Project Title:

Rapid Antibiotic-Resistance Phenotyping of Bacteria from Blood Infections

Principal Investigator(s) (full-time faculty)

Name (PI listed first)	Department	College
William Pitt	Chemical Engineering	Engineering
Robert David	Physics	Math and Physical Sciences
Richard Robison	Micro and Molecular Biology	Life Sciences

Abstract

Over 38,000 people died in 2014 in the U.S. alone due to septicemia, a bloodstream infection capable of eliciting the life-threatening condition known as sepsis. Rapid identification and effective treatment of septicemia are essential to prevent severe sepsis and overcome the alarming mortality rates observed today. Current diagnostics require 24 to 72 hours for blood culture, followed by antimicrobial susceptibility testing (AST), to determine bacteria resistance profiles. Reducing the time to diagnosis would offer significant benefits to patients while lowering mortality rates. Fluorescent detection of metabolic activity in bacteria can be utilized to measure AST in as little as 2-3 hours compared to the current 6-24 hours. This, in combination with a rapid bacteria separation and concentration step, to avoid lengthy times required for initial blood culture, could provide a much faster route to the infecting organisms resistance profiling. This in turn could allow for more effective treatment days sooner.

In this proposal we seek to evaluate the performance of AST of bacteria separated from blood using magnetic nanoparticles and confined to nanoliter droplets for fluorescence detection of bacteria viability. Our long-term goal is to develop an automated, multiplexed phenotypic resistance assay. To evaluate the feasibility of this approach, we aim to demonstrate positive identification of bacterial growth in nanodroplets within 3 hrs and provide a correlation to conventional AST testing for discrete antibiotic concentrations.

This research may enable the future development of a rapid sample preparation platform that integrates with an accelerated multiplexed antimicrobial susceptibility testing technology that could be used by hospitals and labs. This would enable healthcare providers to identify and treat a higher percentage of patients with septicemia before severe symptoms are manifest. Earlier treatment would reduce the frequency of sepsis, decrease days in hospital for infected patients, and ultimately diminish septicemia mortality rates.

The cross-disciplinary research team is composed of Dr. Pitt from Chemical Engineering, Dr. Davis from Physics, and Dr. Robison, who have worked together for 3 years on similar projects. However, this current proposal is a large deviation from what has been done previously. This is a totally new strategy to obtain phenotypic response to antibiotics, which is sorely needed and requested by the NIH. Combined these faculty have experience to tackle this problem, and to obtain the preliminary results needed to write a large proposal to the NIH.

Summary of Plans for External Funding

These preliminary data will be used towards 3 proposals. First, in combination with Precision Membranes LLC, a Provo Company, we will help them write an SBIR proposal with BYU as a subcontract. This will obtain future funding to collect more preliminary data and establish a commercial plan and platform. Secondly, we will use the data to write a large R01 to the NIH with Drs. Pitt and Davis as PIs. Third is a similar proposal to DARPA, who is also interested in phenotype assessment.

Project narrative (up to 5 pages).

Background and Significance:

Antimicrobial resistance is among the nation's most serious health threats. While the development of resistance mechanisms in bacteria cannot be stopped, it can be significantly slowed (CDC 2013). Long turnaround times (at least 24 to 72 hrs) for diagnosis of bacterial infection in blood are a major challenge in the battle to successfully treat infections (Diekema and Pfaller 2013). When a bloodstream infection is suspected, the risk of withholding treatment for these durations is intolerable – bloodstream infection, known as septicemia, progresses rapidly into a deadly sepsis if not treated, has mortality rates between 13-77% depending on the type of organism (Mayr et al. 2014), and is the 11th leading cause of death in the U.S. (killing more than 38,000 individuals annually) (Kochanek et al. 2016). For every hour of septic shock, without effective antibiotic administration, the survival rate decreases 7.6% (Kumar et al. 2006). Hence, physicians have no option but to begin treatment empirically while awaiting identification of the pathogen and its resistance profile.

Broad-spectrum antibiotics are the initial recommended treatment for septicemia, as it increases the probability of combating the infection effectively; but it also increases the risk of creating more resistance (Mayr et al. 2014) and may be totally ineffective. Broad-spectrum antibiotics also deplete natural flora, increasing the risk of developing deadly opportunistic infections such as *C. difficile* (Chalmers et al. 2016). The empiric treatment strategy usually continues until confirmation of the resistance profile of the organism, which may then mandate a change to a more effective antimicrobial such as carbapenam or colistin, antibiotics of last resort.

If the time to resistance profiling of bacteria in blood could be reduced to less than three hours, physicians could begin treatment with targeted, narrow spectrum antibiotics. Treatments would be more likely to be effective, patients would experience fewer side effects, and further development of antimicrobial resistance would be minimized. In addition, fewer cases of septicemia would be overlooked, leading to reduced mortality rates. This is our goal!

Several commercially available technologies exist for rapid genotypic resistome analysis (Opota et al. 2015), but phenotypic analysis remains essential because it conveys more practical information to the clinician (Hughes and Andersson 2017; Piddock 2016). The current state-of-the-art in clinical phenotype resistome diagnosis is liquid broth-based suspension culture in various antibiotics at a range of concentrations, such as commercial VITEK or Phoenix systems (Avesar et al. 2017). These require 6-24 hrs after a liquid culture has been established from a patient, which initial culture may take 24-48 hrs. Faster analysis technology is proposed and published, but not yet translated to the clinic (Avesar et al. 2017; Davenport et al. 2017). Even with dramatic improvements, e.g. 1-hour turnaround, the time required for initial blood culture still dominates the total turnaround time from blood sample to diagnosis. Analysis without prior culture is difficult because the bacterial load in a bacteremic patient's blood is as low as 10 colony forming units per milliliter (CFU/mL) compared to billions of blood cells per milliliter (Yagupsky and Nolte 1990). A typical sample of 7.5 mL would contain at least 75 CFU, sufficient quantity to perform a range of susceptibility tests; however, a compatible detection platform would need to process the full sample volume *and* be sensitive enough to detect growth in a reasonable time frame. This has yet to be demonstrated. *Our approach is significant* because it combines new sample preparation and multiplexing techniques in a way that promises to overcome the long-standing barrier to direct-from-blood phenotypic antimicrobial susceptibility testing (AST), reducing total turnaround times from 24-72 hrs to as few as 3 hrs from blood draw.

Research Team Unique Qualifications:

Dr. Pitt has been working in diagnosing and treating bacterial infections for 25 years. Additionally he has experience in microfluidic devices and fluid mechanics. Dr. Davis has been working on capture of bacteria using magnetic nanoparticles (MNPs) for 3 years. Dr. Robison is an expert in

bacteria and infectious disease, and his knowledge of antibiotic resistance and phenotype is crucial.

Research Plan - Methodology:

To overcome the need for lengthy blood cultures prior to phenotypic antibiotic susceptibility testing (AST), we propose a new sample preparation technique to concentrate bacteria from a patient blood sample (i.e., 7.5 mL) into a small volume. Dr. Jae Lee, our external collaborator at the University of Colorado-Denver, pioneered the use of MNPs modified with zinc-coordinated bis-dipicolylamine (bis-Zn-DPA) for this purpose. Bis-Zn-DPA, a synthetic small molecule targeting ligand, is easy to synthesize and very stable in comparison with antibodies and peptides, and exhibits high selectivity and fast kinetics (~seconds) toward bacteria in whole blood, resulting in the fastest published binding rates in blood (Lee et al. 2014). An initial reduction in volume is achieved by pelleting the MNPs, extracting the blood, and resuspending in 1 mL of buffer. Because this removes nearly all blood cells, further concentration by filtration down to 50 µL or less is possible. We have routinely filtered bacteria-MNP resuspensions (post blood extraction) onto 0.45 um filters without clogging. Fortunately, we observed no inhibition to culture growth for bacteria bound to these MNPs.

Because of this dramatic reduction in volume, rapid nanoliter AST techniques could begin within just 1 h of blood draw. Fluorescence of resorufin, a reduction of resazurin due to bacterial metabolic activity, has been used as a growth indicator for AST. This approach is accelerated when bacteria are confined in nanoliter droplets because resorufin cannot diffuse out of the droplet, generating a high local concentration with detectable signal-to-noise within 2 hours (Boedicker et al. 2008). Generating droplets containing antibiotics is possible by introducing the antibiotics upstream of the droplet generator, but varying the class and concentration is difficult without use of complicated valves and manifolds which require specialized manufacturing (Churski et al. 2012; Mohan et al. 2013). Formation of devices with this level of complexity is incompatible with the high-volume low-cost manufacturing required for a widespread medical test.

We propose to solve this need by creating nanoliter droplets and separating them into multiple channels where they will be passively dosed with antibiotics. As droplets pass by, antibiotics deposited and lyophilized during manufacturing diffuse slowly into the droplets at a controlled rate and in varying concentrations based on the initial amount deposited. Due to the simplicity of the approach, these devices are compatible with high volume manufacturing processes, e.g. injection molding and materials inkjet printing. We anticipate that the success of this technique will be of interest to many in the field of droplet microfluidics, as droplets are commonly used as chemical microreactors (Song et al. 2006) and the need for multiplex analysis is ubiquitous.

We seek to establish feasibility of creating an automated rapid AST assay which employs these innovations. A conceptual diagram of the assay to be developed is presented in Figure 1. Bacteria concentrated from blood using bis-Zn-DPA modified MNPs are resuspended in 1 mL (not represented) and introduced at Fig. 1-A. Unbound particles are separated from bacteria by filtration, enabling further volume reduction. Bacteria bound to particles are backwashed off of the filter using up to 50 µL of solution containing resazurin. This suspension is converted into nanoliter droplets for accelerated growth detection. Reduction to a small volume is critical to reduce the imaging area (50 µL as 1 nL droplets in serpentines with 100 µm walls equates to about 12.5 cm^2). When pumped into final serpentines for image analysis, droplets absorb varying concentrations of antibiotics which are deposited inside channels during manufacturing (Fig. 1, red regions). All serpentines are imaged using a low magnification epi-fluorescence macroscope in real-time to generate growth curves. Droplets containing bacteria with uninhibited growth fluoresce (Fig. 1, yellow circles) due to reduction of resazurin to resorufin (Fig 1-D). Confinement of resorufin within nanoliter droplets yields rapid detection of growth within 2 hours (Boedicker et al. 2008). For purposes of explanation, we show 13 serpentines in the figure. If initial bacterial load is 75 CFU and 80% are captured and recovered during backwash, then 56 CFU remain. To prevent a failed test, we require a 95% probability that each serpentine have at least 4 droplets with bacteria. According to the binomial cumulative distribution function, this occurs when 145 CFU are randomly distributed over 13 channels. Assuming a 30-minute doubling period, more than 145 CFU will be obtained within 1 h. A short incubation

period may be required, or (according to our preliminary data) this much growth may already occur during the capture process which takes about 1 h.

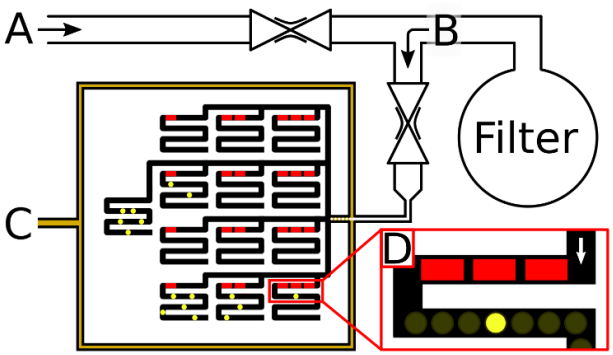

Fig. 1: Schematic of microfluidic concept for rapid phenotypic AST. **(A)** Bacteria captured from blood with magnetic particles are introduced as 1 mL resuspension, and then concentrated on a filter to remove unbound particles. **(B)** Up to 50 µL of solution containing resazurin removes bacteria from filter by backwashing. **(C)** Oil and sample are pumped simultaneously into a junction to produce droplets. **(D)** Expanded view of imaging region after incubation. Droplets with uninhibited bacterial growth fluoresce (yellow circles). On chip dosing of droplets (red regions) supports multiplexing with 12 test channels (4 antibiotics at 3 conc. covering ~12 cm^2) and a

Aim #1 – Capture bacteria and detect bacterial growth from 10 CFU/mL blood in less than 3 hrs.

Rapid isolation and detection of bacteria growth from blood samples is essential for enabling direct and rapid identification of antibiotic resistance in pathogens. This could be possible through MNP capture and filtration to remove excess liquid and unbound MNPs, followed by nanoliter droplet formation and fluorescent detection of bacterial growth. The *objective* of this Aim is to demonstrate growth of bacteria, isolated with our $MNP_{Zn\text{-}DPA}$ separation technique and incubated in nanoliter droplets containing resazurin, in less than 3 h. Our *working hypothesis* is that metabolic activity from bacteria bound with $MNP_{Zn\text{-}DPA}$ will not exhibit growth inhibition and will be detectable by fluorescence in nanodroplets using resazurin in well under 2 h. We will *approach* testing of this hypothesis by 1) demonstrating bacteria isolation and concentration of bacteria initially at a concentration of 10 CFU/mL in fresh, whole blood samples and 2) measuring the time to determine positive growth in nanodroplets with bacteria bound to $MNP_{Zn\text{-}DPA}$. We have selected four species of bacteria to determine feasibility of this approach: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus faecalis*. They are clinically relevant and account for a large percentage of sepsis cases (Mayr et al. 2014). We propose using spiked samples to demonstrate feasibility of our approach and will seek a path forward to use clinical samples from patients suspected to have bloodstream infections. The *rationale* for evaluating growth with bacteria bound to $MNP_{Zn\text{-}DPA}$ is that removing the $MNP_{Zn\text{-}DPA}$ from the bacteria could require significant changes to the growth conditions and introduce a lag phase. Culturing in the presence of the bound $MNP_{Zn\text{-}DPA}$ will keep bacteria capture, concentration, and resistance profiling compatible with bacterial growth, enabling much faster fluorescence growth detection in the nanoliter droplets. When the studies in Aim 1 have been completed, it is our *expectation* that the parameters identified will establish feasibility of using resazurin to evaluate metabolic growth in nanodroplets containing bacteria bound and concentrated from blood using $MNP_{Zn\text{-}DPA}$. This would demonstrate the ability to determine bacteria presence and viability within hours of an initial blood draw.

Only non-pathogenic bacterial strains will be used in this Aim. Serially diluted bacteria will be spiked into 7.5 mL of fresh human blood to a concentration of 10 CFU/mL, unless otherwise stated. The same dilution will be added to fresh cell-free plasma as a positive control (to evaluate enrichment efficiency). $MNP_{Zn\text{-}DPA}$ will be added to the spiked sample and control tubes and then they will be incubated while shaking to allow binding to occur. After incubation, the sample tubes will be placed on a magnetic rack to pellet captured bacteria. After removal of the supernatant, sterilized Mueller-Hinton (MH) broth will be added and removed without disturbing the pellet to remove any remaining cells or debris. MH broth will then be added again and the sample mixed to resuspend the solids

($MNP_{Zn\text{-}DPA}$ and bacteria). At this point, the samples will be filtered to remove excess liquid and unbound $MNP_{Zn\text{-}DPA}$ as described in Milestone 1. For each experiment, a blood test sample and plasma control sample will be vacuum filtered, the filters placed on plates, and cultured to determine bacteria isolation efficiency (calculated by dividing test-plate colony count by control-plate count). To avoid environmental bacteria contamination, only aseptic techniques and sterile reagents will be used for any experiments involving bacteria. These processes will be performed under the direction of Dr. Robison, an experienced microbiologist. Human blood samples will be obtained through the BYU Med Tech teaching lab under a BYU IRB. The blood will be collected in commercial vacutainer tubes supplied with ethylene diamine tetraacetic acid (EDTA) to knock out platelet function. The blood will be used on the same day as collection. Only adults (18+) will be allowed to participate.

Aim #2 – Correlate phenotypic AST in nanodroplets with conventional AST measurements.

Current clinical phenotypic AST techniques are inadequate for timely phenotype diagnosis of the antibiotic resistome, requiring 6-24 h after a liquid culture has been established from a patient, in addition to the initial culture which may take 24-48 h. Nanoliter containment and fluorescence detection of metabolic bacterial growth provides the necessary components for accelerated AST. By guiding the droplets down different channels, multiplexed analysis can be achieved by facile antibiotic dosing of droplets in each channel through contact with pre-deposited antibiotics of prescribed concentrations. Our *objective* for this Aim is to correlate AST as measured rapidly with bacteria captured and concentrated with $MNP_{Zn\text{-}DPA}$ with conventional AST on plates (Kirby-Bauer testing). Our *working hypothesi*s is that we can predict inhibitory concentration using $MNP_{Zn\text{-}DPA}$ captured bacteria, discrete antibiotic concentrations, and nanoliter droplet detection. To *accomplish* this Aim, we will demonstrate that antibiotics can be dosed into droplets at different, discrete concentrations by varying key parameters of the dosing channel, including: 1) The antibiotic concentration (and effect of any matrix co-deposited with the antibiotic), 2) the contact area of the droplet with the antibiotic deposit, and 3) the time of contact between the drop and channel surface. The *rationale* for correlating the AST measurements is that $MNP_{Zn\text{-}DPA}$ on the bacteria could enable either unexpected growth inhibition or less susceptibility if there are combinatorial effects. When completed, this research will establish feasibility of AST with bacteria captured and concentrated with $MNP_{Zn\text{-}DPA}$ and dispersed into nanoliter droplets within 3 hours.

Uptake of antibiotics into droplets will be validated by testing polymeric microfluidic droplet generation chips, with 4 channel cross section sizes: 0.5 mm X 0.5mm, 0.2mm X 0.2mm, 0.1mm X 0.1mm, and 0.05mm X 0.05mm. These channels will be fabricated in SU8 and PDMS in the BYU cleanroom under the direction of Dr. Davis. Before sealing, drops of a cephalosporin antibiotic, cefotaxime (Sigma-Aldrich), will be deposited in each channel and freeze dried. Sometimes a matrix material (polyvinyl acetate-co-polyethylene copolymer, PVA-PE) will be deposited with the antibiotic. For the 4 channel sizes, water droplets with uniform diameters of 0.38 mm, 0.24 mm, 0.12 mm, and 0.038 mm respectively, will be generated in perfluorocarbon oil (Sigma-Aldrich) and pumped through the dosing section of the channel. The diameter of the droplets is larger than the channel width in order to force the droplets to touch the sides of the channel. Negative control will be droplets in a channel with no cefotaxime antibiotic. *Bacteria to be used: E. coli* strains with cefotaxime resistance will be selected from the extensive library of Dr. Richard Robison. Three MIC levels will be selected: susceptible (MIC < 0.2 μg/mL), intermediate (MIC ~ 2 μg/mL), and strongly resistant (MIC > 20 μg/mL) (Kohner et al. 2009). The MIC of the various strains will be measured using the agar plate-dilution method (Waterworth 1982) and broth-dilution method (Wiegand et al. 2008).

Research Milestones:

1. Our first milestone is to demonstrate capture of 80% of bacteria using MNPs, and then concentrate into 50 µL or less. We have already measured 90% capture from Human blood, but we still have to improve concentration. The concentration will be done on microfluidic devices built in Dr. Pitt's lab. Blood containing 10 CFU/mL *E. coli* will be processed according to the protocol defined above and then concentrated onto a sterile 0.45 µm track-etched filter mounted within the 3D printed filter holder. After filtration, the bacteria will be backwashed off of the filter with 50 µL of MH broth. The efficiency of bacteria removal from the filter will be evaluated by culturing the backwashed solution compared to a control sample of the input solution.
2. Milestone 2 is to detect growth of *E. coli* captured on the MNPs and grown in the presence of the MNPs on their surface. Growth will be assessed by reduction of resazurin, which will produce a fluorescence signal captured by microscope camera. The microscope will be operated inside of the incubator, allowing for real-time growth monitoring and shielding the microscope from ambient light. Image capture (and illumination) will occur periodically to minimize exposure and prevent photobleaching. As detection time is expected to be correlated with the log of droplet volume (Boedicker et al. 2008), droplet volumes below 1 nL will be tested to minimize time to detection. An image processing algorithm will be developed to measure growth over time.
3. Milestone 3 is to demonstrate capture directly from blood, and then detection of growth within 3 hours of 4 different species of bacteria important in blood sepsis. These are *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus faecalis*. We will assemble a benchtop prototyped process that combines the modular backwash chip in milestone 1 with the droplet maker and imaging process developed in milestone 2, to determine the time to positive detection, with 95% confidence, for the 4 strains of bacteria.
4. The 4th milestone is to show we can get different levels of antibiotic taken into microdroplets in the microfluidic flow system. This is essential to do the multiplexing of antibiotics and concentrations to test phenotypic response on the microfluidic device. We will demonstrate feasibility of antibiotic dosing at a rate of 10 droplets per second in each dosing micro-channel. We will quantify uptake as we vary the following control parameters: *antibiotic concentration* by 100-fold, *droplet/antibiotic contact area* by 8-fold (by varying channel diameter), and *contact time of droplet/antibiotic* (by varying the flow rate) by 100-fold.
5. The final milestone is to predict susceptibility levels in the microdroplets and compare with susceptibility levels measured by standard medical techniques, to show that our instrument will produce useful information for the clinician. Feasibility of droplet susceptibility measurement at low bacterial numbers will be demonstrated with MNP bound bacteria and integration of the microchannel dosing into the nanoliter droplet growth test apparatus (described in Milestone 2). The various *E. coli* strains will be grown in MH broth without antibiotic and then further diluted into MH broth containing both 44µM resazurin and cefotaxime antibiotic (in a series of concentrations). The resulting dilution will be used to produce nanoliter droplets with less than 1 CFU per drop.

Mentoring environment:

The **Pitt lab** has 2 graduate students that each act as mentors for 3-4 undergraduate students. He meets individually with the graduate students each week. The **Davis** lab has 1 graduate student and 4 undergrads. He meets weekly with them. The **Robison** lab has 2 grad students and several undergrads, and they have weekly group meeting.

Budget and budget narrative (up to 1 page). Teams can propose up to $60K per year for two years.

To accomplish this project, we request $60,000 per year, for two years.
The funds will be distributed between the three groups, as described below, to support students and to buy chemicals and supplies.

Bill Pitt group: $ 22,500 per year
1 grad student, partial support: $10,000
2 undergrad students, partial support: $5,000
Chemicals and other lab supplies: $4,500
Analytical: SEM, TEM, XPS, SIMS, DLS, zeta potential: $3,000

Robert Davis group: $ 22,500 per year
1 grad student, partial support: $10,000
2 undergrad students, partial support: $5,000
Chemicals and other lab supplies: $4,500
Analytical: SEM, TEM, microscopy: $3,000

Richard Robison group: $ 15,000 per year
1 grad student, partial support: $6,000
2 undergrad students, partial support: $4,000
Microbiological lab supplies: $5,000

We will follow the same distribution of funds for each of the two years.

How will our budget help us be more competitive for external funding?

Our research plan and aims are designed to quickly obtain the essential data that will support hypotheses that we can propose in an NIH R01 proposal. These proposals need solid preliminary data, and this plan will provide the data. We are also working in a research space that is new, and the NIH like an element of novelty and well-thought-out creativity. Of course, this research has great potential for high impact on human health, which is the primary funding goal of the NIH.

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1. **Plans for external funding** (up to 1 page). List of potential external funders and a timeline for submitting proposals.

Summary of Plans for External Funding

The data and expertise generated through this research will be used towards 3 proposals.

1. First, in combination with Precision Membranes LLC, a Provo Company, we will help them write an SBIR proposal with BYU as a subcontract. This will obtain future funding to collect more preliminary data and establish a commercial plan and platform. We plan to work together with Precision Membranes to help them submit an SBIR in January 2020, with a subcontract to BYU with Dr. Pitt as the PI on the subcontract. This will be about a $60K subcontract to BYU to support students and supplies for the Pitt, Davis and Robison lab. We have done similar subcontract in the past on other proposals.

2. Secondly, we will use the data to write a large R01 research proposal to the NIH with Drs. Pitt and Davis as PIs. This will occur near the completion of the proposed IDR project when we have shown that we have a viable technology to do rapid phenotyping of the antibiotic resistance of bacteria. The NIH has requested proposals in this area for the past 3 years. We have already talked to officials at the NIH and they are interested in our approach. This area of research is a very active concern to the NIH and CDC (Center for Disease Control), and they have set aside money specifically for phenotyping antibiotic resistance.
The size of this R01 proposal would be on the order of $500K per year for 5 years, with 3 investigators at BYU (Pitt, Davis, Robison), support for several graduate students and many undergraduates, and perhaps a subcontract to Precision Membranes.
This proposal would be submitted in June of 2021, or earlier if we obtain the needed data earlier.

3. DARPA (Defense Advanced Research Projects Agency) has an active program (Friend or Foe program) in the area of phenotypic bacterial identification. We have met with the DARPA program manager Paul Sheehan, twice (once in person meeting and once by teleconference). These meetings were held in order to understand the program goals and how we might use our approach to meet the aims of the program. We believe that the preliminary nanodroplet data produced with the IDR grant will put us in a strong position to apply for funding from this program. We will submit a white paper to Paul in the fall of 2019 or earlier to initiate the process of being evaluated for this funding opportunity.

Biographical Sketches (up to 2 pages per PI)

BIOGRAPHICAL SKETCH

NAME: William G. Pitt

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brigham Young University	B.S.	04/83	Chemical Engineering
University of Wisconsin-Madison	Ph.D.	12/87	Chemical Engineering
University of Minnesota-Twin Cities	Sabbatical	1994-95	Antibiotic delivery to oral bacteria
Montana State University	Sabbatical	2001-02	Antibiotic delivery to oral and industrial biofilms

A. Personal Statement

I have been doing research in drug delivery for 25 years. In my lab we make micelles, liposomes, solid nanoparticles, gas bubbles, and other nanostructures for drug delivery. I also do polymer synthesis to make needed polymers for drug delivery devices. I have over 50 publications in the area of drug delivery.

B. Positions and Honors

Positions and Employment

1982, 1983	Project Engineer, Terra Tek Research, Salt Lake City, UT : corrosion research, geochemistry
1983-1987	Research Assistant, Teaching Assistant, Dept of Chem. Eng., University of Wisconsin-Madison
1987-1993	Assistant Professor, Dept. of Chemical Engineering, Brigham Young University
1988-present	Adjunct Asst, Assoc, Full Professor, Department of Bioengineering, University of Utah
1993-1998	Associate Professor, Chemical Engineering Dept., Brigham Young University
1998-present	Professor, Chemical Engineering Dept., Brigham Young University

Honors

1994-1995	Lasby Visiting Professor, Dept. of Oral Sciences, Univ. of Minnesota, Minneapolis
1998	Outstanding Faculty Award, College of Engineering, Brigham Young University
2007-2012	Pope Professor of Chemical Engineering (endowed chair), Brigham Young University
2010	Outstanding Faculty Award, Department of Chemical Engineering, Brigham Young Univ.
2013	Maesar Research and Creative Works Award, Brigham Young University
2016	Wesley P. Lloyd Award for Distinction in Graduate Education, Brigham Young University

C. Contributions to Science

1. My lab and I have been doing research on infections antibiotic delivery to microorganisms since 1988. The goal of this proposed research is to develop a rapid resistome phenotypic detection system. My contribution to the proposed grant is to serve as the PI on the subcontract to BYU, to continue development of the bacterial separation system and develop the microfluidic system. I have built many novel flow systems during the course of my research, and I have experience with microfluidic devices and 3-D printing of such systems. We have studied

bacterial adhesion to materials and antibiotic delivery to bacterial biofilms for 28 years. I have worked with oral (*S. mutans*) and pathogenic bacteria (*P. aeruginosa, S. aureus, S. epidermidis*). I am a co-PI on an NIH grant to develop a clinical rapid detection system for identifying the carbapenem resistance genes in bacteria from patients with blood infections. I have done NIH-sponsored research, including several grants on treating bacterial infections on orthopedic implants in which I was the PI. Other NIH grants have focused on novel chemotherapeutic delivery systems. We have the equipment, personnel and experience to accomplish this proposed research.

148. C.M. Buchanan, R.L. Wood, T.R. Hoj, M. Alizadeh, C.G. Bledsoe, M.E. Wood, D.S. McClellan, R. Blanco, C.L. Hickey, T.V. Ravsten, G.A. Husseini, R.A. Robison, W.G. Pitt*, "Rapid Separation of Very Low Concentrations of Bacteria From Blood", *J. Microbiol Methods*, **139**, 48-53 (2017).
147. Mahsa Alizadeh, R.L. Wood, Clara M. Buchanan, Colin G. Bledsoe, M.E. Wood, Daniel S. McClellan, Rae Blanco, T. Ravsten, Ghaleb A. Husseini, C.L. Hickey, Richard A. Robison, W.G. Pitt*, "Rapid Separation of Bacteria from Blood – Chemical Aspects", *Colloids & Surfaces B: Biointerfaces*, **154**, 365-372 (2017). http://dx.doi.org/10.1016/j.colsurfb.2017.03.027
144. William G. Pitt*, Mahsa Alizadeh, Ghaleb A. Husseini, Daniel S. McClellan, Clara M. Buchanan, Colin G. Bledsoe, Richard A. Robison, Rae Blanco, Beverly L. Roeder, Madison Melville, Alex K. Hunter, "Rapid Separation of Bacteria from Blood – Review and Outlook", *Biotechnology Progress*, **32**(4), 823-839 (2016).
142. Rafeeq Tanbour, Ana M. Martins, William G. Pitt, Ghaleb A. Husseini*, "Drug Delivery Systems Based on Polymeric Micelles and Ultrasound: A Review", *Current Pharm. Design,* **22**(9), 1 (2016).
141. Chung-Yin Lin, Han-Yi Hsieh, William G. Pitt, Chiung-Yin Huang, I-Chou Tseng, Chih-Kuang Yeh, Kuo-Chen Wei*, and Hao-Li Liu*, "Focused Ultrasound-Induced Blood-Brain Barrier Disruption for Non-Viral, Non-Invasive, and Targeted Gene Delivery", *J. Controlled Release*, **212**, 1-9 (2015).
140. Javadi, M. and Pitt, W.G.*, "Insights into Ultrasonic Release from eLiposomes", *Letters in Applied NanoBioScience*, **4**,(1) 251-258 (2015).
139. Lattin, J.R., Javadi, M., McRae, M., and Pitt, W.G.*, "Cytosolic Delivery via the Endosome using Acoustic Droplet Vaporization, *J. Drug Targeting,* **23**(5), 469-479 (2015). http://dx.doi.org/10.3109/1061186X.2015.1009074
138. Husseini, G.A.*, Kherbeck, L., Pitt, W.G., Hubbell, J.A., Christensen, D.A., and Velluto, D., "Kinetics of Ultrasonic Drug Delivery from Targeted Micelles", *J. Nanosci. Nanotechnol.,* **15**(3), 2099-2104 (2015). http://dx.doi.org/10.1166/jnn.2015.9498
137. Lattin, J.R. and Pitt, W.G.*, "Factors Affecting Ultrasonic Release from eLiposomes", *J. Pharmaceutical Sciences*, **104**(4), 1373-1384 (2015). DOI: 10.1002/jps.24344
136. Pitt*, W.G., Zhao, Y., Jack, D. R., Perez, K.X., Jones, P.W., Marelli, R., Nelson, J.L., and Pruitt, J.D., "Extended Elution of Phospholipid from Silicone Hydrogel Contact Lenses", *J. Biomaterials Sci., Polymer Edn.*, **26**(4), 224-234 (2015). http://dx.doi.org/10.1080/09205063.2014.994947
135. Husseini, G.A.*, Pitt, W.G., Williams, J., and Javadi, M., "Investigating the Release Mechanisms of Calcein from eLiposomes at Higher Temperatures", *J. Colloid Sci. Biotech.* **3**(3), 239-244 (2014). http://dx.doi.org/10.1166/jcsb.2014.1100.

Complete list of published work by W.G. Pitt in MyBibliography (showing 97 of 153 publications): https://www.ncbi.nlm.nih.gov/sites/myncbi/william.pitt.1/bibliography/41164705/public/?sort=date&direction= descending

BIOGRAPHICAL SKETCH

NAME: Robert Davis

POSITION TITLE: Professor, Department of Physics and Astronomy, Brigham Young University

COLLEGE: Physical ad Mathematical Sciences DEPARTMENT: Physics

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Brigham Young University	B.S. M.S.	1989	Physics
University of Utah	PhD	1996	Physics
Cornell University	Postdoc	1998	Applied Physics

A. Personal Statement

The proposed effort is for rapid bacterial separation and detection from whole blood using functionalized nanoparticle separation and nanodrop based detection. I have led efforts at BYU to develop a materials and processes for the successful separation of rare cells (both cancer and bacterial) from whole blood with support from the NSF and NIH and a collaboration with the startup Precision Membranes. My research team at BYU develops and characterizes micro and nanoscale materials and processes for interfacing with fluids and biological tissue for problems in chemical separations and sensing, and healthcare. The four publications listed below are examples of the work.

1. Steven G. Noyce, Richard R. Vanfleet, Harold G. Craighead and Robert C. Davis, "High Surface-Area Carbon Microcantilevers", Nanoscale Advances, DOI: 10.1039/C8NA00101D (2019)
2. Guohai Chen, Berg Dodson, David M Hedges, Scott C Steffensen, John N Harb, Chris Puleo, Craig Galligan, Jeffrey Ashe, Richard R Vanfleet, Robert C Davis, "Fabrication of High Aspect Ratio Millimeter-Tall Free-Standing Carbon Nanotube-Based Microelectrode Arrays" ACS Biomaterials Science & Engineering 4 (5), 1900-1907(2018)
3. David S. Jensen, Supriya S. Kanyal, Vipul Gupta, Michael A. Vail, Andrew E. Dadson, Mark Engelhard, Richard Vanfleet, Robert C. Davis, Matthew R. Linford, "Stable, microfabricated thin layer chromatography plates without volume distortion on patterned, carbon and Al2O3-primed carbon nanotube forests" J. Chrom. A 1257, 195-203 (2012)
4. Song, J., Jensen, D. S., Hutchison, D. N., Turner, B., Wood, T., Dadson, A., Vail, M. A., Linford, M. R., Vanfleet, R. R., Davis, R. C. "Carbon-Nanotube-Templated Microfabrication of Porous Silicon-Carbon Materials with Application to Chemical Separations.", Advanced Functional Materials 21 (6), 1132-1139, (2011)

B. Positions

1996 – 1998 Post Doctoral Research Associate, Cornell University, Applied and Engineering Physics.
1998 – 2004 Assistant Professor, Brigham Young University, Department of Physics and Astronomy.
2004 – 2011 Associate Professor, Brigham Young University, Department of Physics and Astronomy.
2006 – 2007 Visiting Researcher (sabbatical leave), Nanocarbon Research Center, Laboratory for Advanced Industrial Science and Technology (AIST), Tsukuba Japan
2013-present Full Professor, Brigham Young University, Department of Physics and Astronomy.
2013-2014 Visiting Researcher (sabbatical leave), Nanocarbon Research Center, Laboratory for Advanced Industrial Science and Technology (AIST), Tsukuba Japan

Other Experience and Professional Memberships

2002-present Member, AVS Society for Science and Technology

2012-present Member, American Association for Physics Teachers
2014-present Program Committee MEM/NEMS Group AVS International Symposium
2018-present Chair of Program Committee MEM/NEMS Group, AVS International Symposium

C. Contribution to Science

Carbon Nanotube Templated Microfabrication (CNT-M). We have developed this process for microforming materials at BYU. Application examples include: neural probes: chromatography and filtration media, MEMS sensors and actuators.

DNA Templated Nanoscale Circuit Elements. Our work has enabled the development nanoscale structures using bottom-up fabrication of DNA based circuit templates with a goal toward 5 nm scale metallic and semiconducting features.

Permanent Data Storage
I am a key member of an interdisciplinary team at Brigham Young University developing permanent data storage solutions including the development of graphene and other sp2 carbon based "solid state" permanent data storage technology.

The following link will lead to a complete publication list:
https://scholar.google.com/citations?user=0PMZF6gAAAAJ&hl=en

D. Research Support

Externally Funded Projects

NIH SBIR Rapid and Efficient Extraction of Bacteria from Whole Blood for Sepsis Diagnosis
NIH subaward through Precision Membranes PI: Bill Pitt 2018-2919
Rapid and Efficient Extraction of Bacteria from Whole Blood for Sepsis Diagnosis
Role: Co-PI

Recently Completed Closely Related Research Support

BYU-Moxtek: X-Ray Windows and Filters
Moxtek Inc., PI: R. Vanfleet, R. Davis 04/10 – 05/17,
Co-development with company of materials and process for x-ray window applications.
Role: PI/Co-PI

Neural Probe Development
GE global Research, PI: R. Vanfleet, 6/14-6/17
Exploratory development of CNT-M based neural probes and architectures.
Role: Co-PI

AIR-TT: Robust High Porosity Filter for Circulating Tumor Cell Enrichment
National Science Foundation PI: R. Davis 09/15 – 12/17
Development and testing of three dimensional fluid filters for cancer diagnostics

I-Corps: High performance lithographically defined filters
National Science Foundation, PI: R. Davis, 08/14 – 01/16
Company development grant for CNT-M and lithographically defined filter systems.
Role: PI

BIOGRAPHICAL SKETCH

NAME: Richard A. Robison

POSITION TITLE: Professor, Department Chair, and BSL-3 Laboratory Director

COLLEGE: Life Sciences DEPARTMENT: Microbiology & Molecular Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Brigham Young University, Provo, UT	B.S.	04/1978	Microbiology
Brigham Young University, Provo, UT	M.S.	08/1980	Microbiology
Brigham Young University, Provo, UT	PH.D.	08/1988	Microbiology

A. Personal Statement

I have over 35 years of experience working with bacterial pathogens, including their growth, characterization, and detection with molecular genetic assays. I have been the director of the BYU biosafety level-3 (BSL-3) laboratory since its inception in 1998. Between 1998 and 2013, we grew and maintained a collection of CDC select agents for various National Defense programs, most recently for the Department of Homeland Security. Isolates were obtained from local, national, and international sources. Our current archive contains over 1,500 BSL-3 isolates. We also maintain a significant collection of select agent genetic near-neighbors, and other BSL-2 pathogens of humans and animals. During these efforts, we have done extensive work in species identification and genetic characterization, including the development of many unique and useful assays to detect and characterize various pathogens. We have extensive experience in PCR development, including primer and probe design. Below are 4 peer-reviewed publications describing some of these assays.

1. Stewart A, Satterfield B, Cohen M, O'Neill K, Robison R. A quadruplex real-time PCR assay for the detection of *Yersinia pestis* and its plasmids. *J Med Microbiol*. 2008 Mar;57(Pt 3):324-31.
2. Satterfield BA, Stewart AF, Lew CS, Pickett DO, Cohen MN, Moore EA, Luedtke PF, O'Neill KL, Robison RA. A quadruplex real-time PCR assay for rapid detection and differentiation of the *Clostridium botulinum* toxin genes A, B, E and F. *J Med Microbiol*. 2010 Jan;59(Pt 1):55-64.
3. Gunnell MK, Lovelace CD, Satterfield BA, Moore EA, O'Neill KL, Robison RA. A multiplex real-time PCR assay for the detection and differentiation of *Francisella tularensis* subspecies. *J Med Microbiol*. 2012 Nov;61(Pt 11):1525-31.
4. Lowe CW, Satterfield BA, Nelson DB, Thiriot JD, Heder MJ, March JK, Drake DS, Lew CS, Bunnell AJ, Moore ES, O'Neill KL, Robison RA. A Quadruplex Real-Time PCR Assay for the Rapid Detection and Differentiation of the Most Relevant Members of the B. pseudomallei Complex: B. mallei, B. pseudomallei, and B. thailandensis. *PLoS One*. 2016 Oct 13;11(10): e0164006. doi: 10.1371/journal.pone.0164006. PubMed PMID: 27736903; PubMed Central PMCID: PMC5063335.

B. Positions and Honors

Appointments

1986-1991 Associate Director – Biological Sciences, Clinical Research Associates, Provo, UT
1991-1997 Assistant Professor, Dept. of Microbiology and Molecular Biology, BYU, Provo, UT
1997-2004 Associate Professor, Dept. of Microbiology and Molecular Biology, BYU, Provo, UT

1998-present Director, Biosafety Level-3 Laboratory, BYU, Provo, UT
2004-present Professor, Dept. of Microbiology and Molecular Biology, BYU, Provo, UT
2014-present Chair, Dept. of Microbiology and Molecular Biology, BYU, Provo, UT

Honors and Awards

1992 Excellence in Teaching Award, BYU Student Alumni Association
1996-1999 Alcuin Fellowship, BYU Department of Honors and General Education
1998 Excellence in Teaching Award, BYU Student Alumni Association
1998 College Teaching Excellence Award, BYU College of Biology and Agriculture
2001 College Professorship, BYU College of Biology and Agriculture
2003-2006 Alumni Professorship, BYU Alumni Association
2008-2010 Thomas Martin Professorship, BYU College of Life Sciences
2014 Outstanding Research Award, BYU College of Life Sciences

Other Experience and Professional Memberships

1980-present Member, American Society for Microbiology; Branch Councilor to ASM from '07-'08
2000-present Member, American Association for Cancer Research
2010-present Reviewer, Journal of Applied Microbiology and Letters in Applied Microbiology
2012-present Member, Editorial Board, Frontiers in Microbiology and Frontiers in Immunology

C. Contribution to Science

1. Development of novel assays for the detection and characterization of CDC select agents
2. Novel discoveries related to pathogen genetics
3. Pioneering work in microbial disinfection studies and assay development
4. Discovery and characterization of novel antimicrobials

Scholarship summary:

Completed Research Grants ($4,154,000 since 1997)
Scientific Publications (82)
Total Google Scholar Citations (2,952)
U.S. Patents Issued (2)
Professional Consulting Companies (21)
Scientific Presentations (230)

Complete list of published work by Robison in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1lwuTtaO5SNkU/bibliography/49444660/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Completed

1. DHS/NBACC/BNBI BN13-0001 Robison (PI) 01/01/2004-10/31/2013
   Title: A Curated Archive of Viable Select Bacterial Agents and Extracted Nucleic Acids for Bioforensic Casework, Amount: $1,999,100 Role: PI

2. DHS HSHQDC-10-C-00136 Robison & Lee (PIs) 08/09/2010-05/14/2013
   Amount: $686,560, Role: Co-PI

3. NASA NNX15AM89G Austin, Anderson, Huntington & Robison (PIs) 06/15/2015-06/14/2018
   Title: Microorganism Survivability in High-velocity Impacts, Amount: $358,141, Role: Co-PI