Antimicrobial Performance of Surface Modifications Inspired by Plant Polyphenols

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Statement of Purpose: Phenols and polyphenols are ubiquitous in plant tissues, serving a variety of functions including: structural support, prevention of radiation damage, pigmentation, and chemical defense (Quideau S. Angew Chem Int Ed. 2011; 50: 586-621). Given the apparent properties associated with plant-inspired phenolics, materials utilizing such chemistries could afford desirable characteristic in biomedicine and beyond. Our focus was geared towards addressing the overwhelming issues associated with nosocomial and device-related infections, employing the tactics behind the vast molecular arsenal of plant phenolics. We have capitalized on the known antimicrobial properties of soluble phenolic materials and recent techniques for deposition of phenolics-derived coatings to create novel, potent antibacterial surfaces (Sileika TS. Angew Chem Int Ed. 2013; 52:10766-10770). The resultant coating deposition strategies provide nanoscale phenolic coatings, capable of adhering to virtually any surface. While complex phenolic extracts from sources such as green tea and cacao beans can be utilized to achieve a functional antibacterial coating, we further explored the use of defined molecular precursor systems, including tannic acid and pyrogallol. Ultimately, we have devised a single-step dip-coating strategy that can impart antimicrobial properties to any surface, capable of killing bacteria on contact. Additionally, subsequent modifications with poly(ethylene glycol) ligands facilitate antifouling behavior.

Methods: Wells of tissue culture plates were modified with pyrogallol (PG) and tannic acid (TA). Modifications were performed for 48 h using 1 mg/mL precursor in pH 7.8, 100 mM bicine buffer with 600 mM NaCl (buffered saline), under mild agitation. Following modification, the samples were thoroughly rinsed with deionized water and dried under reduced pressure. To impart antifouling character, as-prepared surfaces were further exposed to 1 mM poly(ethylene glycol) methyl ether-thiol (mPEG-SH, 5k MW) or -amine (mPEG-NH_2, 5k MW) in buffered saline for 10 min under mild agitation, followed by rinsing and drying. PG- and TA-modified surfaces, as well as those further functionalized with mPEG, were challenged with gram-negative and gram-positive bacterial strains, including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Inocula ranging from $3x10^6$ to $1x10^8$ CFU/mL were used, in 0.85% NaCl (starvation media) and complete trypticase soy broth, to assess antibacterial and antifouling performance of the materials. Live/dead evaluation was performed using Syto-9/propidium iodide stains.

Results: Mimicking the process of quinone tanning, we have artificially induced oxidation and subsequent oligo- and polymerization of PG and TA through the use of mildly-alkaline, saline solutions. The resultant nanoscale films appear to deposit on virtually any surface, including medically-relevant polymers, metals and ceramics. Upon

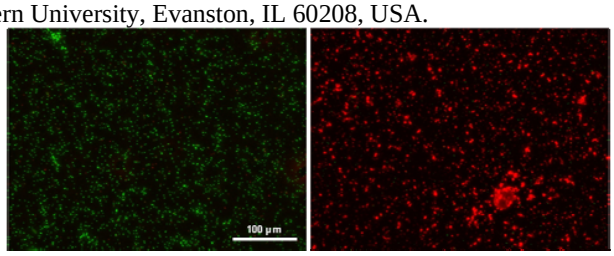

Figure 1. *Staphylococcus aureus* viability following 24 h exposure to bare (left) and TA-modified polystyrene (right). Live (green) and dead (red) signals are superimposed for each respective surface.

contact with the phenolics-derived coatings, bacterial cells exhibit a minimum of 30-fold reduction in viability in as little as 2 h, when compared to untreated controls. We have tested numerous gram-positive and gram-negative strains, including clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Figure 1). While contact-mediate antibacterial properties were observed, no marked increase in cytotoxicity towards mammalian cells was detected. The apparent gradient in toxicity between mammalian and bacterial cells is desirable in creating coatings for implantable medical devices, as well as any components that interface with bodily fluids. Thus, a one-pot approach can be utilized to directly impart contact-mediated antibacterial properties to virtually any substrate.

With further modification by mPEG-SH or mPEG-NH_2, bacterial cellular attachment is abrogated when challenging the surfaces with gram-negative and gram-positive strains. Such antifouling characteristics are valuable in prevention of potential biofilm formation on medical device surfaces, as eradication of latent colonization can necessitate elevated antibiotic concentrations for treatment and potential surgical intervention (Dennis FB. J Vasc Surg. 1991; 13:575-583).

Conclusions: Mimicking the chemical promiscuity of phenolic materials innately found in plant tissues, we have developed a strategy for facile deposition of phenolics-based coatings onto virtually any material. The resultant coatings exhibit exceptional contact-mediated antimicrobial properties towards bacteria, including notoriously-resilient clinical isolates. We have found that subsequent modification of poly(ethylene glycol) ligands can further afford antifouling characteristics to the initial phenolic coatings. In addition to marked antimicrobial properties, we have found that such coatings do not harm interfacing mammalian cells, further potentiating our coating strategy for use in medical and implantable devices.