Dual Antibacterial Drug Loaded Nanoparticles Synergistically Improve Anti-Oral Biofilm Treatments

Kenneth R. Sims Jr.[1], Brian He[1], Julian Maceren[1], Yuan Liu[2], Geelsu Hwang[2], Hyun Koo[2], and Danielle S. W. Benoit[1]
[1] University of Rochester; [2] University of Pennsylvania

Statement of Purpose: Tooth decay, predominantly orchestrated by *Streptococcus mutans,* is the most common disease worldwide and is caused by biofilm formation on the tooth surface [1,2]. We have established pH-responsive nanoparticle carriers (NPCs) capable of loading the membrane disrupting agent farnesol (Far) and binding to tooth, pellicle, and biofilm surfaces [3,4]. However, this approach modestly affected bacterial viability yielding only ~1 log colony forming unit (CFU) reduction against *in vitro* biofilms [3,4]. To enhance anti-biofilm efficacy, we sought to co-load and deliver farnesol plus a potentially synergistic drug to biofilms using NPCs. *S. mutans* secretes glucosyl-transferases (Gtfs), which catalyze biofilm formation by adsorbing to the salivary pellicle and producing exopolysaccharide (EPS) matrix [1]. Therefore, the flavonoid myricetin (Myr), which inhibits Gtf activity and EPS production necessary for biofilm formation [5], was evaluated for incorporation into the NPCs. NPCs co-loaded with Myr and Far to improve anti-biofilm efficacy by first blocking Gtf-mediated EPS matrix production and then killing remaining bacteria were investigated.

Methods: Diblock co-polymer NPCs synthesized via reversible addition-fragmentation chain transfer polymerization containing 2-(dimethylamino) ethyl methacrylate (DMAEMA), butyl methacrylate (BMA), and 2-propylacrylic acid (PAA) (Figure 1A) were loaded with Myr and Far in PBS (Figure 1B). Myr-NPC association constants (K_a) were determined using fluorescence and absorbance spectroscopy (Figure 1C) and loading capacities for each drug were measured as a function of Myr concentration using high performance liquid chromatography (Figure 1D). Gtf inhibition and anti-biofilm efficacy were assessed based on biofilm dry weight (DW) and CFU per DW results (Figure 1E) from *S. mutans* 48-hour *in vitro* biofilms formed on saliva-coated hydroxyapatite disks.

Results: Overall, results revealed that electrostatic interactions occur between Myr and the NPC corona with K_a values from 1×10^4 M^{-1} to 5×10^4 M^{-1} (Figure 1C). This interaction did not inhibit Far loading in the hydrophobic NPC core for Myr concentrations below 2.4 mM (Figure 1D), so NPCs co-loaded with Myr and Far were tested against 48-hour *in vitro S. mutans* biofilms. These studies yielded a ~60% reduction in DW and a ~95% reduction in CFU/DW compared to the control group (Figure 1E), thus revealing the synergy of this dual-loaded NPC approach.

Conclusions: This study confirmed the ability of a pH-responsive NPCs co-loaded with Myr electrostatically and Far hydrophobically to synergistically improve *S. mutans* anti-biofilm efficacy *in vitro*. These findings offer key insights about NPC drug delivery systems as anti-biofilm treatments against oral diseases, such as tooth decay.

References:

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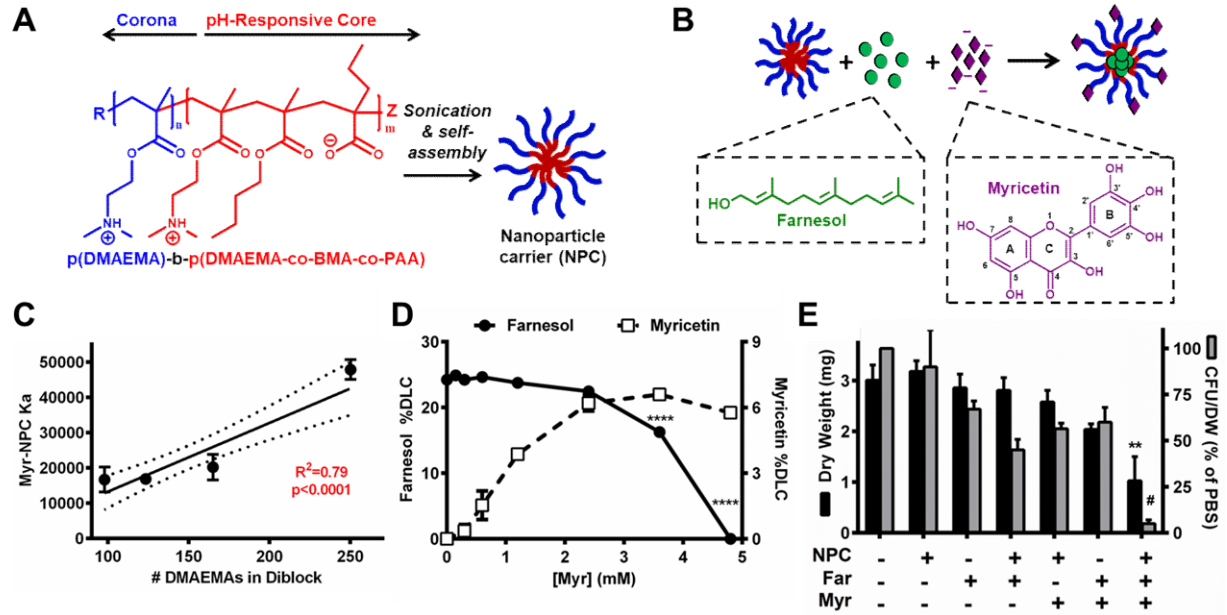

Figure 1. A. Scheme showing NPC polymer composition. **B.** Cartoon illustrating the hypothesized co-loading mechanism where farnesol loads in the NPC core while myricetin interacts electrostatically with the cationic NPC corona. **C**. Scatter plot showing myricetin-NPC K_a values increase with the number of DMAEMA monomers in the diblock co-polymer using NPCs with different Block 1 Mn values and similar Block 2 Mn values. **D.** Myricetin and farnesol drug loading capacity (% DLC) curves for a co-loaded NPC. **** $p<0.0001$ via One-way ANOVA with Dunnett's test. **E.** Anti-biofilm efficacy of NPCs co-loaded with Far and Myr *in vitro* results in ~60% reduction in biofilm dry weight (DW, left y-axis) and ~95% reduction in colony forming units per DW (CFU/DW, right y-axis) against *S. mutans* 48-hour biofilms. ** $p<0.01$ and # $p<0.0001$ via One-way ANOVA with Dunnett's test.