A Novel Core-Shell Microcapsule for Encapsulation and 3D Culture of Embryonic Stem Cells

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Electronic Supplementary Information: Figs. S1-S5 and Table S1 on the following four pages:



Fig. S1. Typical flow cytometry data (symbols) of counts (in natural log scale) versus FITC intensity (in common log scale) for nontreated control cells and cells after incubating with FITC-IgG overnight that were non-encapsulated, encapsulated in plain alginate microbeads, or encapsulated in ACA microcapsules. The vertical green line in the top left panel indicates the maximum FITC of nontreated control cells. The vertical blue line in the top right panel indicates the maximum FITC intensity of nonencapsulated cells after incubating with FITC-IgG overnight. Therefore, cells with FITC intensity between the vertical green and blue lines are taken as FITC-IgG bound cells. Unlike the flow cytometry data of non-encapsulated cells (top two panels), however, the flow cytometry data for encapsulated cells typically had two peaks. The right peak is probably due to the presence of the alginate and/or chitosan complexes that are difficult to completely remove from the sample. To resolve this problem, we fit the flow cytometry data of the encapsulated cells using Origin (v8.6) using two Gaussian peaks shown as the solid red and dashed green lines in the bottom panels. The counts of FITC-IgG bound cells between the blue and green lines were calculated as: $N' = [N' l/(N' l + N' 2)]^*N$, where N represents the original count obtained by flow cytometry, and N'1 and N'2 are the data from fitted left (solid red) and right (dashed green) peak, respectively. The results are reported as the percentage of IgG bound (or positive) cells calculated the summation of N' between the vertical green and blue lines out of total cells calculated as the summation of N' to the left of the vertical blue line.



Fig. S2. Effect of chitosan concentration used for coating (3 min) on the viability of cells encapsulated in the resultant ACA microcapsules



Fig. S3. ATR-FTIR spectra of sodium alginate, chitosan, plain alginate microbeads, AC microcapsules, and ACA microcapsules (liquid core)

Wavenumber (cm ⁻¹)	Alginate	Chitosan
3700-3000	OH stretch	
3360		O-H and N-H stretch
3000-2850	CH stretch	
2873		C-H stretch
1657		amide I
1600	Antisymmetric COO ⁻ stretch	
1590		N-H bending from amine and amide II
1420		-CH ₂ bending
1410	Symmetric COO ⁻ stretch	
1376		CH3 symmetrical deformation

Table S1. ART-FTIR peaks and their assignments for alginate and chitosan



Fig. S4. Phase contrast (A) and fluorescence (B, green and red stain live and dead cells, respectively) images of R1 ES cells 7 days after being encapsulated in ACA microcapsules with alginate hydrogel core in ES cell culture medium showing the formation of multiple cell aggregates of various sizes and shapes: Scale bar, 100 µm.



Fig. S5. Phase contrast (A, E, and E) and fluorescence (B, D and F: green and red stain live and dead cells, respectively) images of C3H10T1/3 mesenchymal stem cells encapsulated in ACA microcapsules with a liquid core under culture at day 0 (A and B), day 28 (C and D) and day 49 (E and F) showing their high viability and proliferation to form cell aggregates at day 30 and 49 from single cells at day 0 under long-term culture: Scale bar (applicable to all panels), 100 µm.