Fiscal Year:	FY 2021	**Task Last Updated:**	FY 09/09/2021
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Project Title:	Immobilization and Stabilization of Biocatalysts for Efficient Pharmaceutical Manufacturing		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2020 TRISH BRASH1901: Translational Research Institute for Space Health (TRISH) Biomedical Research Advances for Space Health
Start Date:	04/01/2020	**End Date:**	03/31/2022
No. of Post Docs:	2	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Bondos, Sarah Ph.D. (Texas A&M Research Foundation)		
Grant/Contract No.:	NNX16AO69A-T0503		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	The significant resources, facilities, and toxic waste produced from traditional chemical synthesis makes production of medicines during long term space exploration impossible. To increase efficiency and environmental safety, the multi-billion dollar biocatalysis industry leverages enzymes to produce fine chemicals, pharmaceuticals, and other industrially relevant compounds. Enzymes are efficient and highly selective reusable biocatalysts that can significantly accelerate the rate of chemical reactions. Biocatalysis offers higher yields, fewer side reactions, elimination of protection and de-protection steps, purer products, easier recovery and separation, and reduced waste. The drawbacks to using enzymes as biocatalysts are that enzymes are costly to produce, easily degraded or inactivated, and difficult to store. Despite the great potential of enzymes in pharmaceutical manufacturing, current approaches to solve the enzyme stability problem are insufficient. Bondwell Technologies has developed a low-cost platform approach to immobilize and stabilize a wide array of enzymes without a time-consuming optimization process. Our biomaterial platform can uniquely incorporate active large, complex proteins via protein fusion, eliminating the need for crosslinkers. Biocatalysis requires both an enzyme, and a mechanism to physically separate the enzyme from product, usually a solid support. In our approach, both of these factors are produced in a single molecule. This rapid, single-pot, single-component approach dramatically reduces the cost of materials synthesis while simultaneously increasing the process' reliability and scalability. This same process can be used for a wide variety of enzymes, eliminating the time-consuming and difficult optimization process required by all other stabilization / immobilization techniques. For systems that chemically cross-link a protein to a surface, one concern is that the protein could leach from the materials if the cross-linked bond is degradable. In contrast, our approach connects enzymes to materials through a stable peptide bond without damaging the enzyme. Additionally, many proteins lose activity when stored dry or at room temperature; however, Bondwell materials can be stored dry at room temperature for nearly 10 years and remain active. Proteins fused to our materials are a million-fold more active than the same protein trapped in hydrogel, and have 1,000-times the binding capacity of protein cross linked to resin beads. We have successfully demonstrated that enzymes remain active when fused to our materials. In this proposal we will demonstrate the unique ability of this technology to manufacturing drugs under storage/use conditions suitable for deep space exploration missions. The proposed plan will produce materials with the ability to produce amoxicillin, cephalosporin, and melatonin. In addition, we will test enzyme efficacy in our system and performance after storage. Our long-term goal is to combine the efficiency, specificity, and broad applicability of biocatalysis with telescoping in a sealed reactor flow chemistry system. Most natural therapies can be catalyzed by enzymes, and enzymologists are using directed evolution and artificial intelligence to rapidly create enzymes that catalyze novel reactions. We envision using each enzyme-material fusion to create a mesh of fibers shaped like a disc ~ 1 cm diameter. The drug is manufactured by placing the correct discs (enzymes) with reagents in the telescoping system and allowing the reaction to occur. Each disk can be rinsed, dried, and re-used as needed to manufacture one or more drugs. This unique system has the potential to produce chemicals, manufacture drugs, prepare food, or even generate biofuels from a small number of precursors.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Biocatalysis is a highly efficient, safe, and sustainable technology for drug manufacturing. The use of enzymes is currently limited by the need to improve enzyme stability using time-consuming processes (multiple scientists working for about 1 year) unique to each enzyme. Current immobilization strategies are expensive and error-prone. Bondwell Technologies has developed a low-cost generalizable approach that can be used to immobilize and stabilize any enzyme. Our approach leverages the unique properties of a novel biomaterial-based platform.The proposed aims will demonstrate the feasibility of this technology for manufacturing therapeutics. Further studies toward our long-term goal will focus on i) building a telescoping sealed reactor flow system for manufacturing in one-pot reactions, ii) expanding the number of immobilized enzymes to manufacture more compounds, and iii) examining long-term storage and reuse of these materials. In addition, membranes composed of our material fused to therapeutic binding proteins (e.g., catalytically inactive b-lactamase to bind b-lactam antibiotics) can also be developed to reclaim drugs from waste streams. Development by NASA of a universal system to produce, immobilize, and stabilize enzymes would create a product that could easily be translated to the rapidly growing multi-billion dollar biocatalysis industry, where it would make a significant impact. Finally, expansion of Bondwell Technologies' unique system to other synthesis processes could allow production of other useful chemicals, food, or even biofuels from a small number of precursors.
Task Progress:	[Ed. note: Reporting as of February 2021] Enzymes have been used in therapeutics syntheses because they offer higher product yields, require fewer steps, create fewer undesirable side products, produce final products with higher purity, and enable easier product recovery than traditional chemical syntheses. However, enzymes have limitations, including easy degradation, loss of activity over time, and the requirement of careful storage and handling, which limit their use in the manufacturing of therapeutics. Bondwell Technologies has developed functionalized enzyme-based materials that address these limitations. These materials can immobilize and safely concentrate enzymes, by 1,000 - 10,000-fold compared to other immobilization techniques. Our platform technology is based on our success in producing fused proteins that can self-assemble into biomaterials in vitro. Proteins with a wide range of physical properties incorporated into our materials are significantly stabilized, while still remaining functional after boiling or autoclaving. Materials functionalized with proteins can be stored dry at room temperature for many years, and survive multiple drying/rehydration cycles, enabling their reuse. Additionally, enzyme-containing fusion proteins have retained their catalytic capabilities. Thus, our materials can be used to improve stability and handling of a variety of enzymes that are used in therapeutics manufacturing. Our long-term goal is to create membranes, each modified with one or more enzymes and sealed in a plastic reaction chamber. Therapeutics would be synthesized by connecting the appropriate series of reaction chambers and adding substrate and any needed cofactors to the first reaction chamber. By the end of year 1, we have established these following main findings: 1. We have successfully inserted the genes of the enzymes into our biomaterial expression cassette. There are 12 total enzymes in the two natural biosynthesis pathways of Penicillin G, cephalosporin C, Amoxicillin, and melatonin. The status of these 12 enzymes are at various states, ranging from at gene synthesis and cloning step to expression, purification, and kinetic characterization step. 2. We have successfully expressed, purified, and formed materials for 4 out of the 12 enzymes of the two natural biosynthesis pathways of penicillin G and cephalosporin C. Those enzyme-materials include Penicillin G Acylase, Isopenicillin N Synthase, Isopenicillin N Acyltransferase, and Deacetoxycephalosporin C synthase.

	3. We have tested the activity of Penicillin G Acylase in our material. Penicillin G Acylase (PGA) is a heterodimeric protein, which carries out two different reactions, i.e., Penicillin G hydrolysis and Amoxicillin synthesis. We demonstrated that the enzyme is fully functional in this material. We have fully characterized PGA in our material and determined kinetic parameters of both Penicillin G hydrolysis and Amoxicillin synthesis reactions. The kinetic parameters are similar to published data in the literature. 4. We have tested PGA functionality with extremely harsh storage conditions. We demonstrated that PGA in our material maintains its catalytic efficiency after being stored with extreme temperature conditions. We also demonstrated the activity and reusability of Isopenicillin N Synthase (IPNS). IPNS is reusable and remarkably active after being left in the open air, at room temperature for 48 hours.
Bibliography Type:	Description: (Last Updated: 01/11/2023)