planktonic and biofilm growth, compared to the control; however, this result is expected since the target of chlorhexidine is to kill bacteria. Although biofilm growth on 2CP-loaded HAp coupons after 3-day elution were not significantly different than growth on unloaded HAp coupons, fully loaded 2CP-HAp coupons significantly reduced biofilm viability, compared to the unloaded control ($p<0.01$).

SIGNIFICANCE/CLINICAL RELEVANCE: Hydroxyapatite coupons loaded with 2CP could be useful for coating titanium dental implants to inhibit biofilm formation near the permucosal extension of the implant, which may be useful for preventing bacterial infection progression into deep tissue and bone. HAp coatings for implants could inhibit biofilm formation and reduce the need for implant removal, tissue debridement, and patient trauma.

REFERENCES: 1. D. Armellini, M.A. Reynolds, J.M. Harro, L. Molly, Biofilm Formation on Natural Teeth and Dental Implants: What is the Difference? The role of biofilms in device-related infections (2009) 109-122.
2. M. Masuda, M. Era, T. Kawahara, T. Kanyama, H. Morita, Antibacterial effect of fatty acid salts on oral bacteria, Biocontrol Science 20(3) (2015) 209-213.
3. R.M. Donlan, Biofilms: microbial life on surfaces, Emerging infectious diseases 8(9) (2002) 881.
4. L.F. Rose, Gum Disease & Systemic Health, Dear Doctor: Dentistry and Oral Health (2019).
5. L. Chen, S. Al-Bayatee, Z. Khurshid, A. Shavandi, P. Brunton, J. Ratnayake, Hydroxyapatite in oral care products—a review, Materials 14(17) (2021) 4865.
6. J.J. Crall, Improving oral health and oral health care delivery for children, Journal of the California Dental Association 39(2) (2011) 90-100.
7. K. Lin, J. Chang, Structure and properties of hydroxyapatite for biomedical applications, Hydroxyapatite (HAp) for biomedical applications, Elsevier (2015), pp. 3-19.
8. O. Adamopoulos, T. Papadopoulos, Nanostructured bioceramics for maxillofacial applications, Journal of Materials Science: Materials in Medicine 18(8) (2007) 1587-1597.
9. S. Bose, S. Tarafder, A. Bandyopadhyay, Hydroxyapatite coatings for metallic implants, Hydroxyapatite (Hap) for biomedical applications, Elsevier (2015), pp. 143-157.
10. D.G. Davies, C.N. Marques, A fatty acid messenger is responsible for inducing dispersion in microbial biofilms, Journal of bacteriology 191(5) (2009) 1393-1403.
11. J.A. Jennings, H.S. Courtney, W.O. Haggard, Cis-2-decenoic acid inhibits S. aureus growth and biofilm in vitro: a pilot study, Clinical Orthopaedics and Related Research® 470(10) (2012) 2663-2670.

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IMAGES AND TABLES:

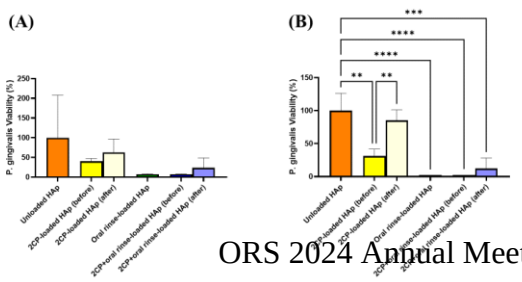

Figure 1. (A) Planktonic and (B) Biofilm viability of P. gingivalis on HAp coupon surfaces. "Before" and "after" refer to before and after 3-day elution. **** indicates significant difference ($p<0.0001$), *** indicates significant difference ($p<0.001$), and ** indicates significant difference ($p<0.01$). Significant differences only shown for comparisons to Unloaded HAp control and between before and after elution groups.