Electronic Supplementary Material (ESI) for RSC Advances.

Edible Polyelectrolyte Microcapsules with Water-Soluble Cargo Assembled in Organic Phase

Houwen Matthew Pan, Arjun Subramanian, Christopher J. Ochs, Jean-Yves Dewavrin, Sebastian Beyer, Dieter W. Trau**

*E-mail: bietrau@nus.edu.sg and sebastian@smart.mit.edu
www.biosingapore.com

Supporting Information

Table of Contents

1. Materials and Method

Maltotriose hydrate 95%, dextran-TRITC (M_w 155000 g mol^{-1}), poly-L-glutamic acid sodium salt (PGA, M_w > 50000 g mol^{-1}), proteinase K, genipin, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), tetramethylrhodamine isothiocyanate (TRITC), ammonium fluoride – hydrofluoric acid mixture 7:1, calcium chloride ($CaCl_2$), hydrochloric acid (HCl) solution 1.0 M were purchased from Sigma-Aldrich, US. ε-polylysine (EPL, M_w 4700 g mol^{-1}) 25% solution in water was purchased from JNC Corporation, Japan. Carboxylated silica particles, (S2605, 5.56 µm ± 0.24 µm) and carboxylated polystyrene particles, (KM28, 5.38 µm) were purchased from Microparticles GmbH, Germany. Hydrochloric acid (HCl) solution 10 M was purchased from VWR, US. Absolute ethanol was purchased from ROMIL, UK. Phosphate buffered saline (PBS) solution 10x, pH 7.4 was purchased from Vivantis Technologies, Malaysia. Ultrapure water was purchased from Invitrogen, US. Transglutaminase (TG) from *Streptoverticillium mobaraense* was received as a free sample from AJINOMOTO, Japan.

1.1. Preparation of Sugar Templates

Maltotriose powder was used as the template material for experiments. Dextran-TRITC (M_w 155 000 g mol^{-1}) was used as a fluorescent tracer. A sugar template and dextran-TRTIC mixture was prepared by mixing in a mass ratio of 99: 1, dissolved in ultrapure water. The solution was freeze dried overnight to obtain a homogenous solid mixture and ground in a mortar until particles in the size range of 10 to 100 µm were obtained. The particles were then re-suspended in absolute ethanol.

1.2. Preparation of ε-Polylysine (EPL) and Polyglutamic Acid (PGA) in Absolute Ethanol

For preparation of PGA ethanol solution, PGA sodium salt powder was first dissolved in a small amount of ultrapure water (5.6 µL per mg of PGA sodium salt). 0.65 µL of HCl (10 M) per mg of PGA sodium salt was added to obtain a non-ionized PGA solution. Next, absolute ethanol was added to obtain a final PGA concentration of 1 mg mL^{-1}. The PGA solution was subjected to heating, sonication and vortex cycles until no more PGA would dissolve. Undissolved PGA particles were filtered and removed. 0.5 µL of HCl (10 M) per mL of PGA ethanol solution was added. For preparation of EPL ethanol solution, EPL solution was diluted in absolute ethanol to obtain a final EPL concentration of 1 mg mL^{-1}. For fluorescent microscopy studies, an EPL-TRITC conjugate in the ratio of 1: 100 (fluorophore: EPL monomer) was prepared.

1.3. Reverse-Phase Layer-by-Layer (RP-LbL) Encapsulation

100-200 µL of particle suspension was incubated with 1 mL of EPL (1 mg mL^{-1}) for 10 min with gentle vortexing, followed by removal of excess polymer by two washing, centrifugation (2800 rpm, 30 s) and re-dispersion cycles with absolute ethanol. The template particles were coated with a second layer by incubation with PGA using similar incubation, washing and re-dispersion procedures, except that ethanol containing 5 mM HCl was used as washing buffer. Alternate

deposition of EPL and PGA was performed until the desired number of layers was achieved. Acidified Ethanol was used as washing buffer for all subsequent layer depositions.

1.4. Hollow Capsule Formation by Sugar Template Dissolution

To observe hollow capsule formation, a small aliquot of encapsulated sugar templates in ethanol was placed between a glass slide and cover slip. A drop of sugar solution (1 µL) was placed on the edge of the cover slip and allowed to spread slowly until the sugar cores are dissolved and hollow shells are formed. Concentrated sugar solution (200 mg mL^{-1}) was used to maintain a stable osmotic pressure.

1.5. Crosslinking Efficiency Study

EPL-TRITC/PGA-coated silica particles (carboxylated) were incubated with 1 mg of EDC, Genipin or TG powder dissolved in 1 x PBS, pH 7.4. Concentration of crosslinker was used in excess. The particles were incubated at 37 °C for 1.5 hrs under gentle vortex. Excess crosslinker was removed by two washing, centrifugation (5000 rpm, 1 min) and re-dispersion cycles with 1 x PBS. 1 mL of ammonium fluoride (NH_4F) etching mixture was added to silica particles and vortexed for 10 min to dissolve silica core to form hollow capsules. Excess NH_4F was removed by two washing, centrifugation (10000 rpm, 5 min) and re-dispersion cycles with 1 x PBS. Used pipette tips and supernatant were disposed in $CaCl_2$ solution. The hollow capsules were added with 5 µL of 5 mg mL^{-1} proteinase K and incubated at 37 °C for 2 hours. Capsule concentration was counted using a hemocytometer.

1.6. Zeta-Potential Measurement

Polystyrene beads (carboxylated) were coated with different number of layers from 1 to 8 via alternating deposition of EPL and PGA using the RP-LbL encapsulation technique (protocol described earlier). Zeta-potential was measured by diluting 10 µL of particle suspension in 1 mL ethanol containing HCl for layers 1 to 8 and absolute ethanol for layer 0 (uncoated particles) with parameters set to 1.359 (25 °C) for refractive index, 1.07 cP (25 °C) for viscosity and 24.3 (25 °C) for permittivity to obtain a valid zeta-potential by the Smoluchowski equation (ZetaSizer Nano, Malvern, UK).

1.7. Fluorescence Intensity Measurement

Silica beads (carboxylated) were coated with different number of layers from 1 to 8 via alternating deposition of EPL-TRITC and PGA using the RP-LbL encapsulation technique. All samples were re-suspended in ethanol containing HCl before measurement. Fluorescence microscopic images were recorded using a CCD color digital camera, Retiga 4000R (QImaging, Canada) connected to a system microscope (Olympus BX41, Japan). Bandpass filters with λ_{ex} 540 nm and λ_{em} 605 nm were used for TRITC detection. Images were captured with QCapture

Pro software (Version 5.1.1.14, QImaging, Canada) and analyzed by ImageJ software (Scion Corp., USA). Approximately 200 fluorescent particles were measured for each data point.

1.8. Atomic Force Microscopy Layer Thickness Measurement

For EPL/PGA multilayer assembly, wafer slides were sequentially immersed into solutions containing 1 mg mL^{-1} of EPL or PGA in acidified ethanol. A period of 20 min was allowed for the deposition of each layer, after which the slides were rinsed with acidified ethanol three times for 1 min and dried with nitrogen. Slides were then scratched with a scalpel blade for AFM analysis and the surface profile was measured. Line scans in random positions on the slide were used to estimate the layer thickness after each deposition step (individual slides).

For AFM on hollow capsules, 5 μL of a dilute particle solution coated with 1 to 4 bilayers was deposited on a clean silicon wafer slide, 1 μL of NH_4F mixture was added and allowed to react. After 1 min, the supernatant was collected carefully and replaced with ultrapure water to wash off excess NH_4F and dissolved silica, the washing procedure was repeated twice and the suspension was allowed to dry. AFM scans were carried out with a Nanowizard II atomic force microscope (JPK Instruments, Berlin, Germany) in AC mode using ultrasharp SiN gold-coated cantilevers (NT-MDT). Line Profiles were drawn across the capsules where the surface morphology indicated collapse of the capsule walls on top of each other and the obtained thickness was divided by 2.

1.9. Infrared Spectroscopy

IR spectra of EPL (free base), PGA (free acid) and EPL/PGA complex were prepared from polymer solutions of 1 mg mL^{-1} in absolute ethanol. IR spectra of EPL-HCl, PGA-Na and EPL-HCl/PGA-Na complex were prepared from polymer solutions of 1 mg mL^{-1} in water. EPL/PGA complex, EPL-HCl/PGA-Na complex, PGA and EPL-HCl were further acidified with 5 mM HCl. The polymer solutions were dried in an oven overnight. The dried materials were ground in a mortar and mixed with KBr at an approximate mass concentration of 2% w/w. Spectra were recorded by the KBr cake method under a nitrogen atmosphere. All samples were analyzed with a FTIR spectroscope (Bio-Rad FTS-3500ARX, Bio-Rad Laboratories, US).

The degree of ionization was calculated from peak areas at 3254 cm^{-1} ($N\text{-}H^+$ stretching vibrations) and 1405 cm^{-1} ($\text{-}CO_2^-$ symmetric stretching vibrations) for $–NH_3^+$ and $–CO_2^-$ respectively. The peak areas were determined from baselines drawn between spectra points 3150 cm^{-1} and 3325 cm^{-1} for $\text{-}NH_3^+$ and between spectra points 1375 cm^{-1} and 1450 cm^{-1} for $–CO_2^-$. EPL-HCl (apparent pK_a = 9) and PGA-Na (apparent pK_a = 5) prepared in ~pH 2 and ~pH 7 aqueous solutions respectively were referenced as completely dissociated compounds. Referencing EPL-HCl and PGA-Na as 100% ionization, the degrees of ionization for EPL/PGA and EPL-HCl/PGA-Na were calculated.

2. Supplementary Figures

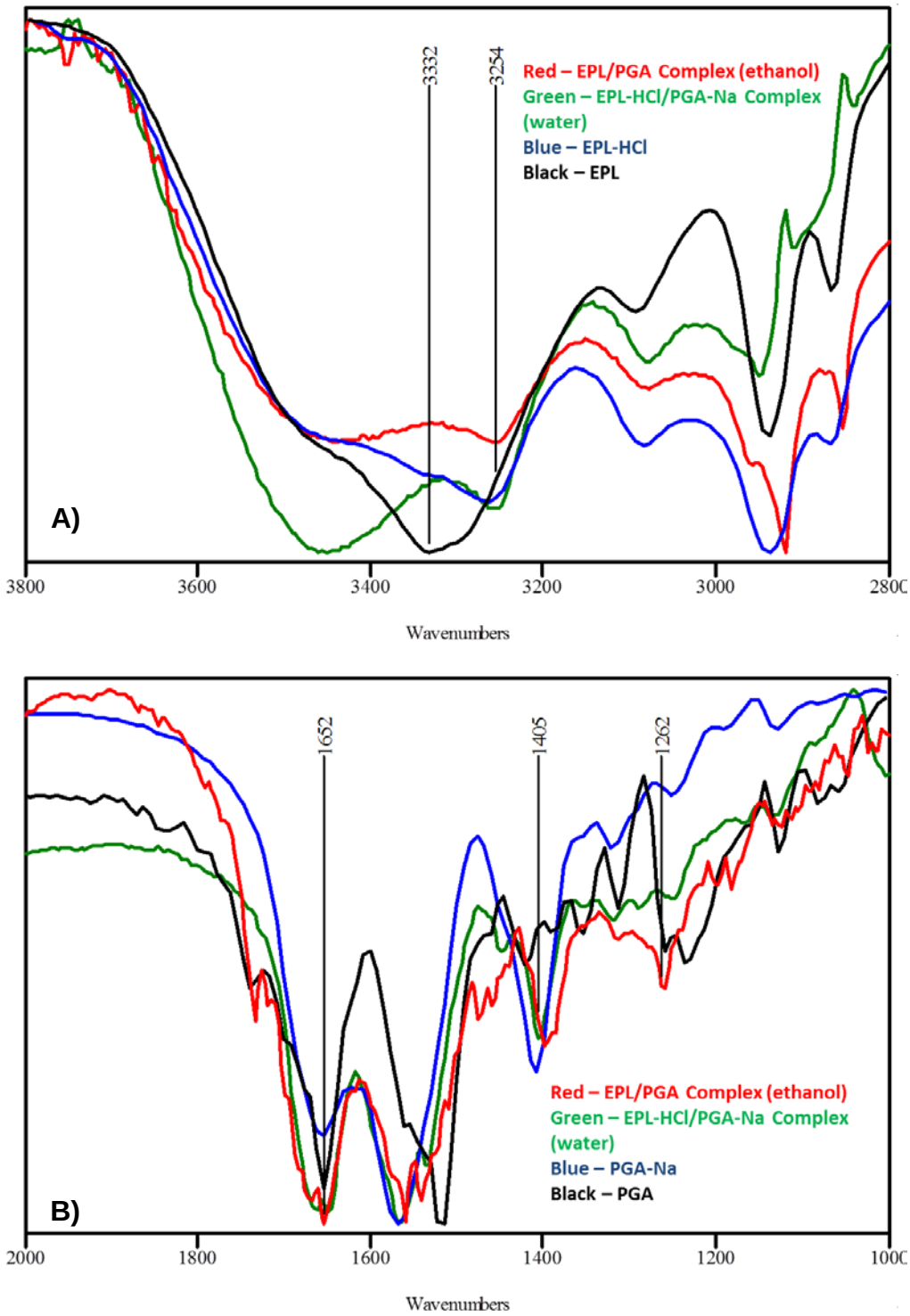


Figure S1. (A) IR spectra overlay of EPL/PGA and EPL-HCl/PGA-Na polyelectrolyte complexes, EPL and EPL-HCl. (B) IR spectra overlay of EPL/PGA and EPL-HCl/PGA-Na polyelectrolyte complexes, PGA and PGA-Na.

Table S1. Allocation of characteristic IR absorption peaks to functional groups.

ε-Polylysine (EPL)
(3450-3160) 3332: -NH_2, N-H stretching vibrations.
ε-Polylysine-hydrochloride (EPL-HCl)
(3350-3100) 3254: -NH_3^+, N-H^+ stretching vibrations. Shifted from 3339, broad band of medium intensity.
Polyglutamic acid (PGA)
1259 and 1236: -COOH, C-O stretching vibrations. A medium to strong absorption band producing a doublet.
1654: -COOH, C=O stretching vibrations. The frequency of C=O stretching vibrations for saturated aliphatic acids decreases in the presence of hydrogen bonding. (from 1740-1715 to 1680-1650). Seems to suggest presence of intramolecular hydrogen-bonded carboxylic acid with very strong absorption band.
Polyglutamic acid sodium salt (PGA-Na)
1406: -CO_2^- symmetric stretching vibrations. Gives rise to broad band of medium intensity and generally has two or three peaks.

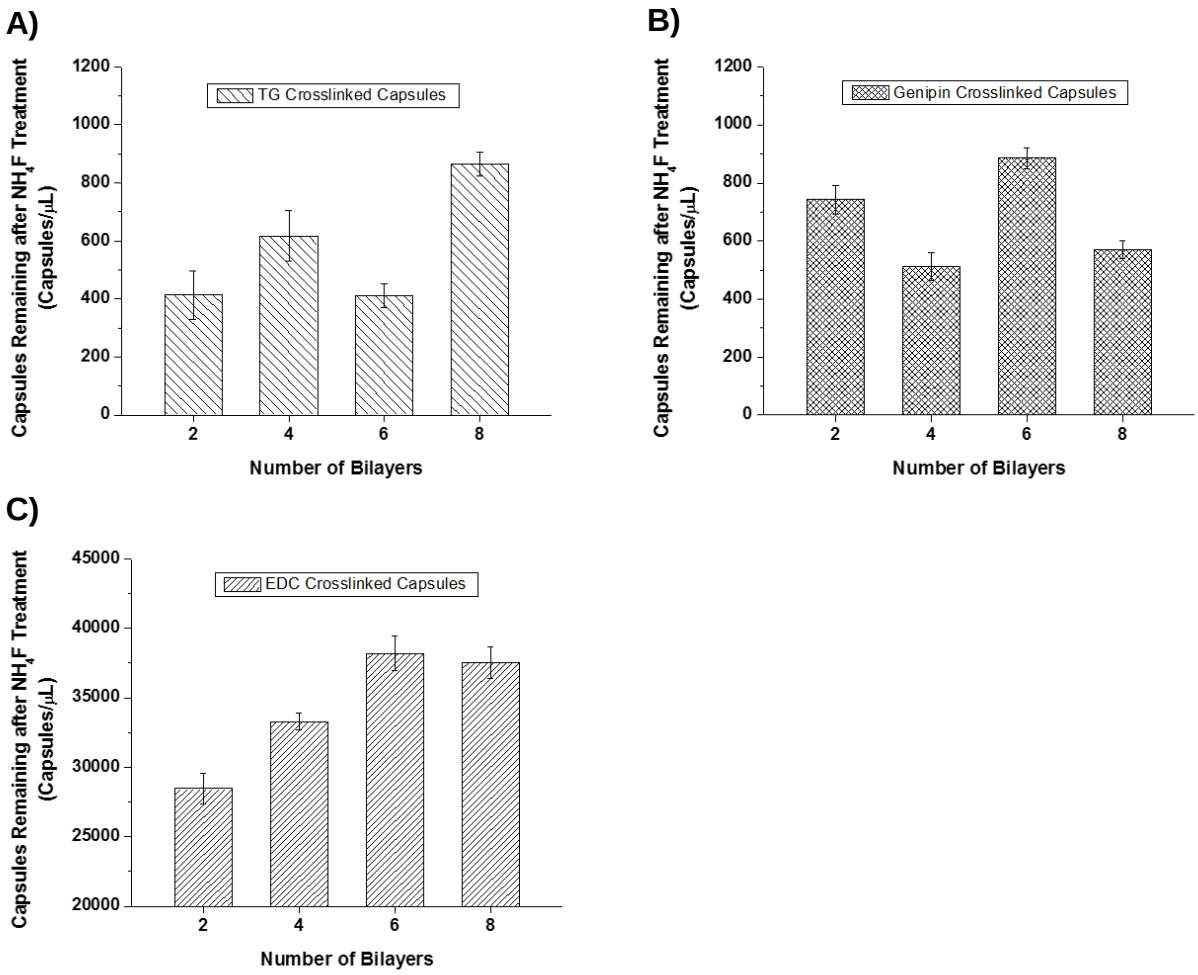
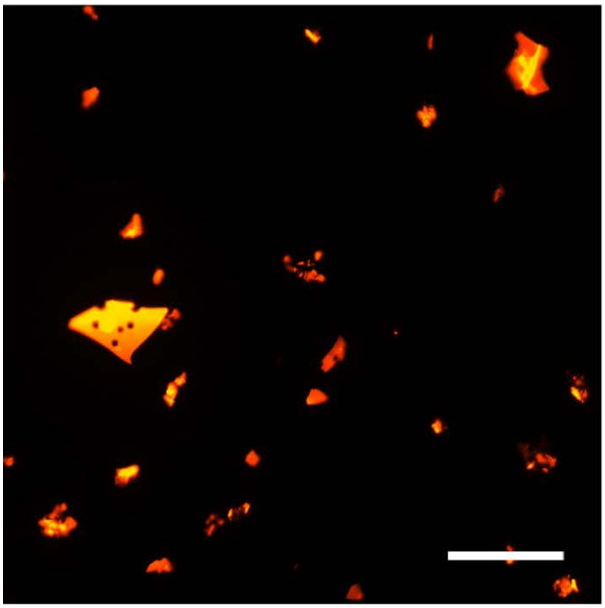
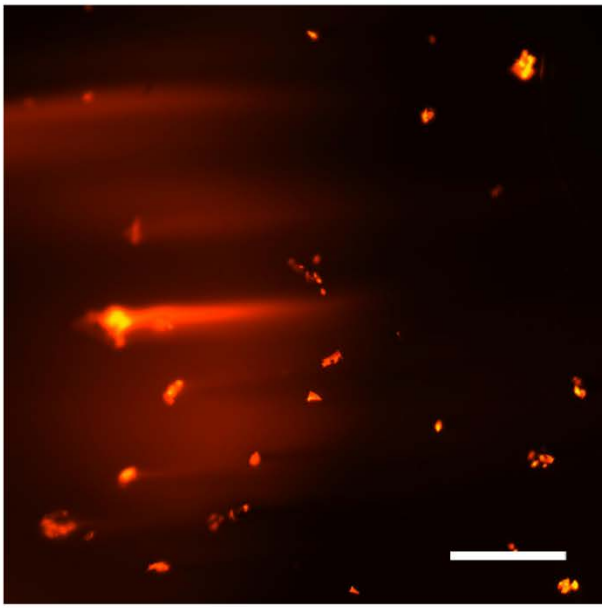


Figure S2. Concentration of capsules after NH_4F treatment for (A) TG-crosslinked (B) Genipin-crosslinked and (C) EDC-crosslinked microcapsules as a function of layer number. The results indicate a general increase in survival rate of capsules as more layers were coated.

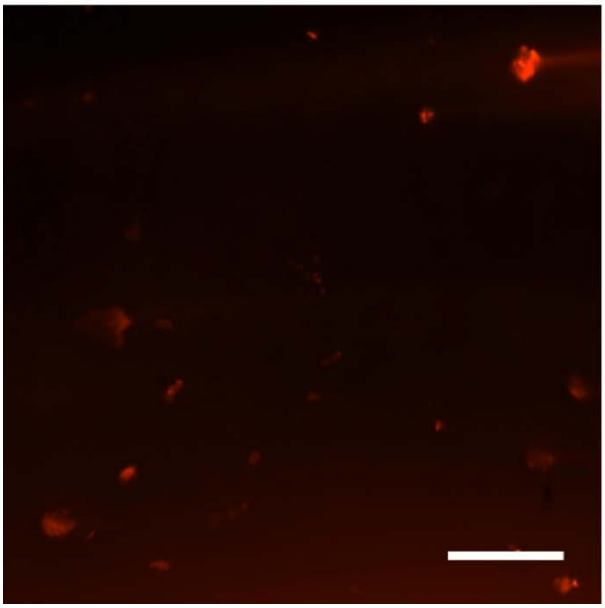
A) EDC-crosslinked particles before adding water



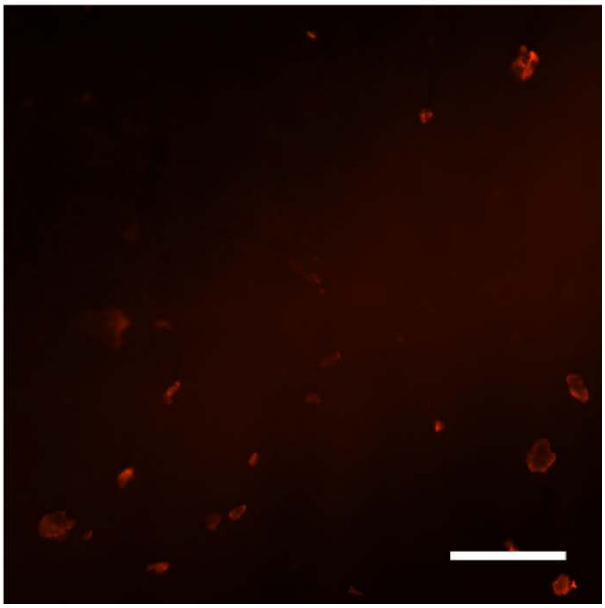
B) Initial addition of water and release of dextran-TRITC



C) Release of dextran-TRITC after ~10s



D) Remaining capsule material



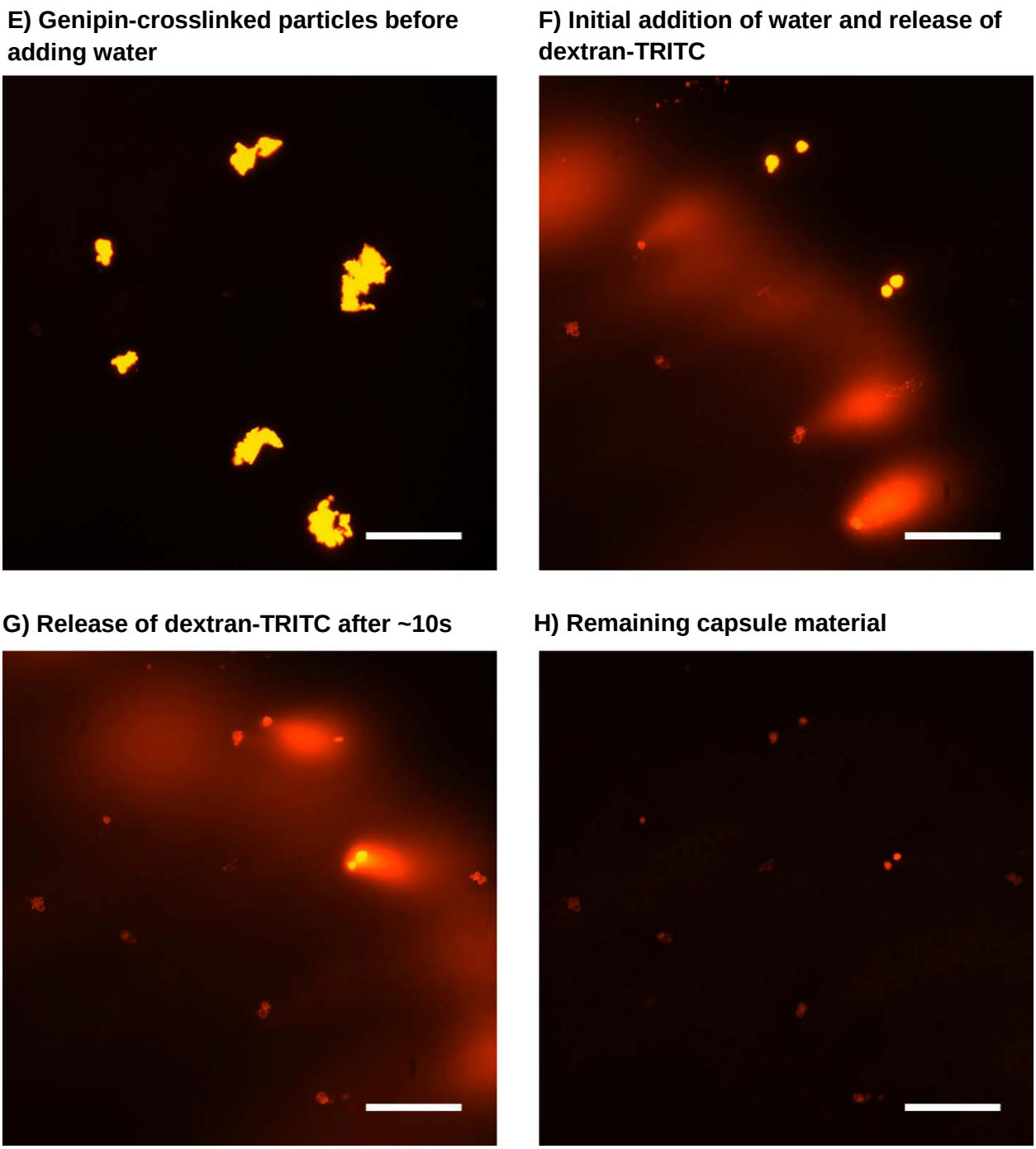


Figure S3. Sequential fluorescent images of EPL/PGA-coated sugar particles (maltotriose) crosslinked with (A-D) EDC or (E-H) Genipin on gradual addition of water. Dextran-TRITC (155 kDa) was encapsulated within the sugar matrix. On addition of water, encapsulated dextran was rapidly released for both (B and C) EDC-crosslinked and (F and G) Genipin-crosslinked capsules. However, Genipin-crosslinked capsules reduced significantly in size as compared to EDC-crosslinked capsules. After release of dextran, (D or H) fluorescent hollow capsules were observed due to adsorption of dextran-TRITC onto the remaining capsule material. All scale bars represent 100 µm.

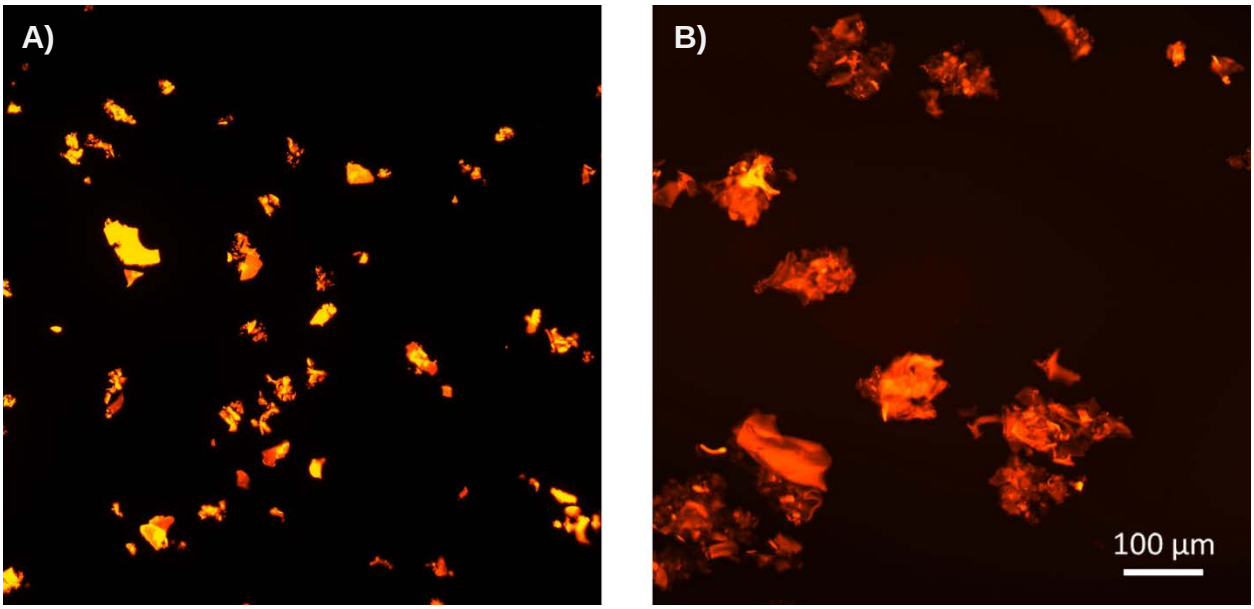


Figure S4. Fluorescent images of EPL/PGA-coated sugar particles (maltotriose) crosslinked with EDC, dispersed in (A) absolute ethanol and (B) ultrapure water. Dextran-TRITC (155 kDa) was encapsulated within the sugar matrix. After complete water re-dispersion, the capsules remained intact but a decrease in fluorescence was observed as a fraction of encapsulated dextran had leeched out.

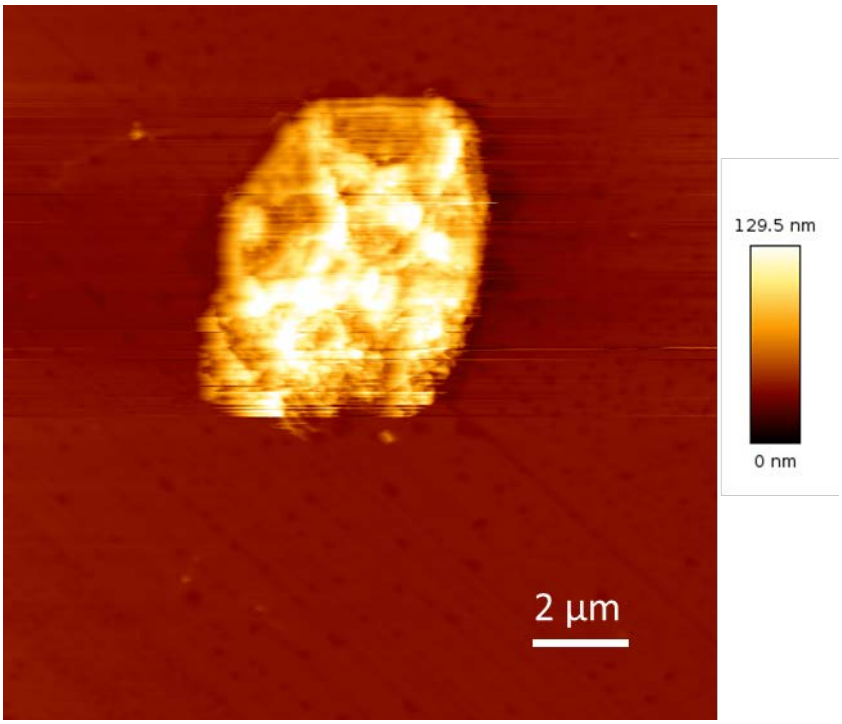


Figure S5. AFM image of a collapsed hollow 8-layer $(EPL/PGA)_4$ microcapsule. The thickness of the PEM capsule is ~30 nm after accounting the double capsule thickness in the AFM image.

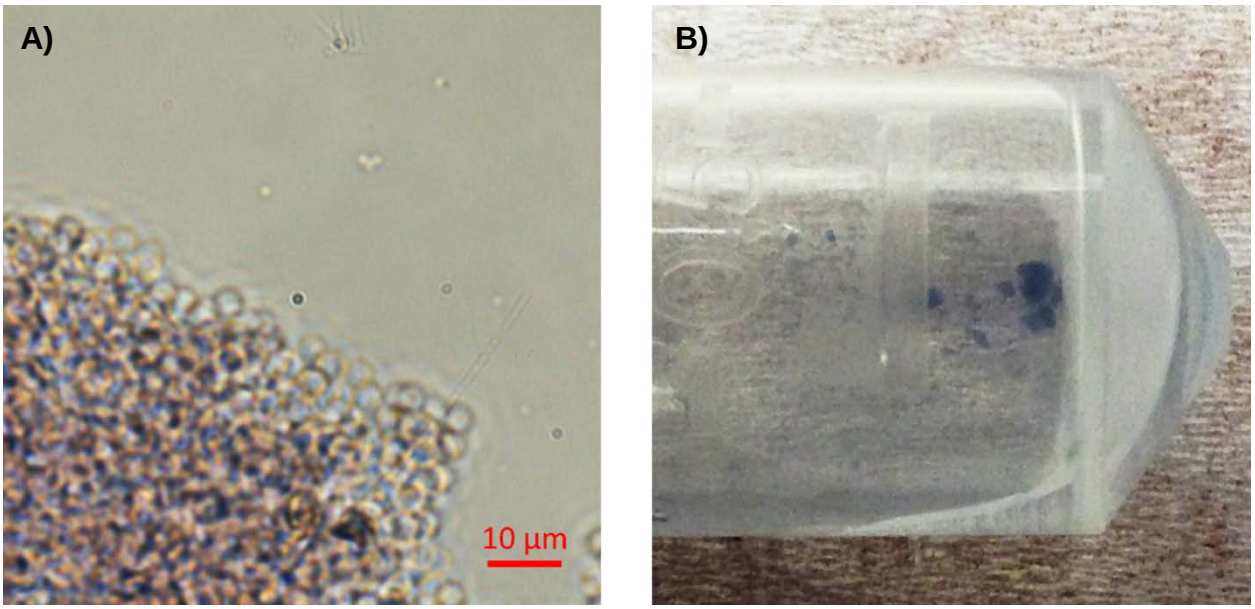


Figure S6. (A) Phase contrast and (B) macroscopic images of blue pigments formed by Genipin crosslinking of EPL/PGA microcapsules.