Research Week 2022

Evaluation of the ability of a reversible switching molecule to attack and defend against bacteria

Guimaraes GMF[1]; Logan MG[2]; Icimoto MY[3]; Patty S[4]; Pfeifer CS[5]

[1]guimarag@ohsu.edu; PhD Student, University of Sao Paulo; Visiting Scholar, Biomaterials and Biomechanics, Oregon Health and Science University
[2]loganma@ohsu.edu; Chemist, Research Assistant, Biomaterials and Biomechanics, Oregon Health and Science University
[3]icimoto@ohsu.edu; Biochemist, Senior Research Associate, Biomaterials and Biomechanics, Oregon Health and Science University
[4]pattys@ohsu.edu; Chemist, Research Associate, Biomaterials and Biomechanics, Oregon Health and Science University
[5]pfeiferc@ohsu.edu Mentor, Associate Professor, Biomaterials and Biomechanics, Oregon Health and Science University

Keywords

Switchable Molecules,

Antimicrobial activity,

Surface chemistry.

Abstract

Biofilm colonization on biomedically-relevant surfaces is one of the major causes leading to reinfection, including in dental restorations. Every time a dental filling is replaced, more tooth structure is lost, complicating prognosis and increasing the chance for more serious systemic infections. The existing antifouling and/or antimicrobial materials are efficient in either preventing attachment or killing the bacteria, but much more effective protection could be achieved if both properties were combined. Thus, our goal was to take advantage of natural pH variations in the oral cavity to design a reversible molecule (carboxy betaine - CB), alternatingly existing in two chemical states: an antibacterial quaternary ammonium cation (through acid-catalyzed ring closure, CB-Ring), and an antifouling linear zwitterion, CB-OH. These molecules have proven to be successful against E. coli, but the switching pH is much lower than physiologically-relevant in the mouth (<1). Derivatives have been synthesized that have moved the equilibrium closer to physiological pH (CB-200), as shown in the figure 1-A/B.

The viability of *Streptococcus mutans* bacteria by bioluminescence (Luciferase Assay) and Crystal Violet (CV) was evaluated by a spectrophotometer (SpectraMax iD3- Molecular Devices). The compounds CB-000, CB-200, CTAC (antibacterial quaternary ammonium control) and MPC (antifouling control) were tested at different pH values (3.0-7.5) either added to mature biofilm grown at pH 7, or to initial biofilms grown at each pH. Results are shown in the figure - 2- A) For Luciferase, as expected, CTAC and MPC showed antimicrobial/antifouling effect. However, for CB's, this phenomenon was not found. B)

Despite a material-limitation for CV for acid pH's, except for CTAC, none of CB inhibitors showed differences.

Conclusions: In all cases, the inhibitors showed no effect suggesting the need to add a long alkyl chain to the derivatives, allowing micellization and charge aggregation to be effective against the gram + S. mutans.

Funding: NIH-NIDCR R35-DE029083.

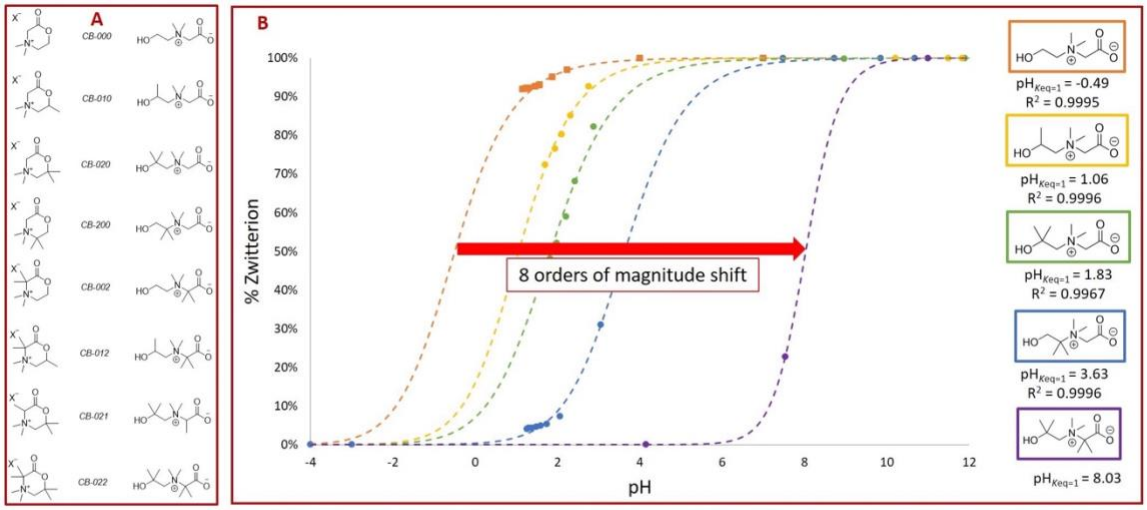
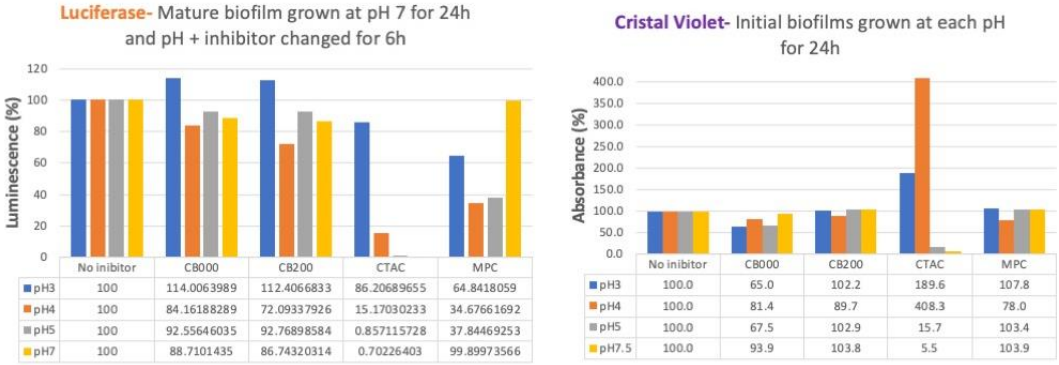


Figure 1 (A) Several possible iterations of the CB molecule. **(B)** Percentage of linear molecule (zwitterion) present in aqueous solution as a function of the measured pH. By varying the number and localization of methyl substitutions in the parent molecule, it is possible to control the pH range at which the switching happens. Ongoing work will aim at further adjusting the substitutions to accomplish the switching at pH 4.5-5.5.



	No inibitor	CB000	CB200	CTAC	MPC
pH3	100	114.0063989	112.4066833	86.20689655	64.8418059
pH4	100	84.16188289	72.09337926	15.17030233	34.67661692
pH5	100	92.55646035	92.76898584	0.857115728	37.84469253
pH7	100	88.7101435	86.74320314	0.70226403	99.89973566

	No inibitor	CB000	CB200	CTAC	MPC
pH3	100.0	65.0	102.2	189.6	107.8
pH4	100.0	81.4	89.7	408.3	78.0
pH5	100.0	67.5	102.9	15.7	103.4
pH7.5	100.0	93.9	103.8	5.5	103.9

Figure 2 **(A)** Luciferase Assay- Mature biofilm grown at pH 7 for 24h and pH + inhibitor changed for 6h and **(B)** Cristal Violet Assay -Initial biofilms grown at each pH for 24h