Protocols for Polymersome

Content

1- Preparation of PMOXA-PDMS-PMOXA polymersome

We followed as described in Corinne Nardin et.al [1], in brief, following steps were carried out,

a. 5ml pear shaped flask is washed with pure ethanol and dried at 120°C for 1hr
b. 1 mg polymer was dissolved in 1mL ethanol
c. Solution was evaporated at 40°C (water bath temperature), 40 rpm (rotation speed at an angle as high as possible, ~ 60°-70°) and a pressure of 150 mbar in rotary evaporator
d. After complete evaporation of ethanol, pressure was reduced using rotary pump vacuum (~10^{-2} mbar) for 30 min.
e. Later 1mL PBS (pH 7.4) was added and stirred overnight at 300rpm
f. In order to remove residual lamellar material and to define particle size less than 200nm, samples were extruded using ultra-filtration equipment (Avanti Polar mini Extruder) with Nucleopore membrane (0.2µm pore size)

2- Preparation of (PEG-b-P(DEAEMA-stat-BMA)) polymersome

We followed as described in Mohamed A. Yassin, et.al [2], in brief, following steps were carried out,

a. Millipore water was used for solution preparation
b. 1mg Polymer was dissolved in HCl (pH 2) with the help of magnetic stirrer at 700rpm
c. Later the pH of solution was reduced from 2 to 8, by adding NaOH drop wise at very slow rate. The pH of the solution should be checked very carefully.

3- Release profile assay

It was performed using cellulose dialysis membrane of MWCO 5000Da and 300KDa for biological entities less than 500Da and Enzymes respectively

a. The beaker was filled with dialysis solution (Milli-Q wáter or Buffer solution)
b. Dialysis tube was cut as per the volume of sample and dialysis tube width.
c. The tube was washed in Milli-Q wáter and pre-incubated in Milli-Q wáter for

20mins.

d. After pre-incubation, the tube was closed on 1 end with magnetic clamp such that atleast 10mm tubing overlaps.
e. Then through the other end, sample was filled inside the tube and air was removed carefully before closing the tube with clamp as before.
f. Dialysis tube was placed in prepared beaker and stirred at low speed (300rpm)
g. Dialysis solution was replaced in beaker after every 2 hour
h. After each hour, sample's were analysed using Beckman Coulter – UV 800 Spectrophotometer for Doxorubicin, enzyme, Tamra dye, fluorescein dye at 480nm, 280nm, 555nm and 490nm respectively
i. After dialysis, the sample was removed carefully after drying the tube with kim wipe.

4- Incorporation and encapsulation of biological entities

Incorporation of DNA channel (15nM conc.) and Encapsulation of enzymes (20% of polymer conc.) was carried out by adding during each polymersome preparation in following ways,

PMOXA-PDMS-PMOXA: DNA channel or Enzyme was added to PBS buffer in last step along with 5µl $MgCl_2$ solution (1 M)

(PEG-b-P(DEAEMA-stat-BMA)): Polymersome solution pH was brought to 5 and DNA channel (15nM conc.) or Enzyme was added along with 5µl $MgCl_2$ solution (1 M). Further, solution pH was reduced further to 8.

5- Preparation of smart Nano-container

Each polymersome was prepared along with DNA channel (15nM Conc.) and Enzyme as follows,

PMOXA-PDMS-PMOXA: DNA channel followed by Enzyme was added to PBS buffer in last step along with 5µl $MgCl_2$ solution (1 M)

(PEG-b-P(DEAEMA-stat-BMA)): Polymersome solution pH was brought to 5 and Enzyme was added and after few minute, DNA channel (15nM conc.) was added along with 5µl $MgCl_2$ solution (1 M). Further, solution pH was reduced further to 8.

6- Characterisation

The following instrument were used to characterize the polymersome,

a. Cryo-TEM (Transmission Electron Microscope
   i) For frozen hydrated specimen preparation, 2µl of sample was deposited on a holey carbon support film attached to a copper grid.

ii) The liquid film was thinned by blotting for 0.5 sec and frozen rapidly in liquid ethane.
iii) After rapid freezing, the sample was loaded to a Gatan cryo transfer holder keeping temperature below -130°C in order to prevent ice crystallization.
iv) TEM inspection was done in ZEISS LIBRA 120, energy filtered images were recorded under low dose electron irradiation conditions.
v) Samples were kept below -130°C during the inspection.

b. Cryo-SEM (Scanning Electron Microscope)
i) Samples were rapidly frozen in liquified ethane according to standard procedure.
ii) Frozen samples were fractured at approximately -130°C in a cryo preparation chamber and transferred to the cooling stage inside a ZEISS Ultra (…..) after coating with 3nm Carbon layer.

c. DLS (Dynamic Light Scattering)

Particle size analysis of aqueous solutions of polymerosomes and gold nanoparticles was performed by dynamic light scattering using a Malvern Zetasizer Nano. Data analysis is based on non negative least squares (NNLS) method [3].

Reference:

1. Corinne Nardin, Thomas Hirt,Jorg Leukel, and Wolfgang Meier, Polymerized ABA Triblock Copolymer Vesicles,Langmuir 2000, 16, 1035-104
2. Mohamed A. Yassin, Dietmar Appelhans, Rafael G. Mendes, Mark H. Rummeli, and Brigitte Voit, Chem. Eur. J. 2012, 18, 12227 – 12231
3. Robert Finsy, Nicolas de Jaeger, Rick Sneyers and Eric Gelade Part. Part. Sys. Charact. 1992, 9, 125