

# **pH-activated Nanoparticles for Controlled Topical Delivery of Farnesol to Disrupt Oral Biofilm Virulence**

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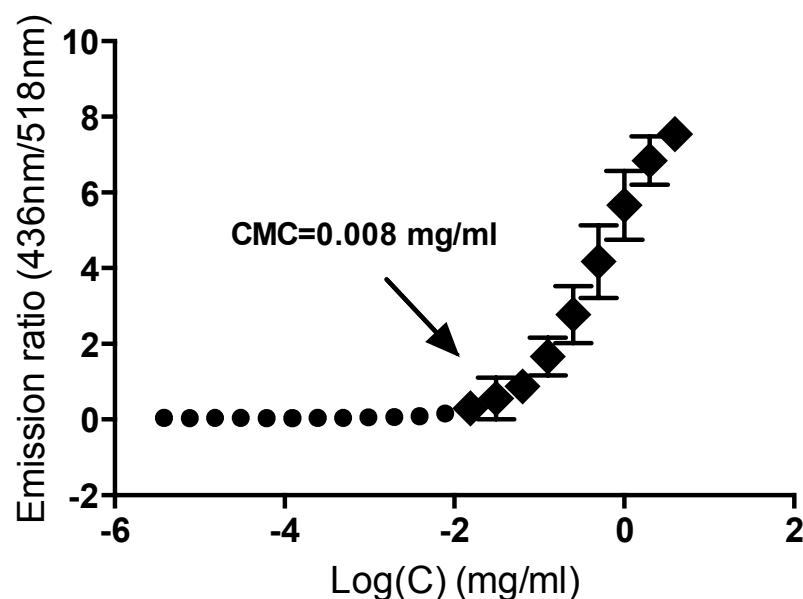
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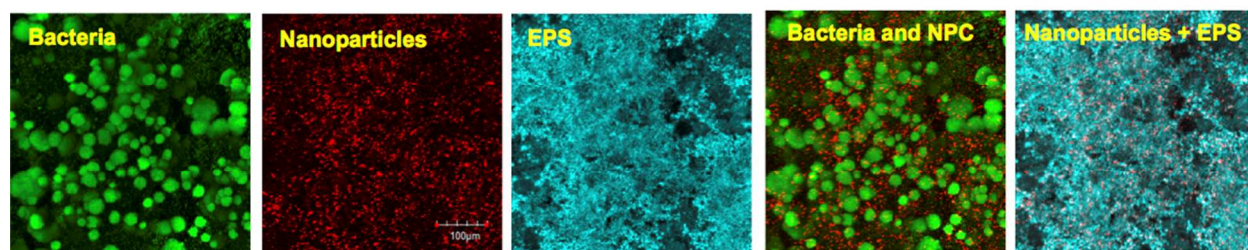
§ Authors contributed equally to this work.

∞ Were involved in *in vivo* efficacy testing herein.

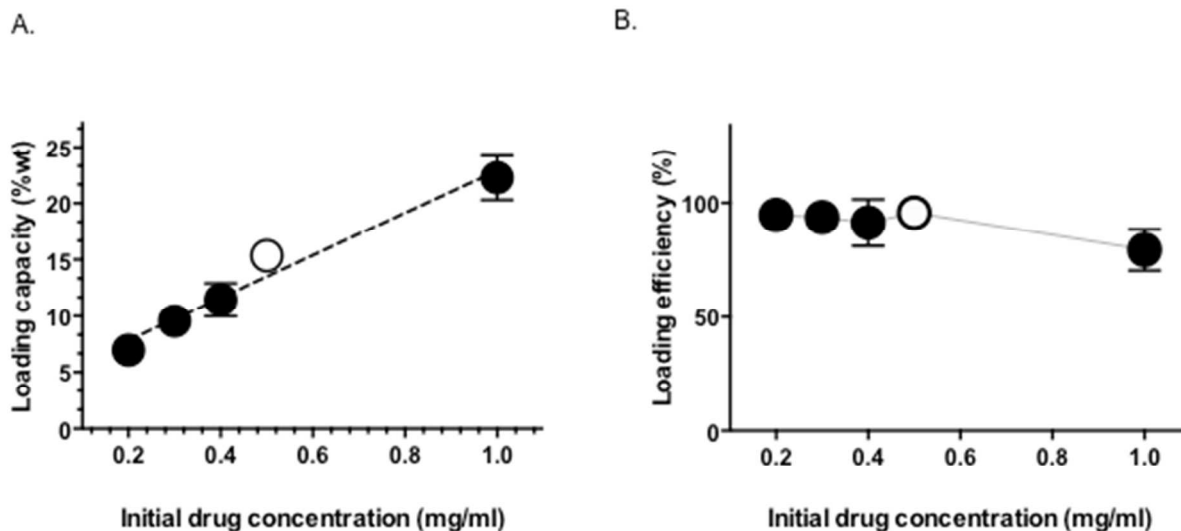
## Supplemental Figures



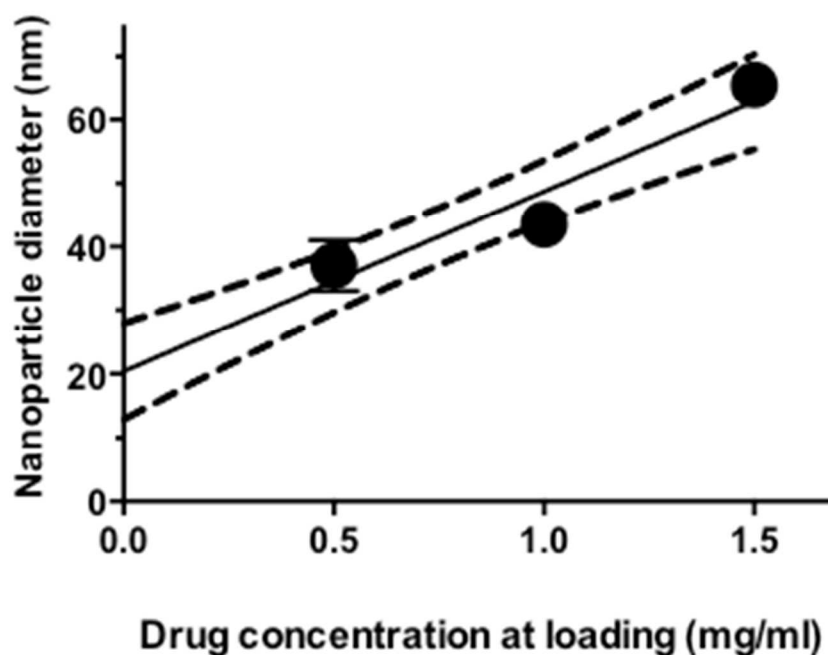
**Figure S1. Critical micelle concentration (CMC) of nanoparticles.** A range of nanoparticles concentrations was incubated with PRODAN<sup>®</sup> and the ratio of fluorescent emissions in hydrophobic phase/hydrophilic phases was plotted versus log(micelle concentration). CMC was determined as a concentration at which the emission ratio begins to increase with polymer concentration. The error bars represent standard error of measurements (n=3).



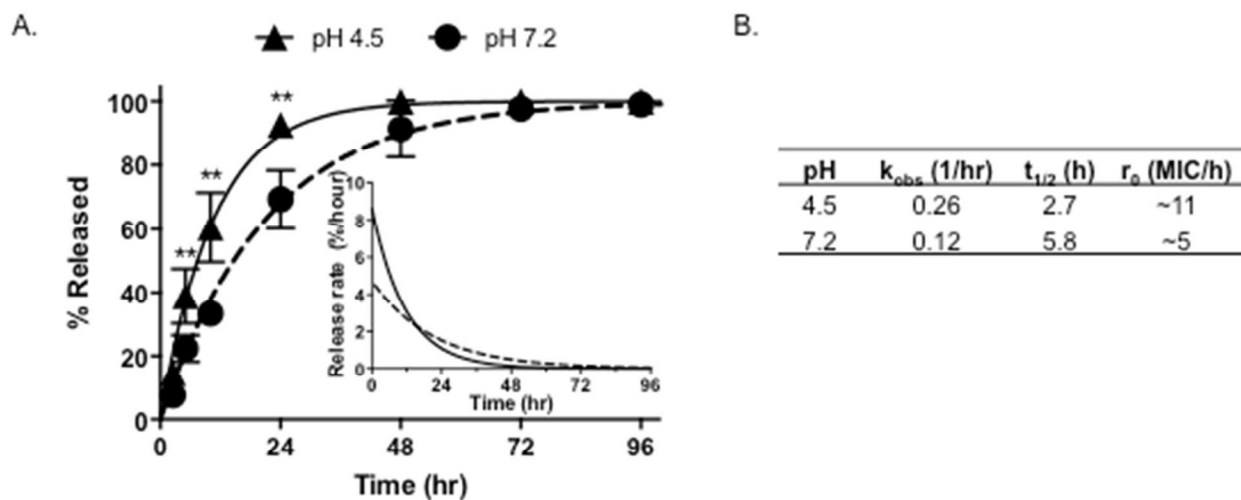
**Figure S2. Confirmation of free nanoparticle attachment to *S. mutans* biofilms treated surfaces.** Bacteria within biofilms forming microcolonies are depicted in green (SYTO 9 labeled), nanoparticles are depicted in red (Texas Red labeled), and EPS in blue (AlexaFluor 647 labeled).



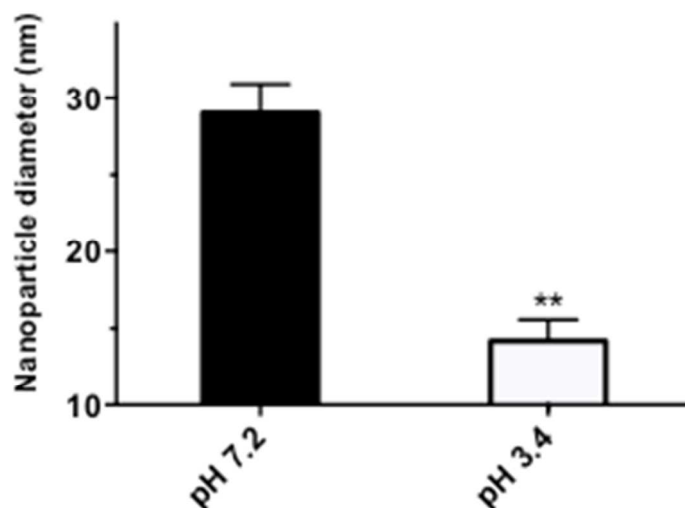
**Figure S3. Nanoparticle loading at a range of drug concentrations.** **A.** Loading capacities and **B.** loading efficiencies of nanoparticles. Blue data points denote loading capacities and efficiencies at which biofilm treatments were performed (15 wt%, 97%). Error bars represent standard error (n=3 independent experiments). As significant Pearson's correlation (dotted line,  $R^2 > 0.86$ ) between loading capacity and initial drug concentration at loading was determined by two-tailed t-test on Pearson's correlation ( $p < 0.0001$ ). The solid line in figure S4B is a guide to an eye.



**Figure S4. Increase in nanoparticle size upon loading.** Nanoparticle sizes were examined by dynamic light scattering (DLS) upon loading at a range of drug concentrations (0-1.5 mg/ml). Error bars represent standard error of measurement (n=2).



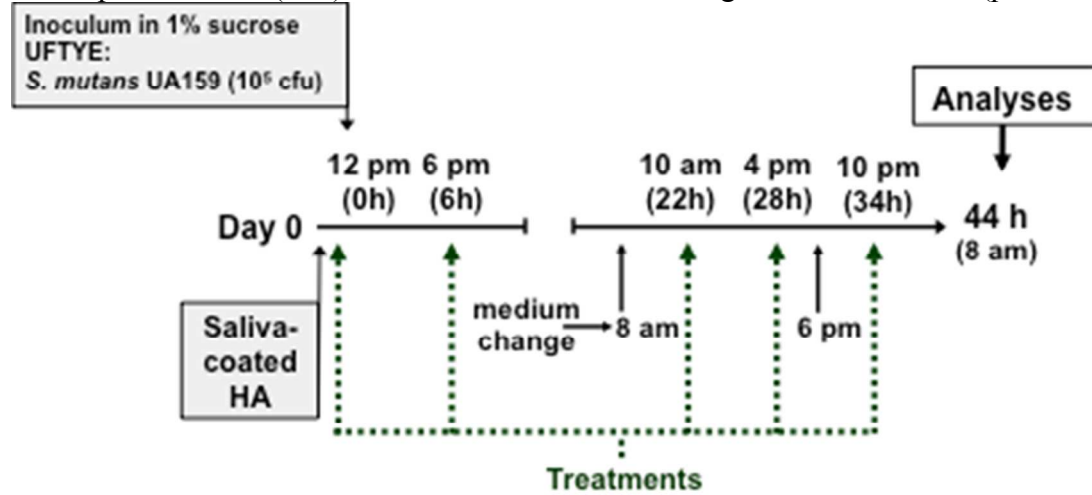
**Figure S5. pH-responsive release of farnesol-loaded nanoparticles in adsorption buffer.** A. Farnesol release profiles at pH 7.2 and 4.5, including farnesol release rates (inset). Solid and dotted lines show fits ( $R^2 > 0.98$ ) to first-order drug release and release rates determined by first derivative of the fits (inset). B. Kinetic parameters of release determined from fits to first order release ( $R^2 > 0.98$ ). Initial release rate (A. inset,  $r_0$ ), release rate constant ( $k_{obs}$ ) and half-time of release ( $t_{1/2}$ ) at pH 4.5 suggest 2-fold faster release at pH 4.5 as compared to pH pH 7.2, similar to data reported for PBS release experiments (Figure 3). Asterisks denote significant differences at  $p < 0.01$ , as determined by two-way ANOVA followed by Tukey's test for multiple comparisons. Adsorption buffer composition: 50 mM KCl, 1.0 mM  $KPO_4$ , 1.0 mM  $CaCl_2$ , 0.1 mM  $MgCl_2$ , pH 6.5.



**Figure S6. pH-responsive mechanism of nanoparticle structure destabilization.**

Nanoparticles demonstrate the pH-responsive structure destabilization at acidic pH. As a result of exposure to extreme acidic pH, ~2-fold decrease in nanoparticle diameter was observed due to

protonation and repulsion of DMAEMA residues within nanoparticle coronas and cores. Error bars represent SEM (n=5) and the asterisks denote as significant difference ( $p < 0.001$ ).



**Figure S7. Treatment regimen during biofilm prevention assay.** Biofilms were formed on sHA surfaces, and treated with either farnesol-loaded nanoparticles (15 wt%) or controls using clinically-relevant treatment regimen of 2-3 treatments per day.