DEVELOPMENT OF AN ALTERNATIVE HARVESTING METHOD USING pH TO DETACH ADHERENT CELLS FROM MICROCARRIERS

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Peripheral nerve injuries are common in Canada, affecting 2.8% of trauma patients treated every year. Current repair strategies are inadequate and repair is often suboptimal with only 25% of patients recovering full motor function and only 3% regaining full sensory function. Because of this, the field is turning toward regenerative medicine to develop a cellular therapy using Schwann cells to repair injured nerves. Schwann cells differentiated from skin derived precursors (SKP-SCs) are a promising cell type as they are easily obtained and allow for autologous therapy. To be able to generate clinically relevant numbers of SKP-SCs, bioreactors need to be used. Since SKP-SCs are an adherent cell type, to be expanded in suspension bioreactors, small spherical beads known as microcarriers need to be used. Our lab has previously shown that these SKP-SCs readily attach to the microcarriers and grow in stirred suspension bioreactors. We have also shown that by controlling the culture parameters, we can increase the maximum cell density compared to conventional static culture methods.

One of the biggest hurdles that remains is an efficient harvesting method that can be scaled up to clinical applications. Current cell detachment protocols use enzymatic based solutions to remove the cells from the surface of the microcarriers. These methods work well in removing the cells, however, they are very labour intensive as they require many washing steps and taking the reactors offline. Therefore, we looked into an alternative method for the detachment of SKP-SCs from microcarriers that will allow for an inline detachment process. This new method is based on previous research done in our lab using high pH solutions to dissociate aggregates.

First we investigated the detachment efficiency in static. Cells were cultured in 6-well plates until confluency and then harvested with solutions ranging from pH 8-9.5. With a pH of 9 and an incubation time of 30 minutes, we were able to recover 75% of cells when compared to traditional enzymatic harvesting. Following this we performed a qualitative analysis on the detachment of the SKP-SCs from the microcarriers to determine if this method has potential. Small 3mL samples were taken and solutions with pHs 8.5, 9, and 9.5 were added and incubated for 30 minutes and agitated every 5 minutes. We found that the cells detached with a high efficiency after 30 minutes with a pH of only 8.5. This was then quantified while maintaining a viability of above 90%. Following this we tested this method in harvesting full 125mL bioreactors. We evaluated different pH, agitation rates, and incubation times. We also assessed the ability of the cells to reattach to microcarriers and continue to expand over several serial passages to ensure there were no negative effects on the cells. Lastly we looked at using this method in our controlled bioreactors to increase the pH without the addition of anything else.

Based on our results, increasing the pH of the culture medium can detach the SKP-SCs from microcarriers at a pH as low as 8.5 which allows for minimal cell damage while still detaching cells. We also noted that when the pH gets too high (>9.5), the microcarriers begin to clump together causing large aggregates of microcarriers which could lead to clogging during the filtration steps. With increasing agitation, higher recovery efficiencies can be achieved indicating that this method of cell detachment has potential for large volume processes.