Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supplementary Information

Gastric-floating microcapsules provide controlled and sustained pelease of multiple cardiovascular drugs

Wei Li Lee[#], Peixin Wee[#], Chandra Nugraha and Say Chye Joachim Loo[*]

[#] Both authors contributed equally to this work.

School of Materials Science and Engineering

Nanyang Technological University

50 Nanyang Avenue, 639798 (Singapore)

Phone: +65 6790-4603

Fax: +65 6790-9081

Email: joachimloo@ntu.edu.sg

Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Experimental Section

Materials: Poly(DL-lactide-co-glycolide) 53/47 (PLGA 53/47, intrinsic viscosity (IV): 1.03, Purac), poly(DL-lactide-co-glycolide) 75/25 (PLGA 75/25, IV: 0.93, Purac), poly(L-lactide) (PLLA, IV: 2.38, Bio Invigor), poly(caprolactone) (PCL, molecular weight (MW): 10 kDa, Aldrich) and poly(vinyl alcohol) (PVA, MW: 30–70 kDa, Sigma–Aldrich) were used without further purification. Drugs (i.e. metoprolol tartrate salt, metformin HCl, fenofibrate), Cremophor® EL, Span 80 and n-hexane were purchased from Sigma-Aldrich. Dichloromethane (DCM), tetrahydrofuran (THF) and acetonitrile (ACN) were purchased from Tedia Company Inc. Olive oil (Pietro Coricelli) were purchased. Phosphate-buffered saline (PBS) at pH 7.4 was from OHME, Singapore. All drugs and solvents were used as received, unless otherwise noted. The marketed tablets used were metoprolol tartrate (Lopressor®), metformin HCl (from East Pharmaceutical) and fenofibrate (Lipanthyl®). The simulated gastric fluid (SGF) (pH 1) was prepared by adding 0.1 M HCl solution (Merck) and 0.02% (w/v) Tween 20 (Tokyo Kasei). The simulated intestinal fluid (SIF) contained pH 6.8 phosphate buffer and 0.5% (w/v) Tween 80 (Sigma-Aldrich).

Fabrication of drug-loaded microparticles: For the fabrication of fenofibrate-loaded PCL particles, fenofibrate (60mg, 30% w/w) was first added to the polymer solution (7% w/v, 0.2 g of PCL dissolved in 2.85 mL of DCM) and the resultant solution was subsequently emulsified in 50 mL PVA aqueous solution (4% w/v) under overhead stirring at 2000 rpm for 4 min. The emulsion was then transferred quickly to rotary evaporator at room temperature (25 ^{o}C) for

solvent evaporation. The hardened particles were centrifuged, rinsed with deionized water and lyophilized. Finally, free fenofibrate were washed away with n-hexane and the particles were dried in vacuum oven.

The metoprolol tartrate-loaded PLGA 75/25 particles were fabricated using Buchi Mini Spray Dryer B-290 with a 2-fluid nozzle of size. A concentration of 2 % (w/v) of PLGA 75/25 was dissolved in chloroform along with metoprolol tartrate (30% w/w). Inlet temperature was set to 50°C at a feed rate of 2.5 mL/min, and an aspirator rate of 100 %. The particles were collected from the collection vessel and lower part of the cyclone. After which, the particles were reconstituted and suspended in 10 mL of PVA aqueous solution (4% w/v), followed by centrifugation, decantation and freeze-drying.

Encapsulation of drug-loaded microparticles in microcapsule: For the encapsulation step, the pre-fabricated particles (0.09 g of fenofibrate-loaded particles and 0.07 g of metoprolol tartrate-loaded particles) were first suspended in 1 mL of PVA aqueous solution (0.1% w/v) containing 0.1 g of metformin HCl (or specified otherwise in the text). The particles/water suspension was then introduced dropwise into the PLGA solution (5% w/v, 0.3 g of PLGA 53/47 dissolved in 6 mL of DCM), followed by the addition of olive oil (0.01 mL) under magnetic stirring. The resultant mixture was added to 50 mL of PVA aqueous solution (0.25%w/v) containing 1 mL of DCM and emulsified under overhead stirring (250 rpm) for 4 min at room temperature (25 ^{o}C). After forming the emulsion droplets, the emulsion was quickly transferred to rotary evaporator with the addition of PVA solution (150 mL) to solidify the microcapsules. The microcapsules

produced were centrifuged, rinsed with deionized water, lyophilized and stored in a desiccator. PLLA and PLLA-PCL (75wt%:25wt%) microcapsules were similarly prepared.

For comparison with the modified encapsulation method as described above, a reference microcapsule (capsule R) loaded with particles (without the addition of 0.1g free metformin HCl) was fabricated through a conventional method in the previous study,[1] in which a higher amount of PVA solution (250 mL, without DCM) was used during emulsification and no rotary evaporator was employed to harden the capsules.

Morphological analysis: The exterior and internal morphologies of the microparticles and microcapsules were viewed under scanning electron microscopy (SEM, JEOL JSM-6360A) at 5 kV. Prior to analysis, samples were first mounted onto a metal stub and cross-sectioned approximately at the centre line using a razor blade. Samples were then coated with gold using a sputter coater (SPI-Module). Measurement of particle/capsule size (in diameter) was performed on the SEM images using the ImageJ software.

Determination of actual drug loading: For the determination of actual loading of water-soluble metformin HCl and metoprolol tartrate, 10 mg of microparticles or microcapsules (n=3) were first dissolved in 1 mL of DCM. Extraction of the drug was then achieved with the use of 10 mL PBS. Hydrophilic metformin HCl preferentially partitions into PBS, whereas the partition coefficient of metoprolol tartrate between DCM and PBS was determined experimentally by

preparing a series of concentrations and was found to be about 2. The drug content in aqueous solution was analyzed using UV-Vis spectrophotometer (Shimadzu UV-250), at the wavelength of 233 nm and 274 nm for metformin HCl and metoprolol tartrate, respectively. For the determination of highly hydrophobic fenofibrate loading, after dissolution of microparticles or microcapsules, 5 mL n-hexane was added to precipitate polymers and other drugs (if any). The mixture was centrifuged and the supernatant was dried. A 10 mL volume of SGF containing 2% w/v Cremophor EL was then added to dissolve the solid fenofibrate for UV-Vis analysis at 292 nm.

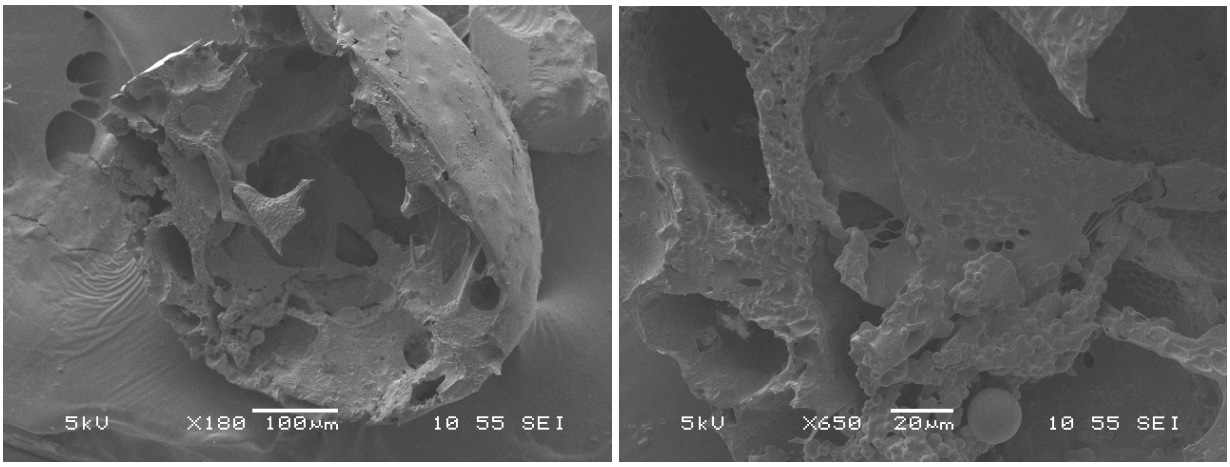
In vitro buoyancy test: The buoyancy of the microcapsules was tested through a visual observation method.[2-4] For each sample of microcapsules, 50 individual microcapsules, in triplicate, were placed into 20 mL SGF filled in the vials (20 mL volume capped bottle). The test bottles were incubated in a water bath at 37 ^{o}C under magnetic stirring at 250 rpm for 24 h.[2] At each pre-determined time, the number of floating microcapsules was counted visually. The percentage of floating microcapsules (an indication of buoyancy) was calculated according to the ratio of the number of floating microcapsules to the total number of microcapsules.

Drug release study: The in vitro metformin HCl and metoprolol tartrate release studies were carried out in SGF and SIF. For poorly water-soluble fenofibrate, the release test was conducted in the medium with the addition of 2% w/v Cremophor EL to maintain a sink condition.[5] Samples (20 mg) were placed, in triplicate, in vials containing 20 mL dissolution medium and were maintained at 37 ^{o}C with a magnetic rotation speed of 250 rpm. At prescribed time intervals,

1 mL of medium from each vial was removed and replaced with fresh medium. The drug content was analyzed using UV-Vis spectrophotometer ($\lambda_{\text{metformin HCl}}$ = 233 nm, $\lambda_{\text{metoprolol tartrate}}$ = 274 nm, $\lambda_{\text{fenofibrate}}$ = 292 nm).

Statistical analysis: Data from different sets of samples were compared by unpaired Student's t-test and the one-way ANOVA analysis coupled with Tukey's multiple comparison tests. Differences were considered statistically significant when $P \leq 0.05$.

Morphological analysis of reference microcapsule (capsule R)



(a) (b)

Figure S1. SEM images of cross-sectional view and close-up view of a reference microcapsule (capsule R). It was found that the encapsulated particles were agglomerated and embedded within the capsule shell.

Formation efficiency of the microcapsules

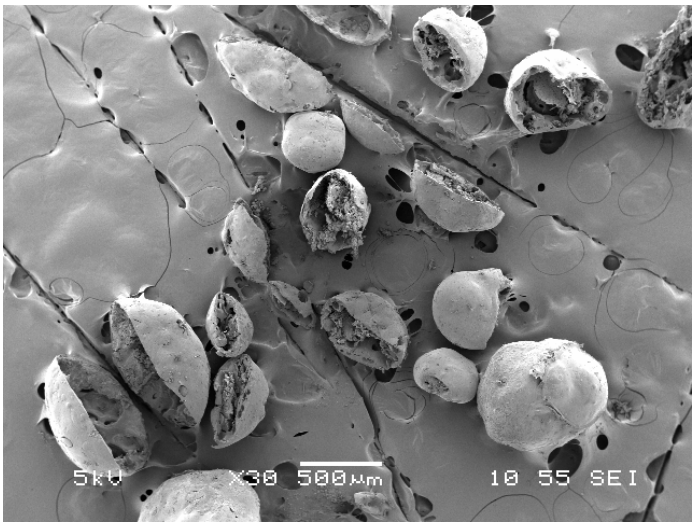


Figure S2. SEM image of cross-sectional view of many olive-oil containing microcapsules is shown to demonstrate homogeneity of the capsule morphology.

Buoyancy Test

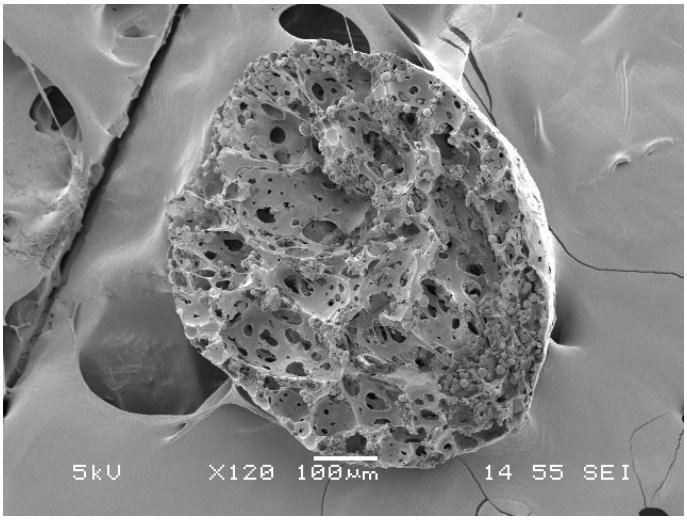
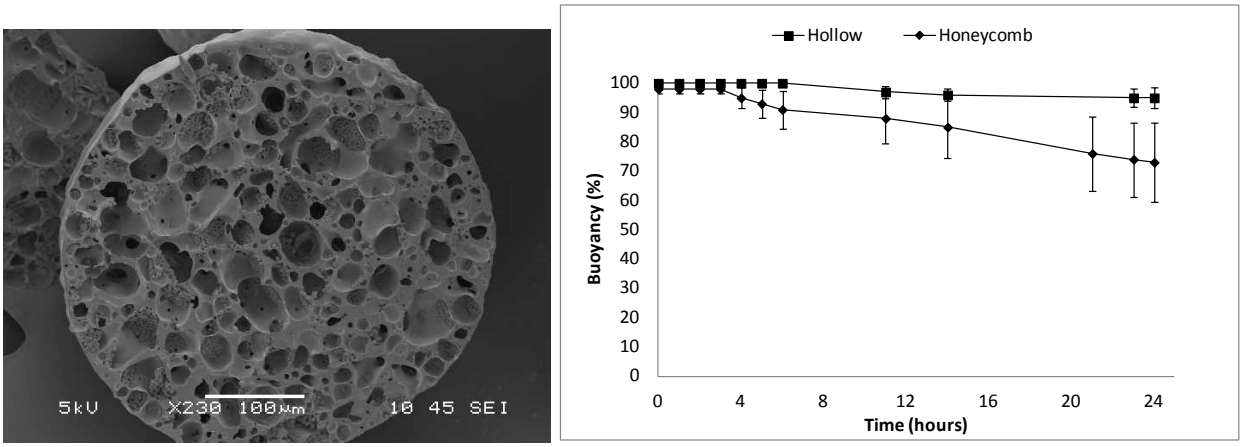
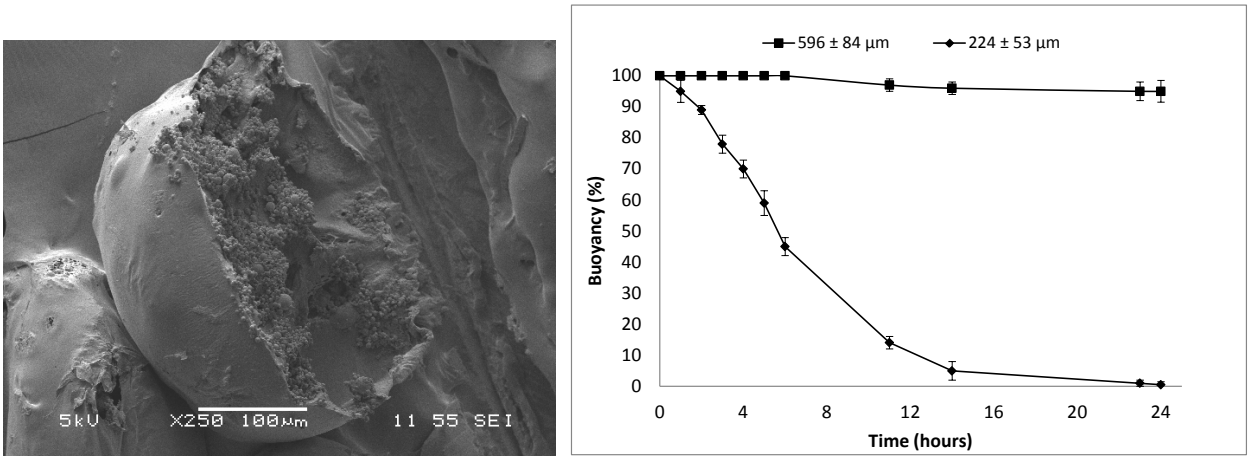


Figure S3. SEM image of cross-sectional view of a non-oil-loaded microcapsule, whereby particles were located within the hollow cavity.

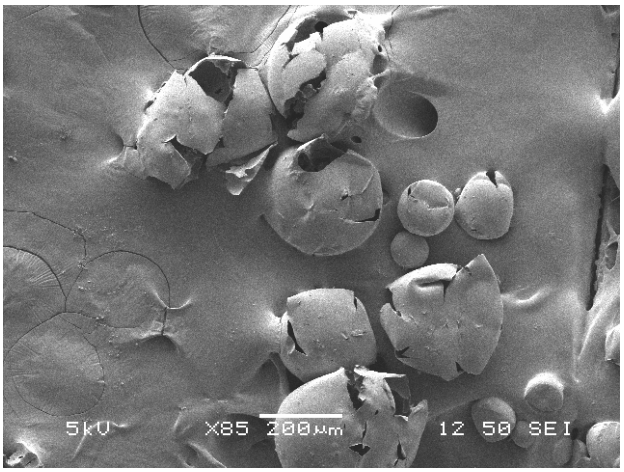


(a) (b)

Figure S4. (a) SEM image of cross-sectional view of an oil-loaded microcapsule with honeycomb structure. (b) Percent buoyancy vs time profiles of the present floating formulation with hollow structure compared to the honeycomb-structured microcapsules (oil-loaded). Higher density due to the decrease in cavity volume, as a result of honeycomb structure, reduced the buoyancy.



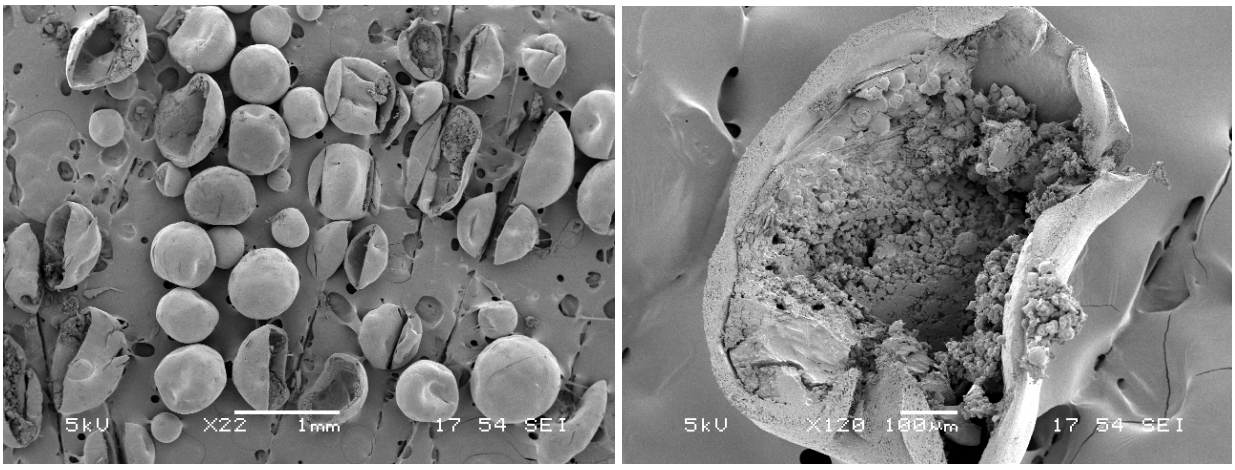
(a) (b)



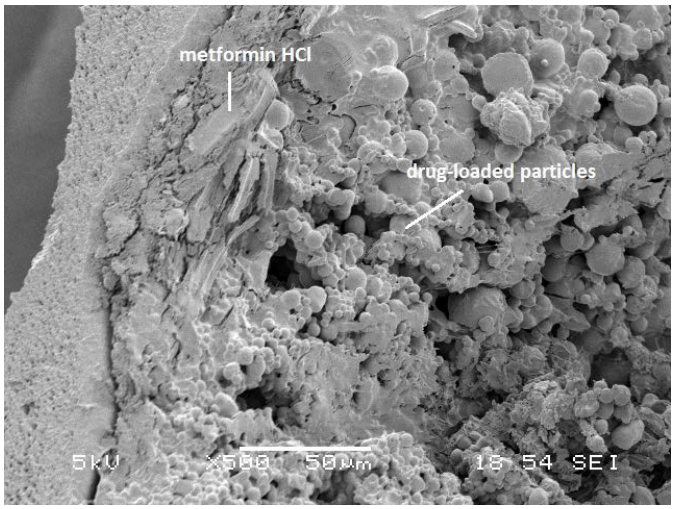
(c)

Figure S5. (a) SEM image of cross-sectional view of an oil-loaded microcapsule of size 224 ± 53 µm. (b) Percent buoyancy vs time profiles of the present floating formulation (596 ± 84 µm) compared with the smaller-sized microcapsules (224 ± 53 µm). (c) SEM image of cracked microcapsules after 24 h in vitro.

Drug Release

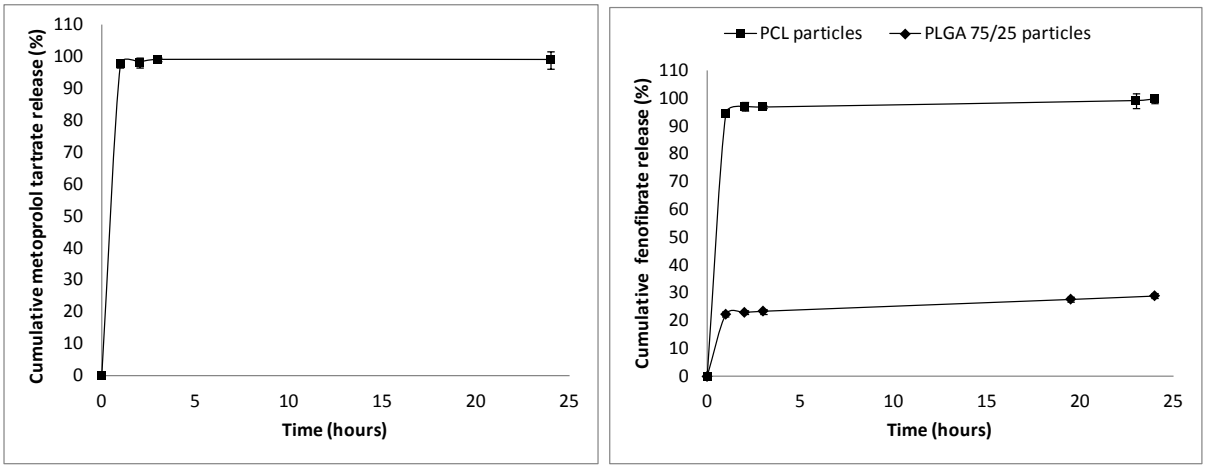


(a) (b)



(c)

Figure S6. SEM images of (a) cross-sectional view of the microcapsules and (b, c) close-up view of a microcapsule, whereby the drug-loaded particles and free metformin HCl were attached onto the inner wall of the microcapsule.



(a) (b)

Figure S7. Release profiles of (a) metoprolol tartrate from PLGA 75/25 particles and (b) fenofibrate from PCL and PLGA 75/25 particles.

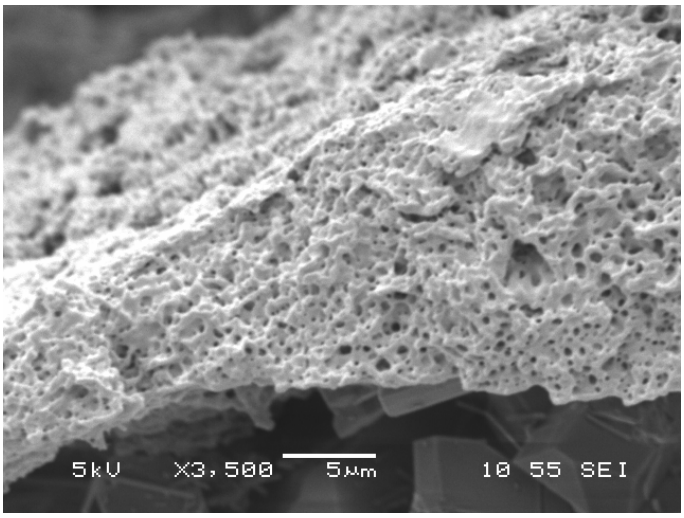
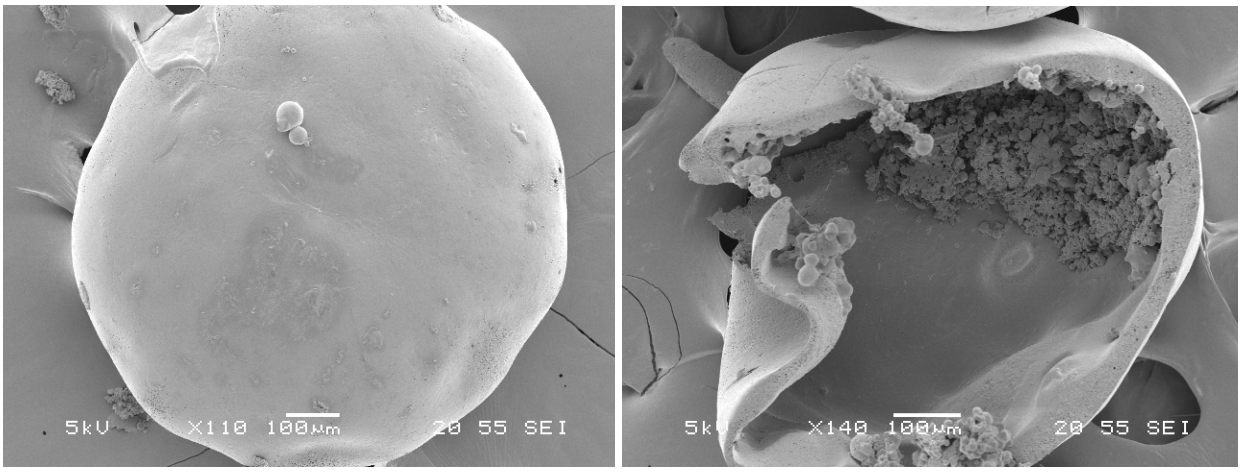
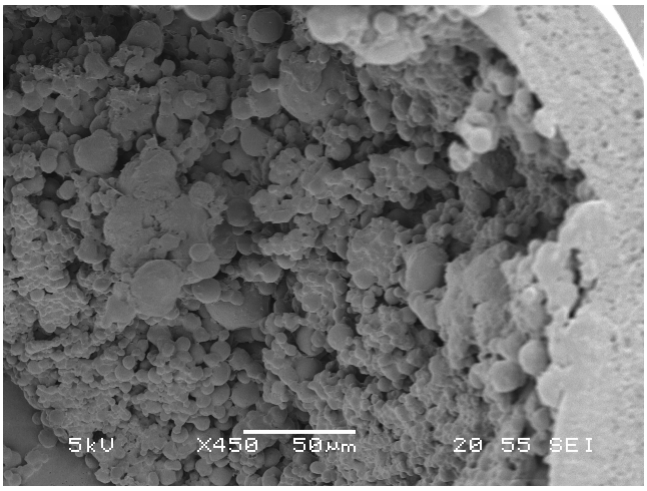


Figure S8. SEM image of cross-sectional view of PLLA capsule shell containing PCL nanoparticulates after dissolution with THF. The dissolution test is based on the solubility differences of the polymers in THF (*i.e.* PCL is soluble in THF; PLLA is not). After THF dissolution, only the PLLA shell remained; the nanopores were identified as PCL.



(a) (b)



(c)

Figure S9. SEM images of the microcapsules after 24 h *in vitro*. (a) Surface view, (b) cross-sectional view, and (c) close-up view.

References

1 Y. L. Khung, W. L. Lee, K. L. Chui, Y. Liu, M. P. Lim, C. L. Huang and S. C. J. Loo, *Adv. Healthcare Mater.*, 2012, **1**, 159.

2 Y. D. Tang, S. S. Venkatraman, F. Y. C. Boey and L. W. Wang, *Int. J. Pharm.*, 2007, **336**, 159.

3 Y. Liu, J. Zhang, Y. Gao and J. Zhu, *Int. J. Pharm.*, 2011, **413**, 103.

4 N. Ma, L. Xu, Q. Wang, X. Zhang, W. Zhang, Y. Li, L. Jin and S. Li, *Int. J. Pharm.*, 2008, **358**, 82.

5 W. He, Y. Tan, Z. Tian, L. Chen, F. Hu and W. Wu, *Int. J. Nanomedicine*, 2011, **6**, 521.