Functional Polymeric Microcapsules for Neural Stem Cell Culture

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Introduction:

Polymeric microcapsules provide a unique means to investigate the influence of physical and biochemical cues on cells in three dimensional microenvironments *in vitro*. Capsule parameters, such as permeability, size, and composition, can influence the viability, proliferation and differentiation of the cells cultured inside microcapsules. We have developed a novel system for generating microcapsules with high reproducibility and good control over size. The microcapsules are fabricated by complex coacervation of cationic methylated collagen (MC) and a polyanionic terpolymer (TP) of methyl methacrylate, methacrylate, and hydroxyethyl methacrylate. The MC/TP system imparts substrate functionality through RGD cellular binding domains on MC, while TP is composed of hydrophobic, hydrophilic and charge carrying components, the relative amounts of which can be tuned to adjust the pore size, permeability and capsule stability.

Methods:

Microcapsules were fabricated using rat neural stem cells suspended in 0.3 mg/ml MC-PBS solution, and a 2.5% TP-PBS solution. The molecular weight (Mw) of TP is 1.93×10^3 kDa. The permeability of the capsules was monitored by the egress of FITC-dextrans of various molecular weights (10, 70, 150, 500, 2000 kDa) and Rh-BSA as a function of time. Bioactivity of FGF2 (20 ng/ml) following ingress was assayed using microencapsulated NR6R-3T3, FGF2-dependent embryonic mouse fibroblasts. The proliferation of the encapsulated rat NSCs was estimated using GFP-expressing rat NSCs by both proliferation rate (WST-1 assay) and morphology on a confocal laser scanning microscopy.

Results and Discussion:

Relatively uniform populations of microcapsules, varying in size from 173 ± 11 µm to 1135 ± 70 µm were fabricated with this method (Figure 1). Microcapsule size can be controlled to be nearly the same as target sizes. Egress of dextrans over a wide range of molecular weights has been quantified to characterize membrane permeability. The time to half-of-maximum release increased with increasing molecular weight of dextran; egress of dextran as large as 2000 kDa was observed. Interestingly, the release kinetics of 70 kDa dextran was nearly identical to that of BSA (67 kDa), suggesting that the effect of charge is minimal for transport of macromolecules. The growth kinetics of the microcapsule membrane, as a function of Mw and concentration of the TP, were examined. The capsule wall exhibited logarithmic growth as a function of time; and, lower Mw led to a thicker membrane. As TP concentration increased, the time to maximum thickness decreased. Rat

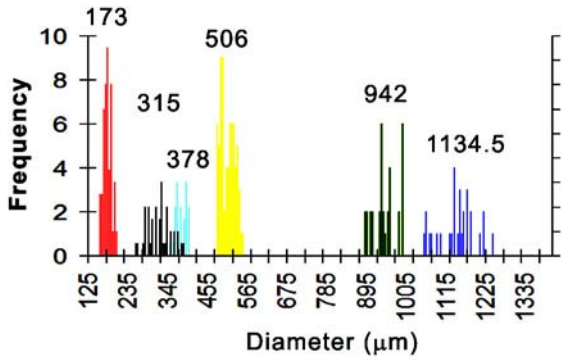

Figure 1. Size distribution of several batches of microcapsules prepared with different target diameters ($p < 0.001$, F-value >> F-critical, single factor ANOVA).

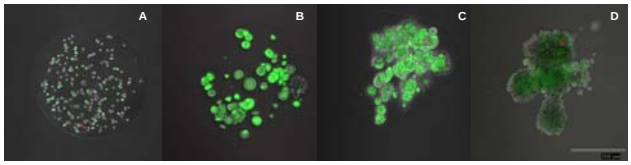

Figure 2. GFP-expressing rat NSCs were cultured in a MC/TP microcapsule. Shown are confocal images of GFP-labeled NSCs on days 1 (A), 4 (B), 5 (C) and 7 (D).

NSCs were encapsulated into these MC-TP microcapsules. NSCs were uniformly distributed in the microcapsules at 4 h after encapsulation. The encapsulated NSCs form multicellular aggregates by day 4 (Figure 2). The NSCs continued to grow in clusters, with a doubling time of ~22.5 hours. By day 7, cell aggregates outgrew and ruptured the capsule membrane.

Conclusions:

This method offers picoliter-scale control over microcapsule size. These microcapsules exhibit high permeability for macromolecules up to 2,000 kDa. FGF2 ingress is sufficient to support the proliferation of NR6R-3T3 fibroblasts and rat NSCs through the ingress of FGF2. This MC/TP microencapsulation system provides a platform to investigate the influence of topographical cues and biochemical signals on the self renewal and differentiation of NSCs.

References:

[1] Yin C. *et al.*, Biomaterials 24: 1771-1780 (2001).