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PI Name:	Howell, David Ph.D.		
Project Title:	Immobilization and Stabilization of Biocatalysts for Efficient Pharmaceutical Manufacturing		
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Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
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PI Email:	dhowell@bondwelltech.com	**Fax:**	FY
PI Organization Type:	INDUSTRY	**Phone:**	979-475-4545
Organization Name:	Bondwell Technologies Inc.		
PI Address 1:	501 Graham Road		
PI Address 2:			
PI Web Page:			
City:	College Station	**State:**	TX
Zip Code:	77845	**Congressional District:**	17
Comments:			
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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:

Despite the great potential for biocatalysis, industrial applications have been hampered because enzymes are easily degraded and can lose activity during storage. Most of the major pharmaceutical companies have set up directed-evolution departments to engineer enzymes and improve enzyme stability. Improved stability generally correlates with improved activity in organic solvents, reduced enzyme degradation, and improved storage; however, directed-evolution is a slow process requiring construction and screening of large libraries that mostly contain variants with reduced or no activity. Additionally, our understanding of the link between protein sequence and function lags well behind our desire for new enzymes. It is not yet possible to predict which protein sequences, or even just sequence alterations, which reliably give rise to whole new, finely tuned catalytic activities limits the ability to rapidly improve, or even to create, enzymes through directed-evolution. The immobilization of enzymes on solid supports can also enhance enzyme stability, although not to the extent required for most chemical transformations. Immobilization primarily allows these expensive bio-catalysts to be easily separated from their chemical product and reused. Immobilization involves entrapment of the enzyme in a polymeric matrix or binding of an enzyme to a prefabricated support, such as an organic resin or silica. Although binding to a support can involve simple adsorption, noncovalent interactions are often too weak to keep the enzyme bound to the carrier under the rigorous industrial conditions required. Bondwell Technologies has developed a low-cost platform approach to safely and reliably immobilize and dramatically stabilize a wide array of enzymes without a time-consuming optimization process. Our unique system has the potential to not only stabilize enzymes but also allow dry storage at room temperature. In this project, we prove feasibility of this novel system for manufacturing pharmaceuticals in space using biocatalysts. This work demonstrates that without the need for directed evolution, facile immobilization of enzymes in our system for the complete synthesis of Melatonin, Penicillin, Amoxicillin and Cephalosporin C. In addition, this project shows that each of these enzymes are functional in our materials and remarkably stabilizes enzymes for suitable for deep space missions. This data is remarkable because any free enzyme would be expected to denature and lose all its catalytic activity if it was to be stored at these same extreme conditions.
To continue development of this technology into a final product suitable for space flight, additional research is required. Specifically:

1. ACV Synthetase (ACVS) is an extremely large gene and toxic to bacteria. We hope to keep trying other cloning options such as Bacillus megatarium. This is important for this pathway but also for building a complete library of drugs.

2. We will continue characterizing the enzymes that are pending. Due to time constraints, the loss of commercially available substrates (COVID related), supply chain issues, temporary closures or employee loss due to COVID, and the troubleshooting process of developing assays for our materials, the characterization process has taken longer than originally anticipated.

3. We will do more aging experiments with different enzyme-Ubx materials in different storage conditions to optimize the activity retention during simulated aging. We will also test different storage conditions to optimize reuse of the enzyme-Ubx materials.

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Our biomaterial platform can 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 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.

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. Product will flow from the last chamber. We envision using each enzyme-material fusion to create a mesh of fibers shaped like a disc ~ 1cm 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.

Task Progress:

With the unique ability of our materials, we demonstrate here that our functionalized enzymatic material is able to catalyze reactions to produce therapeutic drugs under suitable conditions for deep space exploration missions. Our aim was to produce NASA-relevant therapeutics, including penicillin, cephalosporin C, amoxicillin, and melatonin, by incorporating the enzymes from the natural biosynthesis pathways into our biomaterials.

By the end of year 2, 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. We have successfully expressed, purified, and formed materials for 11 out of the 12 enzymes.

2. We have successfully tested the activity of six out of the 12 enzymatic biomaterials. We have demonstrated that these enzymes are fully functional in Ubx materials. We were able to determine kinetic parameters for five of the enzymes while the activity of the sixth was confirmed by mass spectrometry. The kinetic parameters are all consistent with published data in the literature.

3. We have additionally tested Penicillin G Acylase (PGA) functionality with extremely harsh storage conditions. We demonstrated that PGA in our material maintains its catalytic efficiency after being stored in conditions that mimic the extremes expected in long-duration spaceflight.

4. We also demonstrated the activity and reusability of Isopenicillin N Synthase (IPNS) as well as Hydroxytryptophan Decarboxylase (HTDC). IPNS in our material is reusable and active after being left in the open air, at room temperature for 48 hours. HTDC remained reusable and remarkably active even after two weeks at room temperature in open air. We also saw that the materials stored in phosphate buffered saline (PBS) had a higher retention of activity after 48 hours.

5. We showed that a well-characterized enzyme, luciferase, was still remarkably active in our materials after three years of simulated aging, and seven months of actual aging.

6. We demonstrate telescoping synthesis of Melatonin (4 enzymes) is achievable in this system in 2 simple reactions.

In summary, this work demonstrates feasibility of this system as a biocatalysis platform for drug manufacturing in space. The system meets the requirements of spaceflight conditions and duration stated in the solicitation. Further work is required to move from a feasibility standpoint to full product development.

Bibliography Type: Description: (Last Updated: 01/11/2023)

Awards Bondwell Technologies. "BVEDC Launch Award, June 2021." Jun-2021