NIH Project Specific Aims Template

The Specific Aims is required for all NIH applications. It is limited to, one page, and it must follow NIH font, margin, and formatting requirements. Refer to the NIH Application General Instructions and Research Instructions for the full guidelines.

Content:

Specific Aims should be no more than 1 page in length to explain why you want to perform this research, how is it significant, what issue(s) you are addressing, what will you do with your results, and what is the intended impact of your research.

Example

Specific Aims:

This proposal's *objective* is to determine the impact of the spatial structure and mechanics of *Pseudomonas aeruginosa* biofilm infections, in chronic wounds, on virulence, antibiotic resistance, and immune evasion.

Most chronic bacterial infections are caused by biofilms, aggregated bacteria that are embedded in a matrix of polymer and protein. Unlike well-mixed, liquid cultures, biofilm infections have well-defined spatial structure. This spatial structure is given by the sizes of bacterial aggregates, the relative positions of aggregates, and matrix heterogeneity. Aggregates also have viscoelastic mechanical properties that are conferred by the matrix. Basic principles of material transport indicate that the spatial structure of biofilm infections must impact intercellular signaling, virulence, and antibiotic resistance; comparison of biofilm mechanics with known phagocytic forces indicate that resistance to deformation and breakup likely help biofilms resist immunological clearance. However, there is little to no in-depth, quantitative knowledge regarding the impact of spatial structure and mechanics on disease course. Completion of the work we propose here will open new possibilities for therapeutic strategies that specifically target biofilm structure and/or mechanics.

Our *long-term goal* is to find new strategies for remediating biofilm infections by addressing physical properties. Here, our *central hypothesis* is that spatial structure and mechanics are the major *physical* factors controlling the development of pathogenicity, antibiotic resistance, and immune evasion in biofilm infections. This hypothesis is based on a synthesis of our own and others' published work. The *rationale* is that completion will identify key physical targets for preventing, disrupting, or ameliorating biofilm infections for an important biofilm-forming opportunistic human pathogen. The work we propose here will also develop experimental techniques and understanding of an important model system that together will constitute a widely-applicable platform for assessing the impact of biofilm structure and mechanics for other infecting organisms.

We will test our central hypothesis and attain our objective *via* the following *specific aims*:

1: Determine the spatial structure and mechanics of biofilm infections in wounds. For this, we will use sophisticated imaging to determine, in three dimensions, the size, number, locations, and heterogeneous matrix content of bacterial aggregates in a mouse model of chronic wound infection. We will simultaneously measure the density and distribution of neutrophils around the biofilm aggregates. At present, no good technique for measuring the mechanics of biofilm infections exists. We will develop such a technique using AFM microindentation and abradement of *ex vivo* biofilms. *Working hypothesis:* The structure and mechanics of *in vivo* biofilm infections in chronic wounds will follow development trajectories arising from the matrix-producing capabilities of the bacteria and pressure from the host immune defense.

2: Determine how spatial arrangements impact bacterial growth, biofilm microenvironments, antibiotic resistance, and virulence. For this, we will use manipulative techniques that we recently developed to recreate biofilm structures found in *in vivo* and *in vitro* environments and measure the biological changes induced by specific structures. *Working hypothesis:* Virulence and antibiotic resistance will depend on key structural characteristics, such as the sizes of aggregates and the distances between aggregates, through the development of microenvironments that differentiate as a result of the material transport and consumption of growth substrate, bacterial products, and antibiotics.

3: Determine the role of spatial structure and mechanics in biofilm-leucocyte interactions. For this, we will add freshly-isolated human neutrophils to biofilms at different stages of formation and with different

structures and mechanics and monitor the attack by neutrophils and the bacterial response. Working hypothesis: Biofilm tolerance and evasion of neutrophils and their action will depend both on the neutrophils' ability and speed in breaking off and engulfing pieces of biofilm, and on the ability of biofilms to kill immune cells.

The *expected outcome* of this work is a comprehensive understanding of what structures and mechanics develop in biofilm infection of chronic wounds, and the degree to which these structures and mechanics give rise to pathogenicity, antibiotic resistance, and evasion of the immune system. The results will have an important *positive impact* because they lay the groundwork to develop a new class of targeted treatments.