CONFIDENTIAL

Regenerative Medicine Minnesota
Progress Report
Due: May 30, 2016

Grant Title: Mill Creek Life Sciences Cell Kinetics
Grant Number: MRM 2015 BB 005
Requester: Allan B. Dietz, Ph.D
Project Timeline: May 1, 2015 – April 30, 2016
Brief description of project:
Mill Creek provides materials to the regenerative medicine industry. Critical to the success of this industry is the ability to grow stem cells and other cells capable of powerful therapeutic effects. Growing cells as drugs requires materials that meet GMP requirements in a cost-effective and predictable way. PLTMax® is the flagship product from Mill Creek used to meet these needs. While originally developed to be a substitute for FBS, PLTMax proved to be far superior, with improved growth kinetics, better chromosomal stability of cultured cells, more growth factors in a form accessible of cell signaling, and improved function of cells in culture.
PLTMax® is a base ingredient that can be customized to develop an array of media supplementations to serve the growing regenerative medicine industry. However, to reach that goal it was necessary to bring technology to the company to sensitively measure cell growth and factors that affect cell growth when PLTMax® was added to the cell culture media. The Incucyte ZOOM® (Essen Biosciences, Ann Arbor, MI) is the equipment that we needed to incorporate the real time cell imaging technology to our process and optimize the conditions for the production and use of PLTMax®.
Where did this project take place?
This project took place on the premises of Mill Creek Life Sciences in Rochester, MN.
People impacted by project and where they are from:
The development of this project impacted our customers which received important information about our product thanks to the incorporation of the Cell Kinetics assay into the release testing process in our company. Figure 1 illustrates the distribution of our customers.

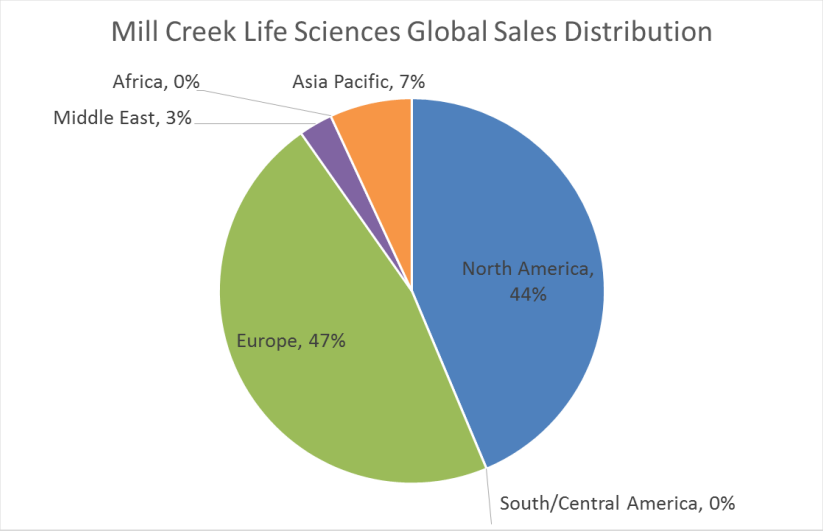

Figure 1: Mill Creek Life Sciences sales by region. Sales are indicated by major geographical region with percentages indicated within the fractions.

What was the outcome of the project?

After being awarded with the Biotechnology/Biobusiness grant from Regenerative Medicine MN in 2015, Mill Creek Life Sciences purchased an Incucyte ZOOM® which allowed real time imaging of cells in culture. Thanks to this technology, cell kinetics is now included as part of the release testing of PLTMax® (Figure 2).

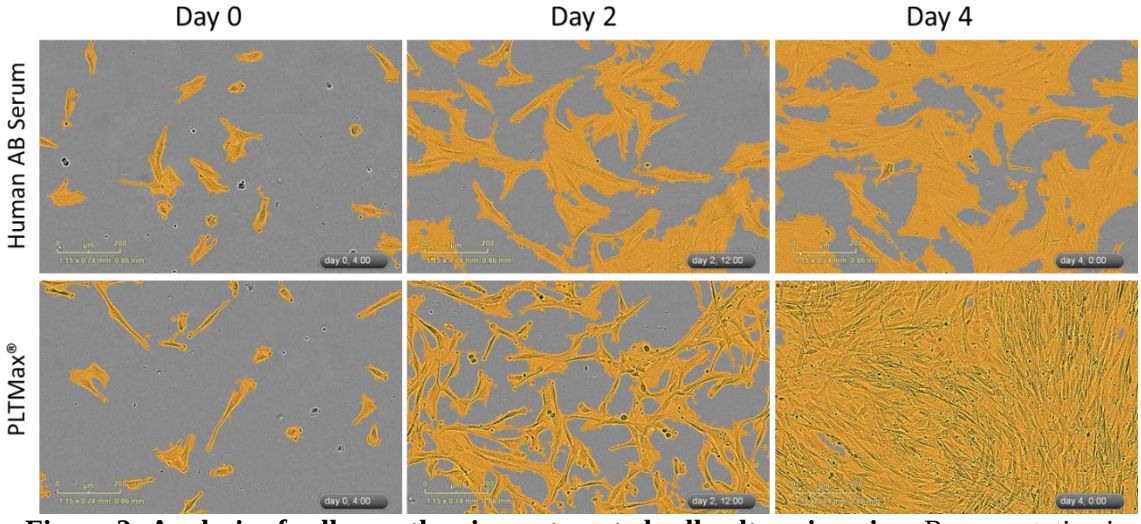

Figure 2: Analysis of cell growth using automated cell culture imaging. Representative images of Adipose derived MSCs at 3 time points of real time monitoring analysis performed using Incucyte ZOOM®. Cells are false color masked by instrument software to measure growth.

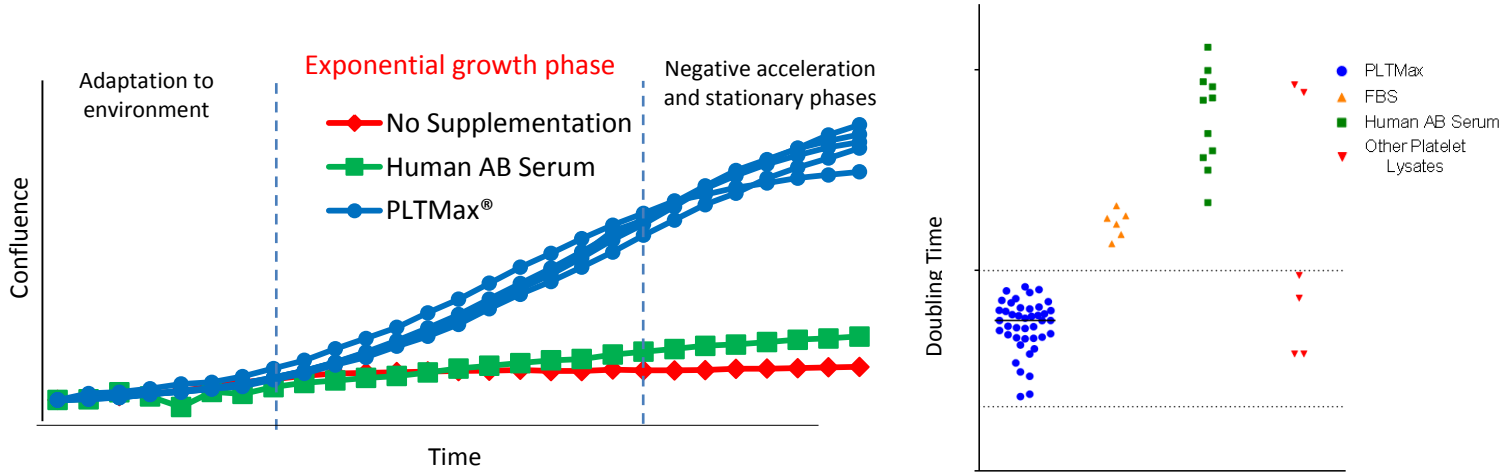

Figure 3: Product analysis used to monitor product quality. A) Lot to lot variation reflected by real time imaging of cell growth using 5 lots of PLTMax® as a media supplement to grow Adipose derived MSCs. The growth of each lot is plotted according to the time in culture and the increase in cell density as measured by confluence. Human AB Serum is the next available human based supplement with differences observed as early as 36 hours. B) Comparison of Adipose derived

MSCs growth after supplementing media with either PLTMax®, FBS, Human AB Serum or different competitor platelet lysates.

The cell kinetics assay provided important information and new data about our product through the analysis of cell growth including lot's performance on the promotion of cell growth (Figure 3B), lot to lot variations (Figure 3A) and differences on cell kinetics based on the cell type used for the study (Figure 4).

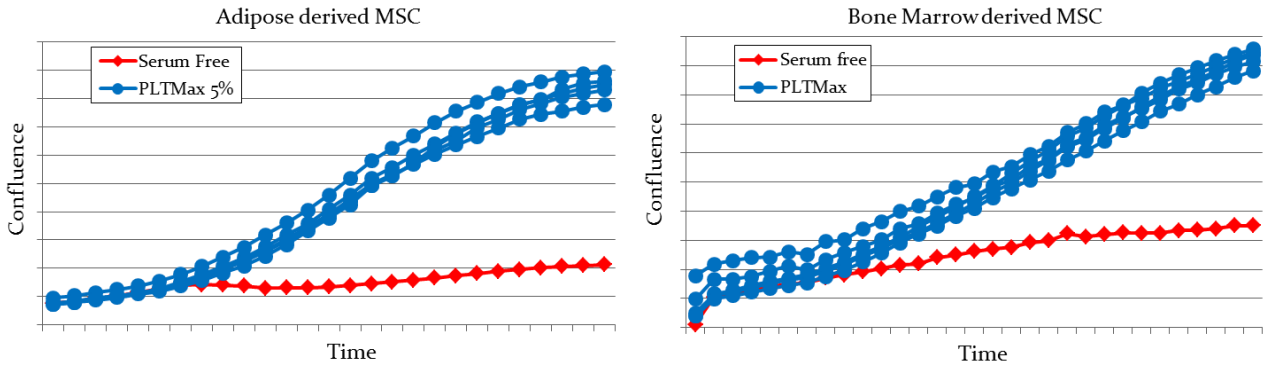

Figure 4: Comparison between cell kinetics of MSC from Adipose and Bone Marrow origin.

As requested by our customers, we also conducted experiments comparing the real time imaging of cell growth with single point cell counts. This comparison allowed us to determine that the cell kinetics assay provides more information about cell growth promoted by PLTMax® than single point cell counts. With single point cell counts, we cannot detect differences on a lot's performance based on number of cells used for the assay once we reach certain number of cells. However, using the cell kinetics assay we increase the sensitivity of the analysis, being able to distinguish between performances depending on the number of cells used in the assay (Figure 5A).

Finally, we were able to answer the request of our customers to show differences on cell growth depending on the percentage of PLTMax® used to supplement the media (Figure 5B) as well as the request to conduct long term stability (Figure 5C) and temperature excursion studies.

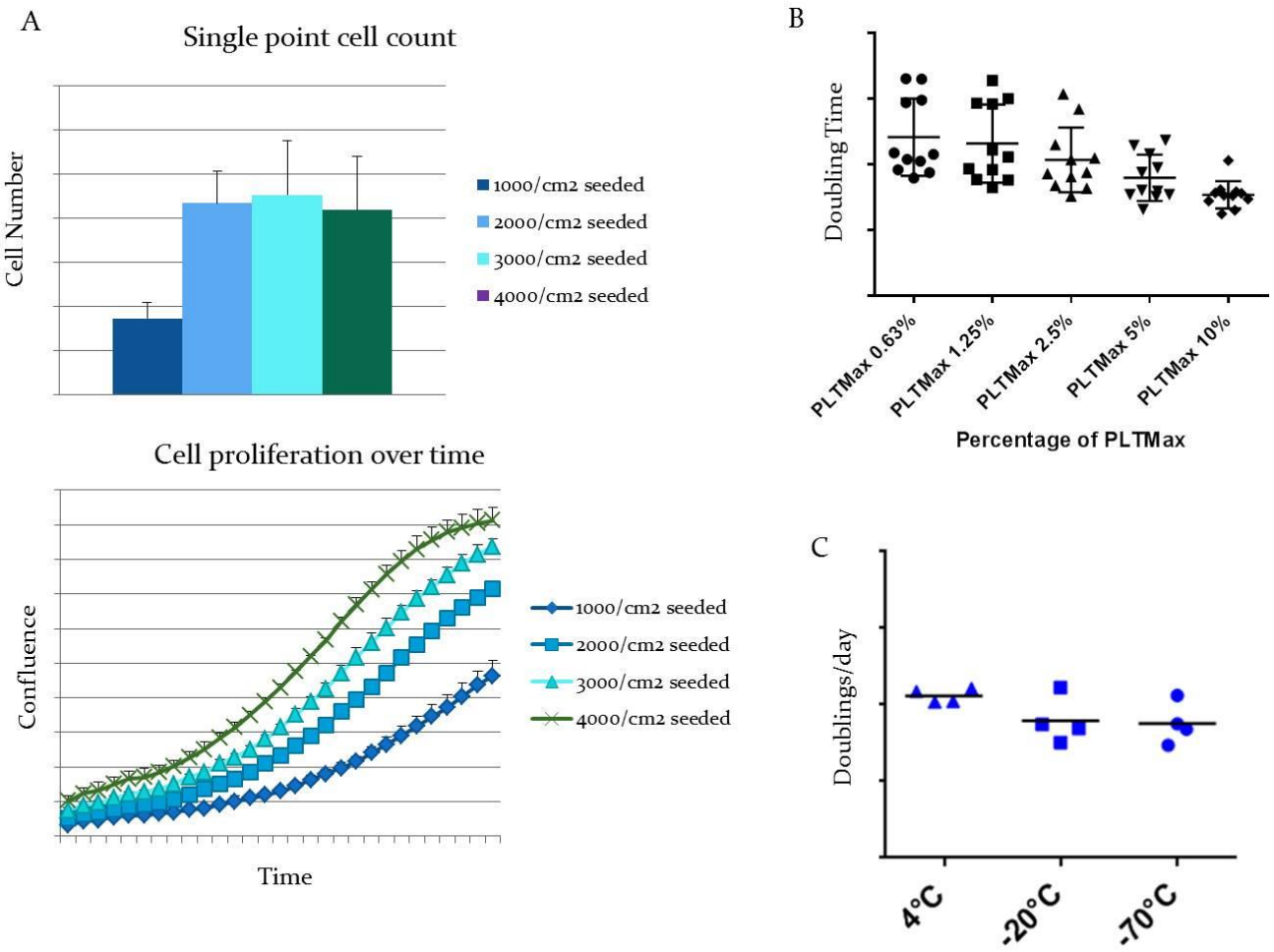

Figure 5: A) Comparison between single point cell counts and Cell kinetics assay. B) Differences on cell growth determined by the percentage of PLTMax® used to supplement the media. C) Results of cell kinetics assay performed using Adipose derived MSCs and media supplemented at 5% with PLTMax® kept at 4°C, -20°C or -70°C for 3 months. This assay corresponds to the first time point of a long term stability study that will be conducted for 5 years.

Responsible Spending:

Mill Creek Life Sciences was awarded with a total funding of $95,600. The total amount of the award was used to purchase an Incucyte ZOOM® ($76,766), Adipose derived Mesenchymal Stem Cells (MSC) and Bone Marrow derived MSC ($1,137), cell culture reagents and vessels ($1,339) and to contribute to the salary of a scientist who conducted the studies ($16,358). All monies were spent on the project, the project was completed, and the project was critical to contributing to meeting customer needs, expanding Mill Creek's business and establishing regenerative medicine in Minnesota.