

Supplementary Information for

Spatially Directed Vesicle Capture in the Ordered Pores of Breath-Figure Polymer Films

Jaspreet Arora, Thiruselvam Ponnusamy, Rubo Zheng, Pradeep Venkataraman, Srinivasa R. Raghavan, Diane Blake and Vijay John

Experimental Details:

Materials: Methylene chloride (organic solvent - ACS grade), Acetic acid (glacial) were obtained from Fisher Scientific, USA. Chitosan medium molecular weight, sodium cyanoborohydride, n-dodecyl aldehyde, carbon disulphide were obtained from Sigma Aldrich. Polystyrene (monocarboxy terminated, approx. M.W. 50,000) was obtained from Scientific Polymers INC., NY. "DiP" the fluorescent lipid 1, 1'-dioctadecyl-3,3,3,3'-tetramethylindocarbocyanine perchlorate was obtained from Invitrogen corporation, CA. L- α -phosphatidylcholine (soy PC 95%) was obtained from Avanti polar lipids. All chemicals were used as received, without further purification.

Synthesis of Hydrophobically Modified Chitosan (hm-chitosan): N-dodecyl tails were attached to low molecular weight chitosan by reacting its amine groups with n-dodecyl aldehyde. Briefly, Chitosan was dissolved in an acidic solution of acetic acid. A solution of the aldehyde in ethanol was then added followed by the addition of sodium cyanoborohydride such that the molar ratio of the aldehyde to the chitosan monomer was 2.5%. The pH was raised to 9 which led to the precipitation of the hm-chitosan. The obtained precipitate was purified by washing with ethanol followed by deionized water. Finally the hm-chitosan was dissolved in a solution of 1% acetic acid.

Fluorescent labelling of hm-chitosan and Chitosan: A solution of chitosan or hm-chitosan in dilute hydrochloric acid was cast in a petri dish and allowed to dry overnight. This film was then neutralized with sodium hydroxide. Then this film was labeled by reacting it with a solution of NHS-fluorescein in ethanol and dimethylformamide in the presence of phosphate buffer saline (PBS) for 30 minutes. After the reaction, the film was rinsed with PBS and lyophilized.

Fabricating the breath figures via a nebulization based process: Thin breath figures films were prepared using a spin coating procedure (spin coater model - WS-400-6NPP-LITE, Laurell Technologies Corporation, North Wales, PA). The polystyrene was dissolved in carbondisulphide to obtain a polymer concentration of 4% (w/v). A 22x22 mm glass coverslip, used as the substrate, was placed on the fragment adapter inside the spin coating chamber. The chamber's fragment adapter was kept under vacuum to hold the substrate while it spun. Through an orifice located on the top of the spin coater, an aerosol mist carrying hm-chitosan/chitosan was introduced at flow rates between 35-40 ml/min. The mist was administered at a room temperature of ~ 25°C

with the mist velocity between 0.087 – 0.099 cm/s normal to the cast polymer solution. The aerosol mist was produced using a nebulizer. The reservoir of the nebulizer was filled with hm-chitosan/chitosan solution and when air at a certain pressure was passed through it, a uniform aerosol mist of water droplets containing hm-chitosan/chitosan was generated. Polymer solution (0.4 ml) was dropped onto the substrate and spun to 2500 rpm for 30 seconds. During the spin coating process, the solvent was allowed to evaporate in the presence of the introduced aerosol mist in order to obtain a porous and opaque film. The films were rigorously rinsed with DI water and 1% acetic acid and further dried at room temperature prior to further analysis and use.

Liposome fabrication: The liposomes were prepared by thin-film evaporation. The phospholipids PC and DiI were mixed in the ratio of about 100:1 (w/w). Phospholipids (0.1g) was dissolved in chloroform and methanol (10 ml) mixture (2:1 v/v). The solution was evaporated inside a round bottom flask using a rotary evaporator (Buchi R-205) for 2.5 hours to obtain a dry lipid film. The lipid film was then hydrated for 1 hour with DI water (5 ml) at 50°C and 125 rpm. The hydrated solution was extruded 11 times through a 400 nm polycarbonate membrane at 50°C followed by the use of a 100 nm membrane (Whatman)

Vesicle capture: Once the polystyrene breath figure films with or without hm-chitosan were fabricated, they were rinsed with ethanol and incubated in a solution of liposomes for 45 minutes. Following incubation, they were rinsed thoroughly with DI water to remove untethered vesicles.

Vesicle release using α -cyclodextrin: α -CD was used to release the captured vesicles from the pores of the breath figures. Once the red fluorescently tagged liposomes were captured in the breath figure films, the films were placed in a small petri dish and a solution of 0.3% (w/v) α -CD (2ml) in DI water was added to the petri dish. At specified time intervals, an aliquot of the solution (150 μ l) was drawn from the petri dish, analyzed for fluorescence using a fluorescence spectrometer (Photon Technology International, New Jersey) and then placed back in the petri dish.

Characterization Methods: The surface morphology of the breath figure polystyrene films containing hm-chitosan/chitosan was characterized with a field emission scanning electron microscope (SEM) (Hitachi S-4800). Prior to the SEM imaging, all the samples were coated with a thin gold layer through sputtering (Polaron SEM coating system) set at 20 mA and 2.4 kV for a duration of 90 sec. The fluorescence of the red fluorescent labeled liposomes and the green fluorescently labeled chitosan were imaged using the 63x lens of a Zeiss LSM confocal microscope system (Carl Zeiss International, Germany).

Figure S1

The aerosol based process was tested to see the breath figure formation. Since hydrophobically modified chitosan (hm-chitosan) was dissolved in 1% acetic acid, an aerosol mist containing 1% acetic acid was created using a nebulizer and used to fabricate breath figures. These breath figures were compared with the ones made when a mist of hm-chitosan was used by scanning electron microscopy (SEM). As seen in FigureS1, the surface morphology of the breath figures created by employing a mist of acetic acid and hm-chitosan is similar with identical pore size.

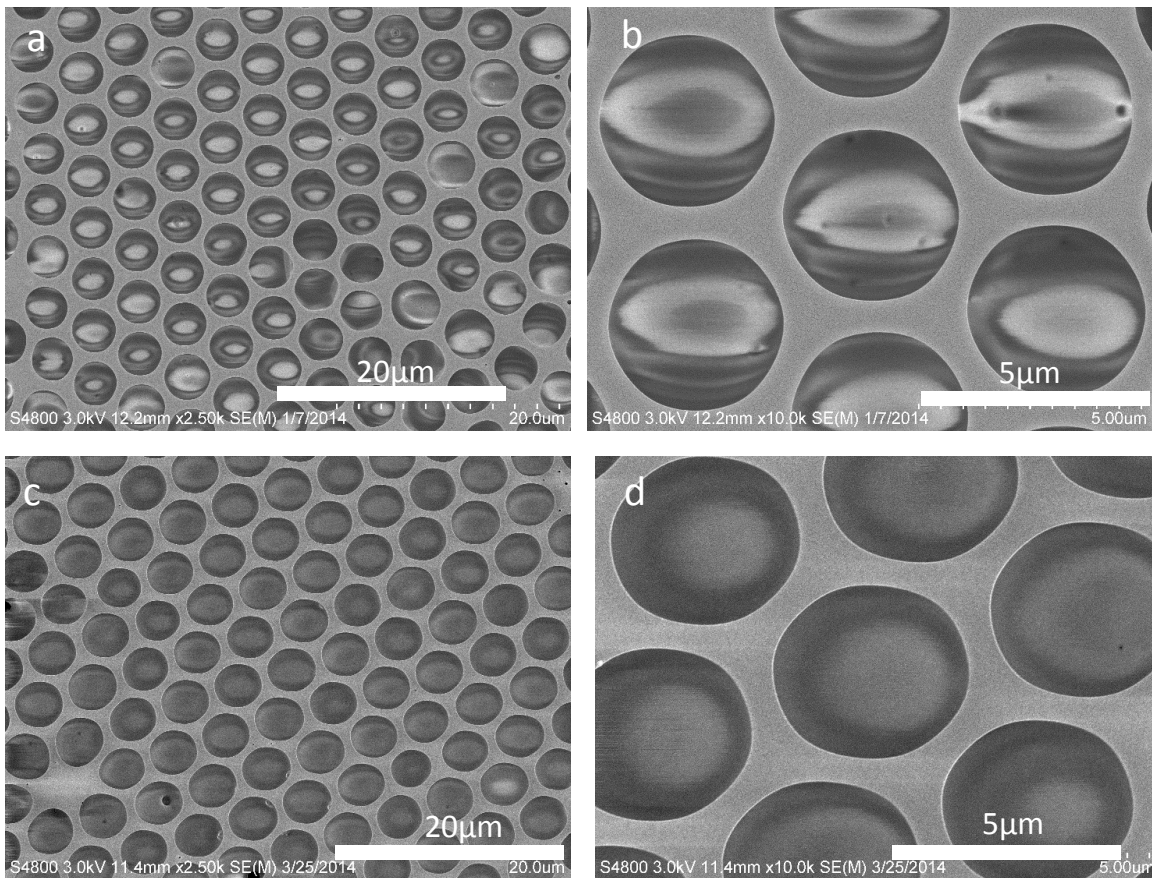


Figure S1: SEM images of the breath figures when a mist of 1%(v/v) acetic acid was employed a) and b) and 1%(w/v) of hm-chitosan was employed c) and d).

Figure S2:

To detect the presence of hm-chitosan in the pores of the breath figures, Fluorescein labeled hm-chitosan was used. Once it was deposited in the pores, confocal laser microscopy was used to detect it along the depth of the breath figures. In Figure S2, images were taken at an interval of 500nm. These images were used to compile Figure 1d (top).

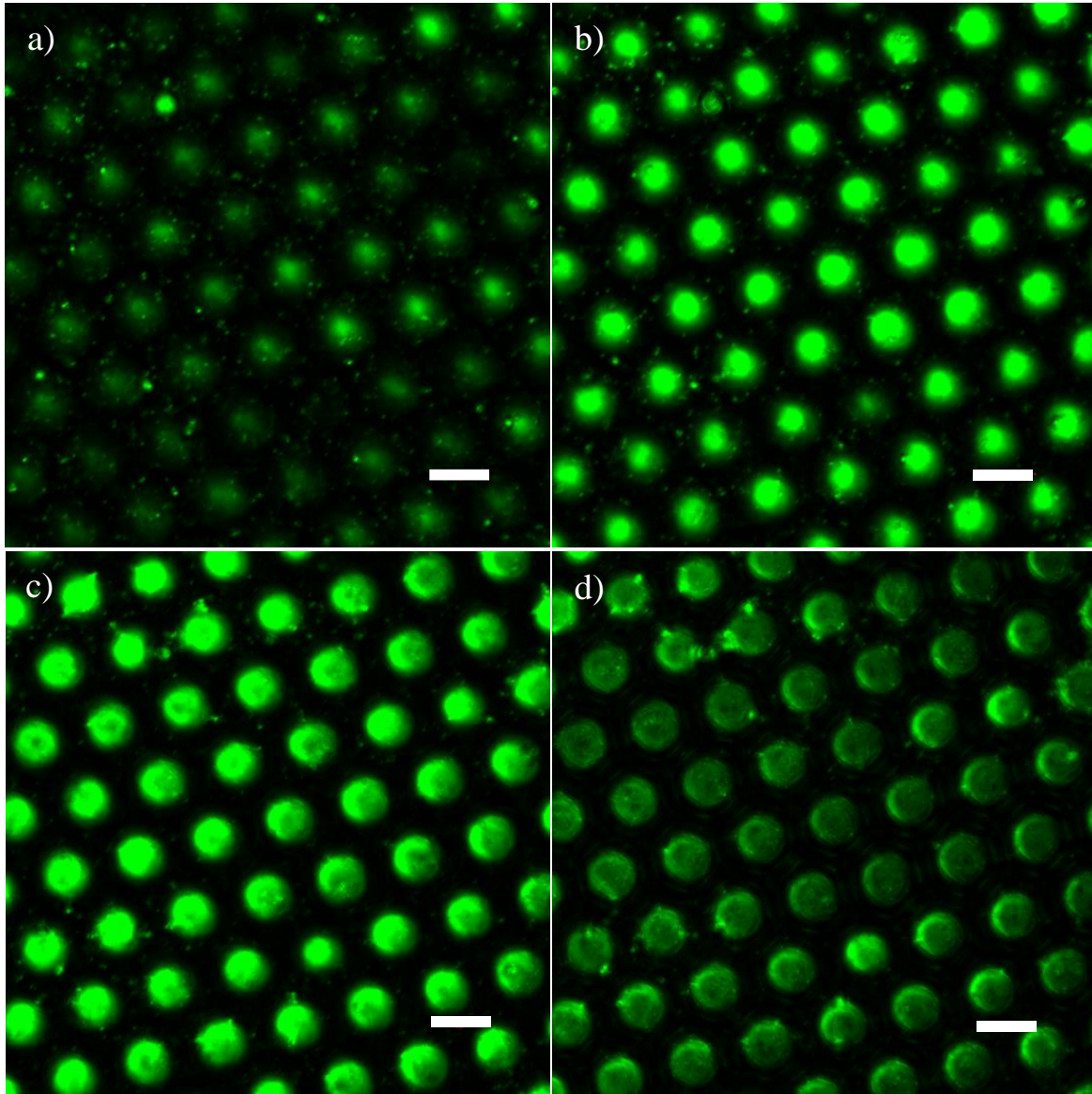


Figure S2: Fluorescein labeled hm-chitosan was employed to fabricate polystyrene breath figures. a) through d) are z-stack confocal microscopic images of the same xy frame of such a sample film. The z-distance between each of the frames is 0.5 μm . Scale bar in all images is 5 μm .

Figure S3:

To test the vesicle capture ability of hm-chitosan, fluorescein labeled hm-chitosan was used to fabricate breath figures. Then, red fluorescently tagged liposomes were incubated on top of these breath figures. The film was then rinsed thoroughly with distilled water to remove the unbound liposomes and analyzed using confocal laser microscopy. The green signal of the hm-chitosan and red signal from the liposomes is simultaneously detected in FigureS3 along the depth of the breath figure film at intervals of 500nm.

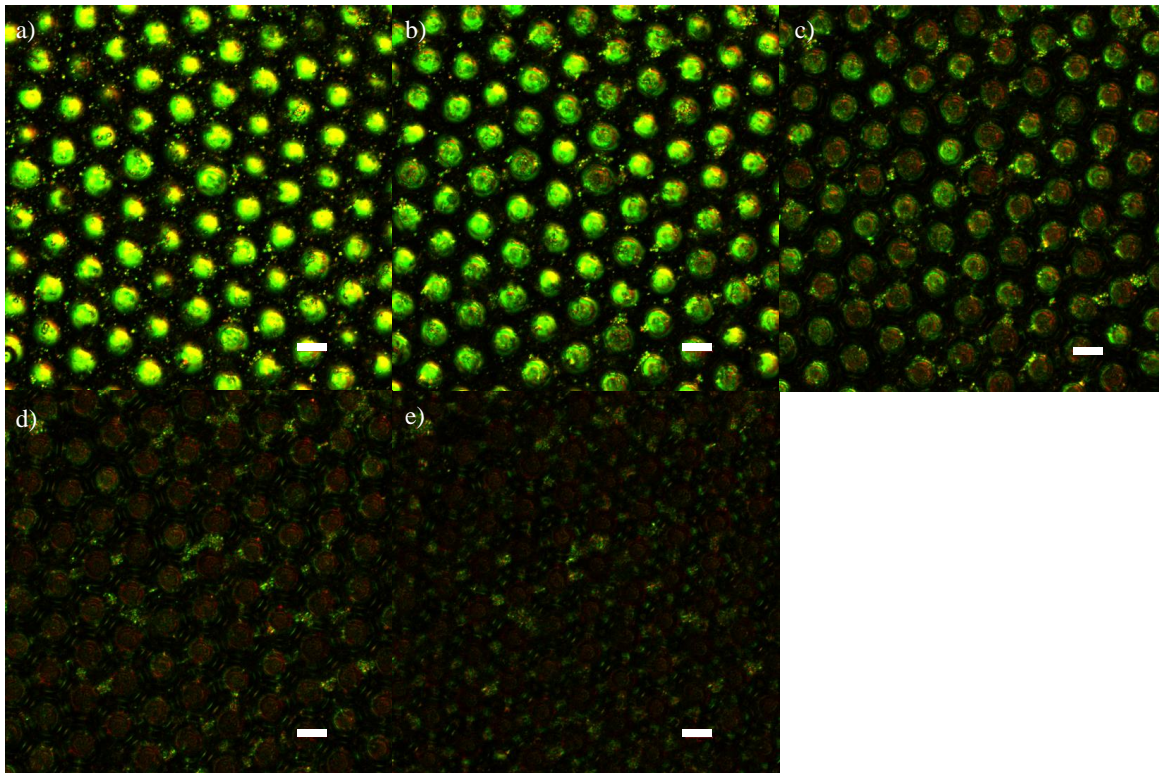


Figure S3: Green fluorescently labeled hm-chitosan was aerosolized to form polystyrene breath figures and red fluorescently labeled liposomes were incubated on these. a) through e) are multi-track z-stack confocal microscopic images of the same xy frame of such a sample film. The green and red fl. Signals are co-localized as in Figure2. The z-distance between each of the frames is 0.5 μm . The scale bar in all images is 5 μm . These images were used to compile Figure2e.

Figure S4:

PC liposomes were analyzed using Cryo-TEM and DLS. From the cryo-TEM image on the left it is clear that the liposomes are mostly unilamellar and spherical. The average diameter of the liposomes was estimated to be 94.27nm with a standard deviation of 22.13nm by Image J analysis. The DLS results of the same liposomes suggest an effective diameter of 118.94nm with a standard deviation of 1.55nm and polydispersity of 0.104. The intensity v/s diameter and the number v/s diameter plots above are in agreement with the value of polydispersity.. The vesicles were prepared via thin film hydration and then they were extruded through a polycarbonate membrane of 100nm to control their size. Extrusion decreases the polydispersity of liposomes to a large extent

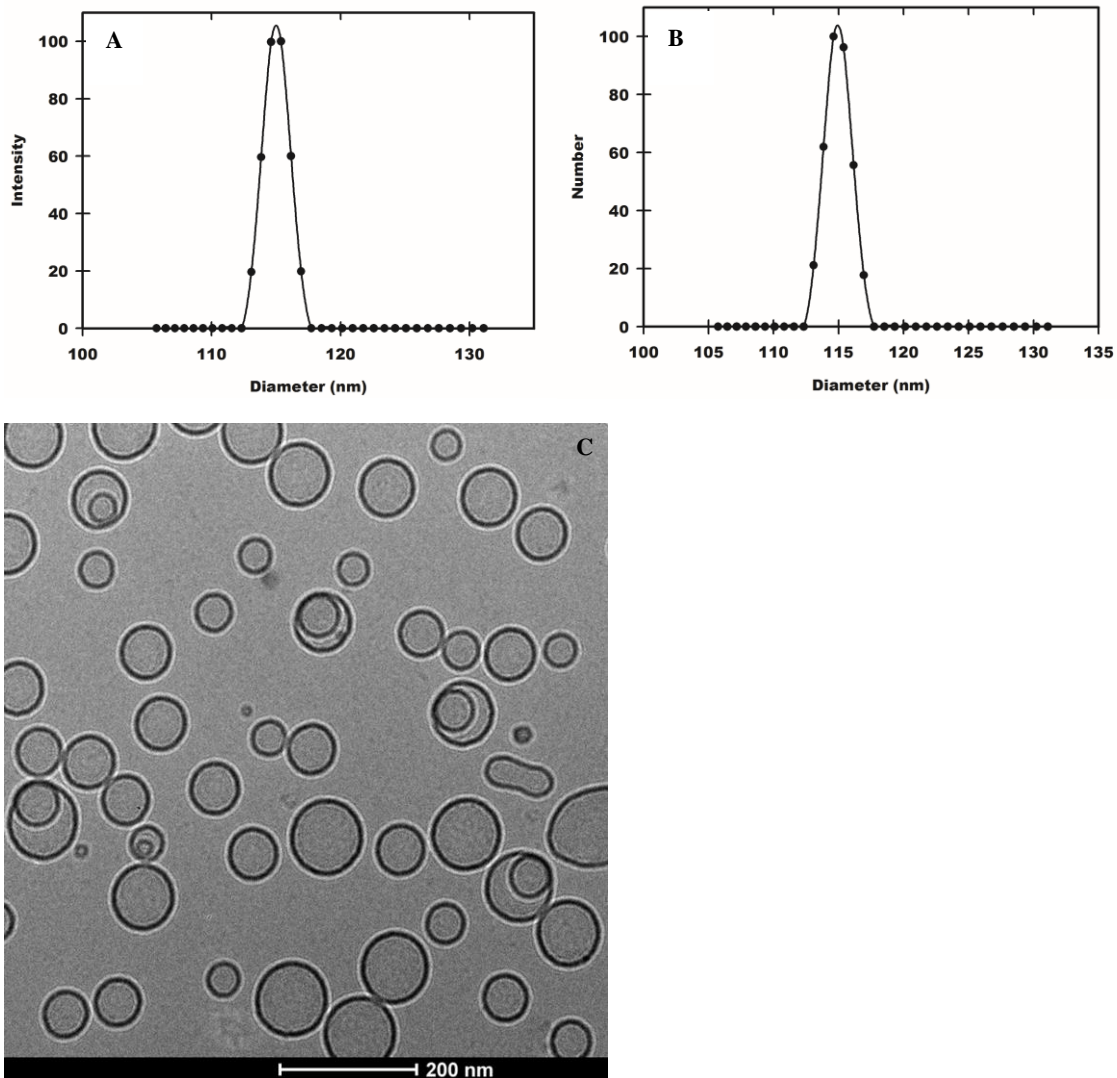


Figure S4: DLS results of the formulated PC liposomes a) Intensity v/s diameter plot, b) Number v/s diameter plot. c) Cryo-TEM image of the PC liposomes.