Reduction of *Pseudomonas Aeruginosa* Biofilms *In Vitro* and Attenuation of Foreign Body Response *In Vivo* by Salicylic Acid-Derived Poly(Anhydride-Esters)

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Statement of Purpose: Bacterial infections caused by adherent bacteria have been observed in numerous artificial and prosthetic implants. Current methods to prevent bacterial adhesion and biofilm formation on the implant imply treatment with high concentrations of antibiotics and in some cases, surgical removal of the infected implant becomes necessary.[1] Biodegradable polymers that deliver non-steroidal anti-inflammatory drugs (NSAIDs) are relevant as potential anti-biofilm therapy that locally inhibit bacterial growth and reduce inflammation. The purpose of this work was to quantify the ability of a salicylic acid-derived poly(anhydride-ester) (**1**) that degrades into salicylic acid (**2**) and adipic acid (**3**), as outlined in Figure 1, to prevent *Pseudomonas aeruginosa* biofilm formation *in vitro* and the effects on the foreign body response *in vivo*.

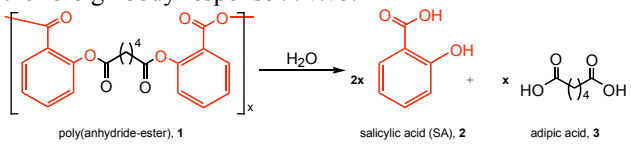

Figure 1

Methods: The ability of poly(anhydride-esters) (**1**) composed of non-steroidal anti-inflammatory drugs that biodegrade to salicylic acid (SA) and adipic acid to prevent colonization by *P. aeruginosa* and their effects on the foreign body response were studied *in vitro* and *in vivo,* respectively. Effect of soluble SA on suspended cultures of *P. aeruginosa* was determined by direct suspended cell count. SA-derived polymers were synthesized using previously described methods[2] and pressed into disks. Polymer disks (13 mm diameter x 1 mm thickness) were submerged in *P. aeruginosa* suspension for less than 3 h to determine bacterial adhesion at different time points. Biofilm formation on polymers disks submerged in sterile medium inoculated with *P. aeruginosa* was analyzed every 8 h over a 3-day period. A biodegradable polyanhydride that does *not* release salicylic acid served as inactive control. A recombinant *P. aeruginosa* pMHLAS, containing a fluorescent reporter gene prior to the *las* regulon[3], was employed to determine whether salicylate-based polymer (**1**) prevents biofilm formation by the released SA inhibiting quorum sensing pathways[3]. For foreign body responses studies, SA-releasing polymer **1** was compared to inactive polymer sub-cutaneous dorsal implantations in 10 control mice (C57/B16 genetic background). Animals were sacrificed at 4 weeks, permitting comparisons of retrieved polymer by Masson's trichrome-stained histological sections.

Results / Discussion: Soluble SA in bacterial medium concentrations up to 300 mg/mL did not affect the growth rate or viability of *P. aeruginosa*, indicating that SA does not exhibit a direct toxicity effect on the bacterium. Short term (3 h) bacterial adhesion studies in agitated batch systems indicated a 47 % reduction in the rate of *P. aeruginosa* adhesion relative to the control polymer that does not release SA upon biodegradation. Long term (3 d) biofilm accumulation studies indicated a dramatic reduction in biofilm formation on salicylate-based polymer versus control polymer (Figure 2). Long-term biofilm accumulation studies with *P. aeruginosa* pMHLAS indicate that salicylate-based polymer (**1**) prevents biofilm accumulation by possibly inhibiting the *las* quorum sensing system. Furthermore, unlike the biodegradable control polymer, salicylate-based polymer implanted sub-cutaneously for a period of 4 weeks resisted cell-mediated degradation and remained intact. Histological and immunohistochemical analysis indicated a reduction in overall encapsulation and paucity of macrophages in the area of the salicylate-based polymer implant.

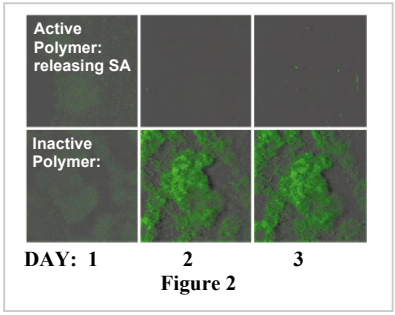

Figure 2

Conclusions: These findings provide important preliminary evidence that polymers of non-steroidal anti-inflammatory drugs (NSAIDs) degrade to produce salicylic acid and reduce or eliminate bacterial biofilm accumulation. Results indicate that the wild-type *Pseudomonas aeruginosa* exhibited slower adhesion rates and significantly less biofilm formation on salicylate-based polymer than on a related biodegradable polyanhydride control.

References:

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3. Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, Rice SA, Eberl L, Molin S, Høiby N, Kjelleberg S, Givskov M. Microbiology 2002;148: 87–102.