Encapsulation of Schwann cells for neurite outgrowth in a microfluidic device
D. E. Mason1,2, B. K. Patel1,3, C. E. Ryan1,4, D. S-K. Yip5, and C. H. Cho6
1New Jersey Institute of Technology (NJIT), Newark, NJ, 2Virginia Commonwealth University, Richmond, VA, 3Lawrence Technological University, Southfield, MI, 4Mercer University, Macon, GA

Introduction: Peripheral neuropathy affects 8% of the world’s population and is associated with diabetes, HIV and chemotherapy. Growth factor therapy has been proposed as a treatment for peripheral neuropathy, requiring frequent injections of nerve growth factor (NGF) or Schwann cells (SC) in order to initiate neurite outgrowth in affected neurons. However, donor SC suffer the risk of being rejected by a patient’s immune system. To overcome these issues, encapsulation of SC in a semi-permeable polyelectrolyte membrane has been applied using an Alginate-Poly-L-Lysine-Alginate (APA) membrane. NGF induced neurite outgrowth of PC12 cells was successfully achieved by co-culture of encapsulated SC (eSC) using a microfluidic device.

Materials and Methods: The cell encapsulation process was performed using co-axial flow of air and solution to form alginate droplets that crosslinked into alginate spheres when immersed in 300 mM CaCl₂. The spheres were incubated in PLL (0.1 mg/mL) for 8 minutes, washed in Hapes Buffer Saline (HBS), and then incubated in alginate (0.04 g/mL) for 5 minutes. Our microfluidic device, consisting of 4 outer wells connected to a center well via canals, was placed in a P35 dish and used for co-culturing PC12 cells (center well) and eSC (outer wells). The center well of the device was coated using PLL (0.05 mg/mL) to promote PC12 adhesion and the co-culture media used was modified SC media containing only 5% serum (1:1 HS/FBS) and 20 ng/mL of NGF.

Results and Discussion: There was a high SC survival rate during the initial sphere creation and coating process as illustrated in the Figure 1(a). AlamarBlue assay showed eSC survival and viability over 7 days; the initial SC count was roughly 54,000 and decreased to 47,000 cells per well plate after 7 days. FTIR analysis of dried alginate and APA spheres indicated noticeable peaks at 1600 λ⁻¹ (N-H stretches) and 2950 λ⁻¹ (N-H bends), characteristic of lysine amine group inclusion into the spheres. PC12 cells co-cultured with eSC had a higher average neurite length of 53.09 μm by day 4 compared to the control of 38.67 μm (p<0.05) shown in Figure 1(b).

Figure 1: a) Live/dead cell assay of eSC. Calcein acetoxymethyl (green) used to represent live cells and ethidium homodimer-1 (red) dye used to represent dead (4x). b) Bar Graph of average neurite growth in micrometers for 4 days. Control PC12 differentiation in 24 well plate at day 3. d) PC12 differentiation in microfluidic model at day 3.

Conclusion: SC encapsulated in APA have yet to be study in relation to how they differentiate neural cells. This study has shown that after encapsulating SC in APA spheres the SC were able to induce neurite outgrowth from PC12 cells. This concept can be applied to the treatment of nerve regeneration in patients affected by neuropathy.

Acknowledgements: We would like to thank the National Science Foundation for funding this REU (grant 1156916) as well as NJIT for providing laboratory space. We would also like to acknowledge Dr. Pfister’s lab for donating materials for our experiments and Dr. Kim’s lab (Rutgers Univ.) for providing the Schwann cells isolated from neonatal rat sciatic nerve.