The role of oxygen and the interaction of human neutrophils with viable planktonic and biofilm Pseudomonas aeruginosa
by Justin Brock Bleazard

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science In Chemical Engineering
Montana State University

Abstract:
Human neutrophils are uniquely capable of generating reactive oxidants when exposed to a variety of serum-opsonized particles or soluble inflammatory stimulants. Neutrophils striking ability to pass high-energy electrons to molecular oxygen (O2) to form the superoxide anion (CF) is central for host antimicrobial defense. This pathway, though effective against bacterial and fungal pathogens, also generates free radicals associated with chronic and acute inflammatory damage of normal neighboring cells. However, bacterial pathogens that employ a biofilm mode of growth present additional obstacles to professional phagocytes such as neutrophils. The biofilm structure, the role of oxygen, and possibly the release of chemoattractants form biological and chemical barriers that may retard the penetration of phagocytes and inhibit host cellular defense mechanisms.

Microelectrode techniques were employed to measure the local mass transport phenomena of human neutrophils exposed to viable unopsonized and 10% autologous serum opsonized Pseudomonas aeruginosa biofilms and planktonic cultures. Both dissolved oxygen consumption and the subsequent production of hydrogen peroxide were examined. Viabilites of planktonic and biofilm Pseudomonas aeruginosa treated with human neutrophils were compared to untreated microorganisms. Also, Scanning Electron Microscopy (SEM) was used to study the surface of Pseudomonas aeruginosa biofilms before and during treatment with serum and neutrophils.

The results of this study indicate that human neutrophils show a decreased antimicrobial efficacy towards Pseudomonas aeruginosa biofilms when compared to their planktonic counterpart. This efficacy was increased by the autologous serum opsonization of bacteria, which agrees with the concept that relevant components of complement are crucial peptide mediators of inflammation. Also, biofilms exposed to 10% normal human serum displayed an immediate marked increase in oxygen consumption that rivaled that of human neutrophils. These results suggest that a dynamic competition between neutrophils and bacteria exists, providing biofilm bacteria with an increased resistance to cellular antimicrobials. Microscopy results indicated both neutrophil plasma membrane stimulation and neutrophil penetration into the biofilm occurred. Further understanding of resistance mechanisms utilized by such bacteria will aid in the treatment of biofilm infection and inflammation.

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APPROVAL

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Justin Brock Bleazard

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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