Inwards Buildup of Concentric Polymer Layers: A Method for Biomolecule Encapsulation and Microcapsule Encoding**

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**Figure S1.** Confocal fluorescence image of an alginate microbead with 3 concentric layers of niPA-FITC/niPA/niPA-FITC. (green/blank/green).

**Figure S2.** (A) Optical transmission and corresponding (B) confocal fluorescence images of Rhodamine123 labeled agarose microbead after incubation with non-fluorescent niPA in 1-butanol. By comparing these two images, reduced fluorescence intensity can be observed to be emitting from the niPA layer (within white arrows). The reduced fluorescence intensity observed within the niPA layer is due to the close interaction of niPA and the Rhodamine 123-agarose polymers (complex formation), and causing a fluorescence quenching effect via an electron-transfer process [1] from the excited fluorescent dyes to the electron deficient \(-\text{NH}_3^+\) groups (data not shown) of niPA.
Figure S3. Confocal fluorescence images of (A) a representative agarose microbead with niPA-FITC layer at Time 0 hours and (B) a representative agarose microbead with niPA-FITC layer at Time 48 hours after incubation in 1-butanol. Insets are fluorescence intensity plot profiles obtained at the yellow line. The average layer thickness (11.0 ± 0.6 μm) and fluorescence intensity intensity (1953 ± 191 pixel values) at Time 0 hour can still be observed (11.3 ± 0.5 μm / 1960 ± 109 pixel values) after 48 hours of incubation in 1-butanol (solvent was not changed).

Figure S4. Confocal fluorescence image demonstrating the deposition of niPA-FITC into the core of agarose microbeads. These microbeads were imaged after incubation with (niPA-FITC/niPA)2/niPA-FITC and the niPA can be observed to have filled the core of the agarose microbeads.

Figure S5. Images of equivalent volume of hydrogel microbeads dispersed in 1-butanol after 2 weeks of incubation with excess niPA dissolved in 1-butanol (left tube/+ve control) or with 1-butanol only (right tube/-ve control). For both tubes, no significant changes in volume were observed but a slight change in opacity could be observed after 2 weeks for the left tube (the change in opacity is caused by the absorption of niPA by the agarose microbeads).
Figure S6. Confocal images of agarose microbeads fabricated with alternating (A) niPA-FITC and (B) niPA-TRITC using 500 μL polymer solution and 15 minutes incubation time; (C) niPA-FITC and (D) niPA-TRITC using 1 mL polymer solution and 15 minutes incubation time; (E) niPA-FITC and niPA using 1 mL polymer solution and 15 minutes incubation time for layers 1 & 2 and 5 minutes for layer 3; (F) niPA-FITC and niPA using 1 mL polymer solution and 15 minutes incubation time for layers 1 & 2 and 45 minutes for layer 3; (G) niPA-FITC and niPA using 1 mL polymer solution and 15 minutes incubation time for layers 1 to 4 and 5 minutes for layer 5; (H) niPA-FITC and niPA using 1 mL polymer solution and 15 minutes incubation time for layers 1 to 4 and 45 minutes for layer 5. Insets are fluorescence intensity plot profiles obtained at the yellow line to highlight the distribution of niPA-FITC or niPA-TRITC. The similar fluorescence intensity observed for each fluorescent layer in each plot highlights that the thicker layers are a result of more niPA packing into the agarose microbeads and not a result of the polymer spreading out within the microbeads.
Figure S7. Remaining percentage of niPA-FITC in the supernatant as a function of layer number. The percentage of niPA-FITC remaining in the supernatant increases when the layer number increases although the same incubation time was used for each layer. This suggests that less polymer is entering the agarose microbeads as more polymer deposits into the agarose microbeads; and is probably caused by previously deposited polymer acting as a diffusion barrier. Briefly, an increase in the layer number probably creates a thicker diffusion barrier for any incoming niPA-FITC and leads to an increase in the percentage of niPA-FITC remaining in the supernatant.

Figure S8. (A-C) Confocal images of agarose microbeads in 0.01x PBS with 5 concentric layers of different colour coding permutations. Fabrication was done in the following order: Layers 1/2/3/4/5 (A) G/B/R/B/G (B) R/G/R/G/R (C) R/G/R/B/G. R – RED, G – GREEN, B – BLANK. (D-F) Confocal images of agarose microbeads in 0.01x PBS with 3 concentric layers (Layer 1/2/3) of the same colour encoding permutation (R/B/G) but with different thickness permutations due to the use of different volumes of polymer. (D) 500 μL /500 μL /500 μL (E) 500 μL /1 mL /500 μL (F) 500 μL /500 μL /1 mL. The insets in the confocal images are magnified images of the fluorescence layers.
**Figure S9.** Confocal fluorescence images showing the distribution of dextran-TRITC (65,000–76,000 Da) within agarose microcapsules with (A) 1 concentric coloured layer, (B) 3 concentric coloured layers and (C) 5 concentric coloured layers. The agarose microcapsules are dispersed in 1-butanol.

**References**