Allegheny-Singer Research Institute

Annual Progress Report: 2014 Formula Grant

Reporting Period

January 1, 2015 – June 30, 2015

Formula Grant Overview

The Allegheny-Singer Research Institute received $42,788 in formula funds for the grant award period January 1, 2015 through June 30, 2016. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Promotion of Bacteriotherapy Adhesion in Chronic Wound Treatment – Bacteriotherapy treatment in chronic wounds has the potential to change current wound management. However, a technique to reliably attach the individual bacteria to wound dressings has yet to be developed. Recently, a bacteria coating polymer which should promote adhesion of the bacteriotherapy to the synthetic mesh has been developed. We propose to investigate the polymer's ability to promote adherence of individual bacteria to the mesh fibers and to determine whether the polymer alters the bacteriotherapy's efficacy. By developing a reproducible application method we can expand the research into an i*n vivo* model with the long term goal being the application of bacteriotherapy in the treatment of chronic wounds.

Anticipated Duration of Project

1/1/2015 – 6/30/2016

Project Overview

Our long term goal is to improve the outcome of wound care, while decreasing the time and cost associated with treatment. Our short term goal is to test a treatment protocol that combines the use of bacteriotherapy, to protect the wound from further colonization while enhancing tissue regeneration, and a polymeric coating to promote bacterial adhesion to the gauze for treatment purposes.

Aim 1: Establish the efficacy of different combinations of bacteriotherapy into planktonic cultures and *in vitro* biofilms. Target Cultures will be composed of clinical and bioluminescent isolates of *S. aureus*, *P. aeruginosa*, or in combination. The three proposed bacteriotherapy pools will be composed of 3, 5, or 8 species of commensal bacteria. We will test the three bacteriotherapy pools on the three microbial cultures at multiple time points and stages of

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growth. Inhibition will be measured using Zone of inhibition (ZOI) metric, as well as region of interest (ROI) based on bioluminescence. Based on these results a single bacteriotherapy pool will be used for Aim 2 and 3.

Aim 2: Determine if the polymeric bacterial coating affects the efficacy of the bacteriotherapy. Polymer modification of the bacteriotherapy will be accomplished by mixing the bacteria and polymers, incubating for 30 minutes, and removing the excess polymer by centrifugation. The bacteriotherapy-polymer mixture will be tested utilizing ZOI and ROI. The efficacy of the mixture will be compared to the efficacy results of Aim 1.

Aim 3: Attach the bacteriotherapy-polymer mixture to the delivery matrix and establish efficacy. In this delivery phase the bacteriotherapy-polymer mixture will be attached to synthetic gauze for application purposes. To coat the gauze dressing, the gauze will be immersed in the polymer modified bacteriotherapy and incubated at various timepoints. Bacteriotherapy adherence to the gauze will be determined through the use of fluorescent *in situ* hybridization (FISH) with species-specific probes for the bacteriotherapy species. The bacteriotherapy coated gauze will be tested as previously described in Aim 1. The efficacy of the mixture will be compared to the results of Aim 2. If all three aims are achieved we plan to pursue funding to test the bacteriotherapy *in vivo*.

Principal Investigator

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Other Participating Researchers

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Expected Research Outcomes and Benefits

Chronic wounds are one of the most costly health care issues, reinforced by the doubling of the aged population at risk in the United States. Chronic wounds affect 6.5 million patients at an estimated cost of $25 billion spent annually on treatment. Sharp rises in the incidence of diabetes and obesity worldwide contribute to the need for treatment. Chronic wounds have a profound effect on quality of life and will require significant shifts in models of care as the treatment shifts from inpatient to outpatient, significantly increasing office visits.

Treatment of chronic wounds over the last 30 years has remained unchanged, with limited success. Wound bed debridement, revitalization of tissue and multiple antibiotic combinations, gauzes/dressings/gels containing various silver molecules of various concentrations has been the standard. Bacteriotherapy has been used in a multitude of diseases, but has shown clinical

success in oral health, especially in the treatment of infected root canals. Additionally an exploratory study of burn wounds found bacteriotherapy application on the burn wounds appeared to decrease the bacterial burden of the wound and promote formation of granulation tissue. Based on these findings we will investigate the role of bacteriotherapy for chronic wounds. If all three aims are achieved we could initiate *in vivo* studies with the long term goal of developing a cost effective, easily applied treatment option for wound care.

Summary of Research Completed

Due to the delay in the signed contract delivery, executed contract was received June 8th, 2015; very little progress has been achieved to date. A project team meeting occurred in late June for project planning. The following progress has been achieved:

Aim 1: Bacteriotherapy pools have been ordered for the project. We decided to change the bacteriotherapy pools from a mix of 3, 5, and 8 bacteria to 1, 4, and 4 bacteria after reviewing the literature, discussing the need for a baseline control, and determining the different bacteriotherapy pools that were commercially available at this time. The bacteriotherapy pools will be composed of the following organisms (Table 1).

All three pools were received. The Lactobacillius reuteri pool was resuspended in 1-2ml of MRS broth. The broth culture was aseptically placed into sterile 15 ml test tubes containing 10 ml of sterile MRS broth. The tubes were then vortexed at room temperature on a vortex mixer (Fisher Scientific™ Digital mixer) for two minutes until the mixture was homogenous. The samples were then kept at room temperature for 30 minutes to assure rehydration of the freeze-dried powder. The samples were then returned to the vortex machine and vortexed for an additional two minutes.

For the LB and SLB pools, the therapeutic bacteria arrived within a capsule. MRS broth was aseptically placed into sterile 15 ml. test tubes containing 10 ml of sterile MRS broth. The capsules were opened and deposited into a test tube. The tubes were vortexed at room temperature for two minutes until the mixture was homogenous. The samples were kept at room temperature for 30 minutes to assure rehydration of the powder. The samples were then returned to the vortex machine and vortexed for an additional two minutes. All three pools were incubated at 37° C for 48 hours.

After incubation frozen stocks were created, 5mL of glycerol was added to each 10mL of culture. The culture was vortexed. 1mL of the culture was aliquoted into cryovials and the stocks were frozen at -80°C. At this time we have frozen stocks of all three therapeutic bacterial pools. Additionally we have completed basic CFU calculations for the three therapeutic bacterial pools at different concentrations. 10uL of frozen stock was added 1mL of MRS broth in a 15mL conical tube. The tube was incubated overnight at 37° C. An optical density measurement was taken, serial dilutions were created, and plates on MRS agar. These serial dilutions were incubated 24 hrs and then colonies counts performed. Based on the colony counts the CFU was calculated for the different concentrations (Table 2).

In summary we have identified the therapeutic bacterial pools for the project. These pools have been received, frozen stocks have been created which will be used throughout the duration of the project, and basic concentrations have been calculated at various time points.

Table 1: Components of Therapeutic Bacteria Pools

Therapeutic bacteria pool	***Components of the pool***
LB	Lactobacillus rhamnosus
	Lactobacillus casei
	Lactobacillus acidophilus
	Bifidobacterium longum
SLB	Saccharomyces boulardii
	Lactobacillus rhamnosus
	Bifidobacterium bifidum
	Bifidobacterium breve
LR	Lactobacillus reuteri

Table 2: CFUs Calculations for the Therapeutic Bacterial Pools

Therapeutic Bacterial pool	LB	SLB	LR
OD reading	CFU		
0.034	9.00E+06		
0.039		1.40E+07	
0.043	1.38E+07		
0.071		4.00E+07	
0.853	7.80E+07		
1.030		1.28E+08	
1.080			1.16E+08

Aim 2: No progress to report.

Aim 3: No progress to report.