SUMMARY STATEMENT

PROGRAM CONTACT: [redacted]

(Privileged Communication)

Release Date: **02/27/2017**
Revised Date:

Application Number: **1 R01 AI121500-01A1**

Principal Investigator

GORDON, VERNITA

Applicant Organization: UNIVERSITY OF TEXAS, AUSTIN

Review Group: **BMBI**
Biomaterials and Biointerfaces Study Section

Meeting Date: **02/15/2017**
Council: **MAY 2017**
Requested Start: **05/01/2017**

RFA/PA: **PAR16-242**
PCC: **M36**

Project Title: **Assessing the roles of biofilm structure and mechanics in pathogenic, persistent infections**
SRG Action: **Impact Score:15 Percentile:1**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested	Estimated Total Cost
1		
2		
3		
4		
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.
NEW INVESTIGATOR

1R01AI121500-01A1 Gordon, Vernita

NEW INVESTIGATOR

RESUME AND SUMMARY OF DISCUSSION: This application proposes to determine the mechanics and structure of biofilm infections of the opportunistic pathogen Pseudomonas aeruginosa in chronic wounds and how these physical properties impact disease course. The impact of these studies, if successful, will address the major physical factors controlling virulence, antibiotic resistance, and immune evasion in biofilm infections and is expected to give rise to new types of treatments and diagnostics for chronic biofilm infections that specifically target structure and mechanics. The strengths of the application are the dramatically improved focus; the supportive preliminary data; and the novel mechanism to treat biofilms. Concerns areb that it will require a large amount of coordination between groups. Overall, the application receives much enthusiasm in the area of biofilm infections.

DESCRIPTION (provided by applicant): What spatial structure and mechanics develops in biofilm infections, and how such spatial structure and mechanics impact the persistence and virulence of biofilm infections, is not known. The long-term goal is to find diagnostic and treatment approaches that address the structure and mechanics of multicellular, three- dimensional biofilm infections within the host. The objective of this application is to determine the mechanics and structure of biofilm infections of the opportunistic pathogen Pseudomonas aeruginosa in chronic wounds, and how these physical properties impact disease course. The central hypothesis is that spatial structure and mechanics are the major physical factors controlling virulence, antibiotic resistance, and immune evasion in biofilm infections. The rationale underlying this application is that completion will identify key physical targets for preventing, disrupting, or ameliorating biofilm infections for an important biofilm-forming pathogen. The proposed work will also develop a widely-applicable platform for assessing the state and impact of biofilm structure and mechanics for other infecting organisms. The central hypothesis will be tested by pursuing three specific aims: 1) Determine the spatial structure and mechanics of in vivo biofilm infections; 2) Determine how spatial arrangements differentiate into distinct microenvironments; 3) Determine the role of spatial structure and mechanics in biofilm-neutrophil interactions. We will pursue these aims using an innovative combination of analytical and manipulative techniques from both biological and physical sciences. These include both recently-developed techniques specific to biofilm studies, and more-established techniques that have been applied very little to the study of biofilm materials. The proposed research is significant, because it will determine which structural and mechanical characteristics should be therapeutic targets. It is also significant because it will develop a platform that can be extended to study other pathogens (or commensals) and synergies to open new avenues for biofilm therapies. This work will develop foundational resources that will be used by other researchers, for P. aeruginosa and other organisms. The proximate expected outcome of this work an understanding of which biofilm structural and mechanical characteristics contribute to clinical impact. The results will have an important positive impact immediately because they will establish better understanding of biofilm infection, virulence, and resistance to antibiotics and the immune system for an important pathogen, and long-term because they lay the groundwork to develop a suite of techniques for better treatment of biofilm infections.

PUBLIC HEALTH RELEVANCE: The proposed research is relevant to public health because understanding what biofilm structures and mechanics exist and the degree to which they influence the medical outcomes of biofilm infections is expected to give rise to new types of treatments and diagnostics for chronic biofilm infections that specifically target structure and mechanics. Thus, this application is relevant to the part of NIH's mission that pertains to fostering fundamental creative discoveries and innovative research strategies as a basis for ultimately protecting health.

CRITIQUES: The written critiques of individual reviewers are provided in essentially unedited form below. These critiques were prepared prior to the meeting and may not have been revised afterwards.

CRITIQUE 1:

Significance: 2
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 1

Overall Impact: This application is to investigate the structural and physical properties of biofilms using a wide range of novel techniques developed by the team and how these properties affect infections, antibiotic resistance, resistance to immune invasion and virulence. The proposed study is very novel in several aspects including novel techniques used, different properties to be studied, new insights into biofilm development, etc. This application has been improved substantially from the previous applications and the team has made several progresses with published records to support the current study. A few concerns still exist including coordination of the research activities, unclear description of budgets, inconsistence in description of research activities and justification of animal to be used.

1. Significance:

Strengths

- It is novel to investigate the structural and physical properties of biofilms.
- Better understanding of such properties will likely lead to develop novel control strategies.

Weaknesses

2. Investigator(s):

Strengths

- All investigators have published extensively in the areas of proposed research.

Weaknesses

- It is not well defined specifics tasks for each investigator.

3. Innovation:

Strengths

- Novel techniques will be developed to study biofilms.
- The application focus on the structural and mechanical properties of biofilms.
- The application will also study interaction of immune cells with biofilms.

Weaknesses

4. Approach:

Strengths

- The application will use both in vivo and in vitro models for characterization of biofilm.

- Several cutting-age techniques have been proposed for the study.
- End-point measurement are clearly defined.

Weaknesses

- In aim 1, how to image biofilms in specific sites of wounded area over time is not clearly described.
- In aim 2, in vitro antibiotic resistance test will be performed. Why not use in vivo model?
- In aim 3, in vitro model will be used; however, mouse is needed in the animal protocol section?

5. Environment:

Strengths

- Excellent environment.

Weaknesses

Protections for Human Subjects: Not Applicable (No Human Subjects)

Vertebrate Animals: NO, animal welfare concerns or incomplete

- Mouse is still required for the aim 3 but not described in the approach section

Biohazards: Acceptable

Resubmission:

- It is a much-improved submission comparing to the previous one with a more focused mission. However, there are several inconsistences in the application including animal use, the model to be used for aim 1 studies.

Resource Sharing Plans: Unacceptable

- The proposed meetings and data exchange is not sufficient for such complex projects

Authentication of Key Biological and/or Chemical Resources: Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Certain portions of budget justification are missing. Support for several graduate students/postdocs is requested without clearly defined tasks on the project.

CRITIQUE 2:

Significance: 1

Investigator(s): 1
Innovation: 2
Approach: 1
Environment: 1

Overall Impact: The central hypothesis to be tested in this resubmission of an R01 application is that spatial structure and mechanics are the major physical factors controlling the development of pathogenicity, antibiotic resistance, and immune evasion in biofilm infections. To test this hypothesis, they will use *Pseudomonas aeruginosa* as a model microorganisms and determine the spatial structure and mechanics of biofilm infections in wounds; determine how spatial arrangements impact bacterial growth, biofilm microenvironments, antibiotic resistance, and virulence; and determine the role of spatial structure and mechanics in biofilm leucocyte interactions. This is a much-reduced set of objectives compared to the original application, which was to examine two microorganisms in three environments. Other significant changes have been made in response to SS's critique, including a reorganization of the research team. The overall effect is a tighter application with high probability of success.

1. Significance:

Strengths

- The scientific premise of this application is that biofilm microarchitecture and mechanics are key to the development, antibiotic resistance and immune evasion in biofilm infections. Understanding this may prove to be key in preventing and treating biofilms.
- The data resulting from this study will be important to the field and for the development of drugs that target novel sites, leading to prevention, disruption or amelioration of biofilms.

Weaknesses

- None

2. Investigator(s):

Strengths

- This is an outstanding team of investigators, each bringing critical expertise to the problem.
- The team is already interactive.

Weaknesses

- None

3. Innovation:

Strengths

- The approach that will be used for this study is innovative and will result in new tools for studying biofilms. The team will develop a new technique for measuring the mechanism of biofilm infections.
- Sophisticated imaging will be used to characterize biofilm structure and manipulative techniques we be employed to recreate these structures to better understand their role.
- A fresh approach will be used to examine the interplay between neutrophils and bacteria.

Weaknesses

- None

4. Approach:

Strengths

- Use of in vivo biofilms rather than in vitro biofilms.
- Selection of a panel of mutants to examine the role of alginate, Psl, and Pel.
- Multiple assays for disease outcomes: systemic spread of bacteria, quorum sensing, antibiotic effectiveness, inflammation (neutrophil invasion), wound closure and healing.
- Physical structures of biofilm will be determined by CLSM and quantitative image analysis.
- Use of quantum dots to assess diffusion characteristics.
- AFM and microrheology will be used to assess biomechanics.
- In vivo data will be compared to biofilms grown in vitro.
- Aim 2 will take advantage of novel methods for building biofilms that allows monitoring while testing different microarchitectures on biofilm development, etc. This will enable them to examine different properties noted in the mouse model.
- Aim 3 will add neutrophils to the equation. The three aims build on each other nicely.

Weaknesses

- The study relies on close coordination among laboratories.

5. Environment:

Strengths

- The environments at the two partnering institutions are mutually synergistic.

Weaknesses

- None

Protections for Human Subjects: Not Applicable (No Human Subject)

Vertebrate Animals: YES, all four points addressed

- Care has been taken to ensure the least number of animals will be used for the studies.

Biohazards: Acceptable

- Pseudomonas will be handled in appropriate biosafety hoods.

Resubmission:

- The application has responded well to SS critique. Where appropriate, they have explained their rationale for the choices they have made.
- The application is much more focused than originally.

- Preliminary data are now published.

Resource Sharing Plans: Acceptable

Authentication of Key Biological and/or Chemical Resources: Acceptable

Budget and Period of Support: Recommend as Requested

CRITIQUE 3:

Significance: 2
Investigator(s): 2
Innovation: 1
Approach: 2
Environment: 1

Overall Impact: This application seeks to test the hypothesis that virulence, antibiotic resistance, and immune evasion in biofilm infections of the pathogen *Pseudomonas aeruginosa* within chronic wounds are influenced by the biofilm's physical properties. Three aims are proposed to test this hypothesis. In Aim 1, *P. aeruginosa* applied to surgical excisions (in mice) will form biofilms; systemic bacterial load will be evaluated along with quorum sensing, antibiotic efficacy, and inflammation. Biofilms will be evaluated for a comprehensive set of outcomes including mass transport, heterogeneity, and mechanical properties. Aim 2 seeks to determine how spatial structures within the film develop microenvironments that influence biofilm disease, affect antibiotic resistance, and virulence. These two aims are well founded in the literature that was reviewed within the Research Strategy, and both aims were also well supported by a robust body of preliminary data. Aim 2 is particularly compelling as the application and team seek to recreate what are effectively biomimetic biofilms (based on observations made in Aim 1) and then evaluate these 'synthetic' biofilms for controlled studies of the influence of biofilm structure. Finally, in Aim 3, how properties including elasticity and yielding relate to immunological clearance are evaluated in a systematic manner. Overall this is a strong team with a strong application that has clearly benefitted from focus added as a result of the initial review. This work has high potential translational potential as a better understanding of factors that contribute to virulence, persistence, and function of biofilms may lead to identification of new therapeutic targets.

1. Significance:

Strengths

- Chronic infections affect millions of Americans and may result in death and sometimes result in life-limiting comorbidities including amputation.
- The influence of biofilm physical properties and structures are considered important to the survival and persistence of the biofilms, yet comprehensive characterization of these factors has not been performed and so the influence of these factors on virulence, antibiotic resistance, and immune evasion remain unknown. Yet elucidating these factors will enable identification of potential therapeutic targets.
- The scientific premise of this application is that the structure and mechanical properties and behavior are critical to the persistence and antibiotic resistance of biofilms. Elucidating the role

of biofilm structure and mechanics may provide insight into novel approaches to treat and mitigating chronic biofilm infections.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- This investigative team is led by an exciting new investigator (Dr. Gordon), an Assistant Professor in the Department of Physics at UT Austin, with expertise in characterization of physical properties and biological manipulation of biofilms.
- The collaborative and interdisciplinary team members bring expertise in bacterial pathogenesis in wound and soft tissue infections (Runbaugh), advanced biomedical imaging a rheology of complex fluids (Christopher), and imaging and visualization of bacteria (Shear).

Weaknesses

- None noted. This is a strong team.

3. Innovation:

Strengths

- Relating the physical properties (i.e., spatial structure and mechanics) of *Pseudomonas aeruginosa* biofilms in chronic wounds to the virulence, antibiotic resistance, and immune evasion is novel and holds potential impact for improving patient outcomes and reducing development of antibiotic resistant infections.
- Successful completion of the proposed work would enable development of novel therapeutic strategies with a specific focus on disrupting the biofilm structure and/or mechanics.

Weaknesses

- None noted.

4. Approach:

Strengths

- A key strength of this application is the assessment of only one pathogen *Pseudomonas aeruginosa*. This tight focus will enable the team to dig deeply into the questions posed in this application without losing focus as would likely happen with multiple strains of bacteria.
- Aim 1 – development of a new imaging technique using fluorescent beads and quantum dots to measure transport properties of in vivo infection. This technique will extend beyond studies of biofilms. Also, new technique in Aim 1 to evaluate heterogeneity of the matrix content. Both are strengths of this application.
- Aim 2 is particularly compelling as the application and team seek to recreate what are effectively 2D biomimetic biofilms (based on observations made in Aim 1), e.g. using optical trapping to control heterogeneity, and then evaluate these ‘synthetic’ biofilms for controlled studies of the influence of biofilm structure.
- Aim 3 makes a nice progression from in vitro to in vivo studies with a clearly defined and effective experimental design.

Weaknesses

- Adhesion is likely to occur between the AFM tip and biofilms (e.g., see Activity 1.2); however, this is poorly accounted for in the research strategy. The citation #156 used in this context describes a soft film on a stiff substrate and does not review or provide reasonable solutions to deal with adhesive contact between a probe and a half space.
- With regards to AFM testing, how will films be removed from the animal without disrupting the film structure or causing viscous dissipation (and thus affecting the measured properties)?

5. Environment:

Strengths

- The environments at UT Austin and in the lab of the application and her collaborators (at both UT Austin and at Texas Tech University) are excellent. The equipment and facilities needed to excel at the proposed research are available.

Weaknesses

- None noted.

Protections for Human Subjects: Not Applicable (No Human Subjects)

Vertebrate Animals: YES, all four points addressed

Biohazards: Acceptable

Resubmission:

- The application and her team appear to have responded to the previous reviewer's criticisms in a thorough and careful manner.
- Alterations from the original application should be but were not clearly denoted within the Research Plan.

Resource Sharing Plans: Acceptable

Authentication of Key Biological and/or Chemical Resources: Acceptable

Budget and Period of Support: Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMAL: ACCEPTABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R01 AI121500-01A1; PI Name: Gordon, Vernita

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.