Nanoparticles for targeted, pH-responsive delivery of anti-biofilm drugs

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Statement of Purpose: In the US alone, $81 billion was spent on diagnosis, prevention, and treatment of oral biofilm-associated diseases in 2006[1]. Treatment or prevention of dental biofilms requires delivery of high concentrations of anti-bacterial agents at the dental surface. Thus, diblock copolymers composed of poly(2-dimethylamino)ethyl methacrylate, butyl methacrylate (BMA), and 2-propylacrylic acid (PAA) (pDMAEMA-b-p(DMAEMA-co-BMA-co-PAA)), were explored for their ability to target the dental surface and entrap and deliver the anti-biofilm drug, farnesol. Diblocks self-assemble into ~20 nm positively-charged nanoparticles with hydrophobic cores capable of drug loading[2]. Moreover, the diblocks have established pH-responsive behavior [3] that may provide a means to trigger drug delivery, as dental biofilm microenvironments exhibit greatly reduced pH (~4.5). We assessed adsorption of diblock copolymer nanoparticles to surfaces that emulate the dental surface. Nanoparticle loading capacities and release profiles of farnesol, an anti-biofilm drug were investigated.

Methods: Diblock copolymers were synthesized by reversible-addition fragmentation chain transfer (RAFT) polymerization to achieve mono-disperse polymers (PDI<1.3) of ~20 kDa, with equivalent first and second block molecular weights. Reaction conditions were degrees of polymerization (DP) of 200 and 250 for 1st and 2nd blocks, respectively, and 1:10 initiator to chain transfer agent (CTA) ratios. Nanoparticles were labeled with Texas Red-isothiocyanate, to quantify adsorption to HA (hydroxyapatite), sHA (hydroxyapatite coated with saliva proteins), and gsHA hydroxyapatite coated with saliva proteins and glucans), surfaces that emulate the dental surface. Adsorption of the nanoparticles and several control polymers including pDMAEMA alone, nanoparticles with pDMAEMA coronas and hydrophobic BMA cores, and nanoparticles with neutral external PEG blocks with p(DMAEMA-co-BMA-co-PAA) cores was performed. Finally, nanoparticles were loaded with farnesol using sonication. Drug loading capacities and release from the nanoparticles were measured by high performance liquid chromatography (HPLC).

Results: The role of pDMAEMA in the adsorption of nanoparticles to dental surfaces was examined (Figure 1.A). For adsorption experiments performed at 1 μM polymer, ~75% of pDMAEMA only (no core), ~55% of pDMAEMA-b-p(DMAEMA-co-BMA-co-PAA) and ~55% of pDMAEMA-b-BMA nanoparticles adsorbed as compared to ~27% of nanoparticles formed from PEG-b-p(DMAEMA-co-BMA-co-PAA). As they exhibited the greatest adsorption, have inherent pH-responsive behavior [3], and formed drug-loadable nanoparticles, more sophisticated adsorption experiments were performed on nanoparticles composed of pDMAEMA-b-p(DMAEMA-co-BMA-co-PAA) (Figure 1.B). According to Langmuir fits of the data, the maximal capacity of nanoparticles on the dental surfaces was ~21 μmol/m^2 and the adsorption affinity constants (K_a) were ~215 L*mmol^{-1}. These values did not differ statistically between the various dental surfaces. Nanoparticles were loaded with farnesol with maximal loading of 27 wt %. TEM images of the unloaded and loaded nanoparticles show slight (~10 nm) increase in size upon loading (Figure 2, inset). Drug release experiments showed that at pH 4.5, ~37% of farnesol was released after 5 hours with ~100% released after 48 hours, as compared physiologic pH, when ~24% release was achieved after 5 hours and ~70% after 48 hours (Figure 2).

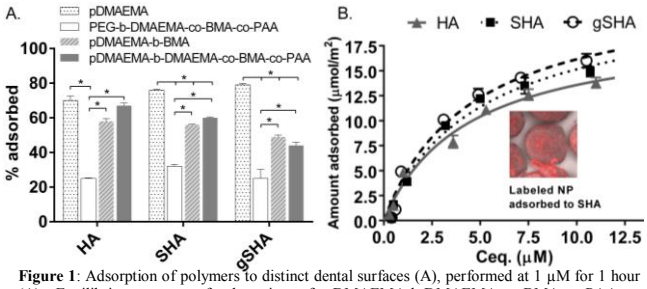

Figure 1: Adsorption of polymers to distinct dental surfaces (A), performed at 1 μM for 1 hour (A). Equilibrium curves of adsorption of pDMAEMA-b-DMAEMA-co-BMA-co-PAA at increasing concentrations (B). Two-way ANOVA was followed by Tukey's test for multiple comparisons (α=0.0001). The error bars represent SEM (n=3).

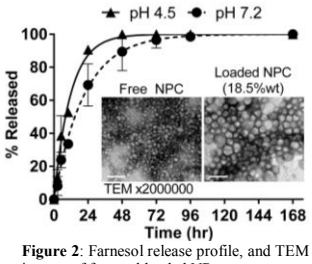

Figure 2: Farnesol release profile, and TEM images of free and loaded NP.

Conclusions: pDMAEMA-b-p(DMAEMA-co-BMA-co-PAA) nanoparticles strongly adsorb to dental surfaces with affinities ~20 times higher than the affinities of bisphosphonates to hydroxyapatite [4]. Nanoparticle affinity is likely due to positively-charged amine residues of the external pDMAEMA block that interact with the overall negative charge of dental surfaces[5], as similar adsorption behavior was observed with pDMAEMA alone, pDMAEMA-b-p(DMAEMA-co-BMA-co-PAA), and pDMAEMA-b-BMA. Farnesol, which is insoluble in water, can be loaded and delivered at ~26.7-fold greater concentrations from nanoparticles than its minimal inhibitory concentration (MIC) for common oral bacteria such as Streptococcus mutants (S. mutans)[6]. Rapid and pH-responsive farnesol release from nanoparticles indicates that therapeutic delivery may be advantageous for the relatively short windows of dental treatment and low pH microenvironments of pathologic dental biofilms. Current experiments are focused on the anti-bacterial activity of pH-responsive delivery, where preliminary data show 3-log and 4-log reductions of *S. mutants* concentrations at pH 7.2, and pH 4.0 respectively.

References:

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