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Degenerative central nervous system (CNS) diseases such as spinal cord injury (SCI) affect approximately 260,000 Americans annually [1,2]. As used current treatments are not curative, cell based therapies promise to be effective in the long-term. In cell based therapies, the diseased tissue is replaced by transplanting stem cells or other functional cells required in the body [3]. Studies have shown the capability of skin-derived precursor cells (SKPs) to differentiate in vitro into neural crest-derived cell types such as Schwann cells and other neurological cells [4]. Their proliferative capacity and differentiation potential makes them an attractive source for surgical nerve repair.

The main objective of the present study was to develop protocols to maintain and expand rodent SKP cells in static culture. Serial passaging was used as a technique to successfully cultivate SKPs. These free-floating cells were seeded as single cells in tissue culture dishes, and the cells divided for a period of time, growing in aggregates. Prior to passage SKPs, cells were (1) harvested from the tissue culture dishes and (2) dissociated to break up the connection between the cells in the aggregates. The remaining cells were (3) frozen down at -192 °C for long-term storage.

Two different segregation procedures were tested: chemical and enzymatic dissociation. Chemical dissociation was accomplished by changing the environmental pH of SKP aggregates. Enzymatic dissociation was carried out by analysing growth kinetics of SKPs after treatment with different enzymes. Segregation of SKPs using chemical dissociation proved to be unsuitable, causing cell death. Out of the three tested enzymes, Collagenase XI was deemed appropriate to dissociate SKPs into single cells, due to high growth rate and viability of the cells.

Two freezing protocols were tested: freezing SKP cells as single cells and as aggregates. Freezing as aggregates improved cell survival through better sphere formation.

Using these developed protocols, expansion of the cells was achieved over an extended culture period with high cell viability.

References

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4. Fernandes *et al.* 2004, 2006; McKenzie et al. 2006