Pre-treatment with Poloxamer Surfactant Augments Estrogen-Mediated Bacterial Clearance by Phagocytes in an *In Vitro* Model of Chronic Wound Biofilms.

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Chronic wounds are common in the elderly and lead to substantial morbidity and mortality. Evidence suggests that age-related impaired healing may result in part from a decline in estrogen-mediated signalling with increasing age. Biofilm formation in chronic wounds affecting the elderly can often impede delivery of antibiotic therapy and enable bacteria to evade innate host immune responses. Unresolved biofilms typically lead to pronounced bacterial survival and spread, increased risk of infection by wound pathogens and delayed wound closure. Moreover, given antibiotic resistance is commonplace there is an urgent need to develop new therapeutic strategies to resolve wound infections with reduced reliance on antibiotics. Topical use of surfactants to disrupt established or developing biofilms alongside steroid hormone therapy to stimulate host immunity represents one such emergent strategy to combat biofilms in impaired wound healing states. The aim of this project was to investigate the combined use of poloxamer surfactants with targeted stimulation of innate immune responses by local estrogen replacement therapy as a potential novel dual strategy to resolve wound biofilms in the elderly.

Poloxamer surfactants (P188 and P407: 0.1, 1.0 and 10.0 mg/ml) were investigated alone and in combination with hormone supplementation (estradiol: $1x10^{7}$M) to assess their ability to promote biofilm disruption and inflammatory cell clearance of two key wound bacterial pathogens, *Staphylococcus aureus* (Sa) & *Pseudomonas aeruginosa* (Pa). The crystal violet (CV) assay was used to determine biofilm mass following treatment of Sa and Pa biofilms (n=6) with poloxamers, with or without combined estradiol supplementation for 24 hours. Host phagocytes (U937-derived monocytes and macrophages) stimulated with estradiol for 24 hours were assessed *in vitro* (n=6) in terms of host cell viability and clearance of Sa or Pa following pre-treatment of biofilms with poloxamer. Host-pathogen interactions were visualised by fluorescence and scanning electron microscopy (SEM).

Findings indicated estradiol ($1x10^{7}$M) had no detrimental effects on host phagocyte proliferation or viability. Both poloxamers were tolerable to host inflammatory cells (with no significant effect on cell proliferation or viability) when applied at the lower dosage of 0.1 mg/ml, so this concentration was selected for subsequent investigation in the study. Both poloxamer P188 and P407 significantly ($P<0.05$) reduced biofilm mass when applied directly to Sa and Pa biofilms for 24 hours at a concentration of 0.1 mg/ml. Estradiol had no effect on biofilm mass when applied directly to biofilms but hampered the poloxamer-mediated reduction in biofilm mass when applied concomitantly as a combined treatment with P188 or P407. This interaction between poloxamers and estradiol confirmed sequential (rather than concurrent) dual therapy was more effective at

biofilm reduction. Pre-treatment of established biofilms of Sa or Pa with 0.1 mg/ml poloxamer (P188 or P407) for 24 hours significantly ($P<0.05$) increased subsequent phagocyte-mediated bacterial clearance compared to regimens lacking poloxamer pre-treatment. Similarly, a single therapy approach involving treatment of phagocytes with estradiol alone significantly ($P<0.01$) reduced bacterial recovery from Sa and Pa biofilms compared with untreated control phagocytes. More importantly however, sequential dual therapy involving pre-treatment of biofilms with 0.1 mg/ml poloxamer (P188 or P407) for 24 hours significantly ($P<0.05$) enhanced subsequent estradiol-mediated clearance of Sa and Pa by phagocytes compared to corresponding findings observed with single therapy approaches involving either estradiol or poloxamer pre-treatment alone.

Overall, this study shows that initial disruption of biofilms with poloxamer surfactants enhances subsequent host-mediated clearance of both Gram-positive and Gram-negative bacteria *in vitro*, particularly when phagocytes have been exposed to estradiol. These findings suggest that sequential treatment of biofilms with poloxamer followed by stimulation of phagocytes with estradiol may be an effective dual strategy to resolve wound biofilms in the elderly that warrants further investigation.