Long Term Viability of Alginate-Microencapsulated Schwann Cell Line RT4-D6P2T

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Statement of Purpose: The biomaterial alginate is gaining extensive usage for microencapsulation and *in vivo* immunoprotection of foreign cells that produce and secrete important therapeutic biological factors. Maintenance of survival and functionality of these cells is vital to the implantable system. Schwann cells are known to enhance the regeneration of damaged peripheral nerves partly via expression and release of supporting neurotrophic factors. However, non-autologous Schwann cell sources will be rejected by the host immune system; thus the need for microencapsulation. Hence, it is worthwhile to investigate whether the viability of alginate-encapsulated Schwann cells can be maintained up to a period of 1 month *in vitro*.

Methods: Dissociated RT4-D6P2T rat Schwann cells were mixed with sterile 1% sodium alginate (Sigma-Aldrich, St. Louis, MO) in PBS. Microencapsulated cells at low, medium, and high initial densities (about 300, 3000, and 30000 cells per microsphere, respectively) were formed by dropping the alginate cell suspension in various concentrations (10, 50, 100, and 200 mM) of buffered $BaCl_2$ cross-linking solution using a small diameter needle. After washing and rinsing, the capsules were incubated with culture medium (DMEM with 10% fetal bovine serum) at 37°C, 5% CO_2, and 90% relative humidity. Culture medium was replaced twice a week. Cell and microsphere morphologies were viewed under an inverted microscope. Viable cells were detected using Live/Dead® (Invitrogen, Carlsbad, CA) fluorescence microscopy. To determine if the encapsulated Schwann cells can still divide and proliferate, MTT assay was performed. Cells were evaluated at 3, 7, 14, 21, and 28 days in culture.

Results and Discussion: Alginate microspheres exhibit mean diameter sizes of 2.4, 2.6, 2.5, and 2.5 mm when polymerized using 10, 50, 100, and 200 mM Ba^{2+}, respectively. Encapsulated Schwann cells show spherical and clustered morphologies (Fig. 1) unlike cells growing on tissue culture plates with flattened and spread appearance. Higher [Ba^{2+}] leads to lower microsphere transparency; therefore cells were harder to view under visible light conditions. Live/Dead® fluorescence staining alleviates this problem. Viable cells were evident as shown by the green fluorescence emission in all samples until the 4-week incubation (Fig. 2). This proves that Schwann cells can be maintained long term in alginate microcapsules. MTT results additionally reveal that cells can proliferate while entrapped within the alginate biomaterial, particularly at the low and medium initial cell densities (Fig. 2). After 28 days of *in vivo* culture, the microspheres still remain intact and their shapes still resemble that of the newly fabricated capsules.

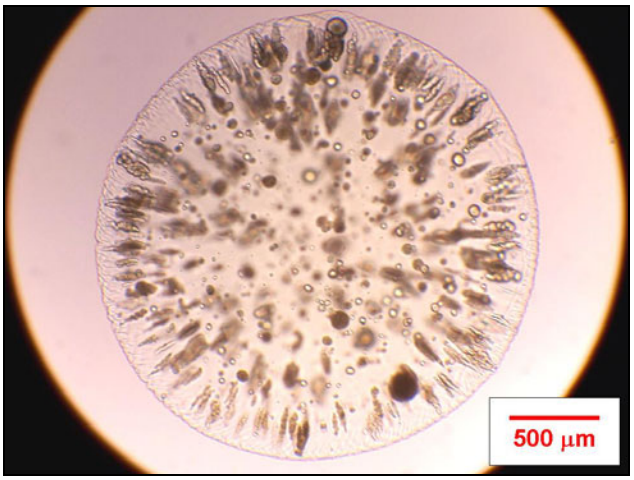

Figure 1. Alginate-microencapsulated Schwann cells

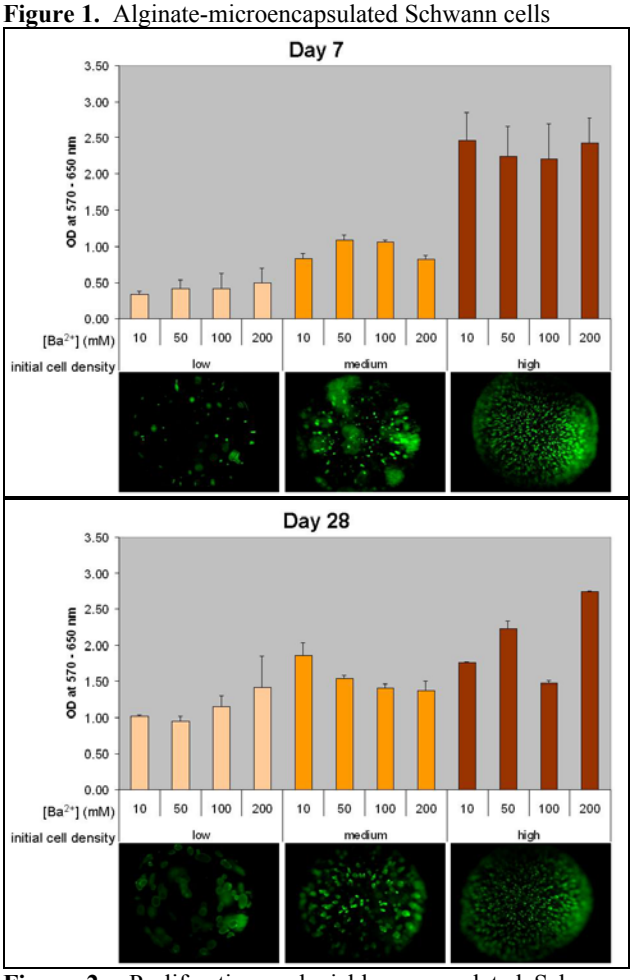

Figure 2. Proliferating and viable encapsulated Schwann cells

Conclusions: RT4-D6P2T Schwann cells can be maintained viable in alginate microspheres for a long period of time. To decrease the microcapsule volume for improved nutrient diffusion and cell survival electrostatic fabrication is recommended.