Regenerative Medicine Minnesota
Progress Report

Grant Title: Molecular farming to reduce cost of recombinant proteins important to regenerative medicine
Grant Number: RMM-2017-BB-09
Requester: James Thompson, PhD
Project Timeline: 5/30/2017 – 5/29/2018

Brief description of project:

MNPHARM, SBC used the Biotechnology Grant funding from Regenerative Medicine Minnesota's to develop manufacturing protocols for two human proteins critical for Regenerative Medicine. With this project, the human cytokines Bone Morphogenetic Protein-2 (BMP-2) and Vascular Endothelial Growth Factor 165 (VEGF) were made successfully. The products made were of high purity and quality. BMP-2 promotes the differentiation of mesenchymal stem cells into bone-forming osteoblasts. The VEGF protein stimulates angiogenesis and vasculogenesis, as well as osteoblastic differentiation, migration and bone mineralization. A combined delivery of BMP-2 and VEGF will promote greater bone formation. We now desire to supply customers, especially researchers who aim to deposit these cytokines with stem cells by 3D printing to make prevascularized bone tissue for clinical needs.

MNPHARM's breakthrough relates to the development and ownership of a technology that allows commercial-scale production of proteins from plants grown indoors, a process called "Molecular Pharming". The cost of proteins used in regenerative medicine and tissue/bone engineering can be reduced greatly with this approach.

Where did this project take place?

MNPHARM moved into a new laboratory space within the 4Front Technology Campus in Oakdale, MN, only 3 months before this grant period began. All of the effort and new infrastructure built was located inside this ~3000 sq. ft. office and laboratory facility. The entire project took place within Minnesota near Hwy. 694.

What was the outcome of the project?

In the grant application, goals were defined as 1] the expression, purification and characterization of plant-derived, glycosylated human BMP-2 and VEGF proteins, 2] a repeated generation of several >10 mg pure protein samples to insure the method's robustness, and 3] a gift of pure protein samples to willing collaborators in Minnesota that would share data with us regarding the product's characterization and/or function. We are collaborating with several Minnesota entities. We completed all these Aims.

(Did the project work the way you expected it to?
What were the successes? What were the failures?)

The expression levels of human VEGF 165 in plants was much higher than expected, while the expression of BMP-2 was roughly about that anticipated. VEGF was >40% of the soluble protein extracted from the plant using our methods! We knew ahead that human BMP-2 is a poorly soluble protein at the pH of leaf extracts; BMP-2 aggregates and precipitates at the pH of plant extracts. However, we developed a very workable, cheap means to deal with this issue. We do have some optimization remaining for certain steps of production for both proteins. There exists infrastructure for protein purification that MNPHARM would have liked to have and tested during this pilot project but could not yet afford. An example is a continuous chromatography system that costs just over $800K. We are altering our expression technology further as a result of this project to discover whether recombinant protein yields could be induced to go even higher. We moved away from deconstructed viral expression vectors, for which MNPHARM had exclusive use, to a technology of our design.

When the study began, we thought that plants with gene knock-outs that produce human-like glycosylated proteins would be in hand a year later. Both VEGF and BMP-2 are glycoproteins. We must wait several more months for seeds from these engineered plant strains. The intended Cas9-CRISP mediated genomic alterations appear to have been successful and plant embryos were made, but there remains much lots of characterization to do. In a few small (pre-)clinical studies published, other plant-derived, glycosylated proteins were injected into animals or human patients… and interestingly there were no significant adverse side-effects. However, MNPHARM will produce and sell fully human-like proteins for regenerative medicine in the future.

How did it impact regenerative medicine in Minnesota?

Given this pilot project's success, MNPHARM is busy now converting our Minnesota lab into a small GMP facility for recombinant protein production. An expert Compliance Officer was hired, Tom Martin (an ex-3M VP). We expect to finish the conversion to adopting GMP-level training, methods and documentation within one year. A GMP facility is required so our products can be utilized in pre-clinical and clinical studies. We want to be ready to seek FDA approval for any protein products meant for regenerative medicine.

MNPHARM will use the results from this pilot to attract more investor financing. Funding is required to build a large production ability in Minnesota. MNPHARM wants a facility able to process tons of plant biomass to yield kg-levels of pharmaceutical-grade proteins with every 2-day batch. Importantly, the costs of goods sold (COGS) are predicted to be around $0.12/mg protein when given this degree of production scale-up and our methods, significantly less than for other techniques of recombinant protein

manufacturing, such as mammalian or bacterial cell culturing carried out at similar scales.

Please list any of the following that have resulted from your Regenerative Medicine Minnesota grant funding:

- *Publications and/or manuscripts submitted for publication*

No publications were submitted yet but some are planned. We will be happy to update RMM later with all our citations.

- *Disclosures/patents*

We decided to keep the novel methods developed with this funding a trade secret rather than seeking patent protection. The main rationale is that we're not interested in licensing to other businesses at this time.

- *Other grant applications and/or awards*

Based on these pilot results, we obtained a second Biotechnology grant from Regenerative Medicine Minnesota for $100K. We will improve upon our methods while manufacturing several more proteins important for regenerative medicine in Minnesota: BMP6, BMP7, fibronectin, a fibronectin domain and two BMP-fibronectin fusion polypeptides. We will evaluate functionalized scaffolds made from attaching these two proteins and BMP2 by chemical means that involve covalent bonds.

We plan to submit other grants based on these results. We will update RMM when that occurs.

Responsible Spending:

We spend more than the grant and absorbed those costs ourselves. Below is an accounting of the invoices against the grant.

This grant was extremely helpful for us to purchase needed equipment. Thank you.

Grant spending by category:	
Lab Supplies	$ 4,067.91
Labor & OH	$ 50,421.45
Equipment	$ 35,510.64
Indirect	$ 10,000.00
Total	$ 100,000.00