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Fabrication of monodisperse liposomes-in-microgel hybrid microparticles in capillary-based microfluidic devices

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ABSTRACT

This study introduces a drop-based microfluidic approach to physically immobilize liposomes in microgel (liposomes-in-microgel) particles. For this, we generate a uniform liposomes-in-water-in-oil emulsion in a capillary-based microfluidic device. Basically, we have investigated how the flow rate and flow composition affect generation of emulsion precursor drops in micro-channels. Then, the precursor emulsion drops are solidified by photo-polymerization. From characterization of hydrogel mesh sizes, we have figured out that the mesh size of the liposomes-in-microgel particles is bigger than that of bare microgel particles, since liposomes take space in the hydrogel phase. In our further study on drug releasing, we have observed that immobilization of liposomes in the microgel particles can not only remarkably retard drug releasing, but also enables a sustained release, which stems from the enhanced matrix viscosity of the surrounding hydrogel phase.

1. Introduction

The molecular geometry of the unit amphiphiles, which is typically determined by the packing parameter ($P = V/aL$), can be correlated with their assembled architecture: P is the packing parameter, V and L are the effective volume and length of the amphiphile hydrophobic chain, and a is the amphiphile optimal area at the interface [1,2]. For a packing parameter of ~1/3, a micelle structure is generated. A bilayered vesicle is made, when the packing parameter is 1/2–1. For a packing parameter of ~1, a planar bilayer, commonly referred to as lamellar, is formed. The lipid is a sort of amphiphile molecules having both hydrophilic and hydrophobic properties. The packing parameter of lipids ranges 1/2–1, so that they form a lipid membrane with different curvatures, which leads to a structurally defined vesicle geometry after the molecular assembly.

Liposomes have a bilayered vesicular structure. This structural uniqueness allows them to encapsulate the hydrophilic molecules within the interior aqueous core as well as the hydrophobic molecules within the bilayered lipid shell, respectively [2]. Hence, they are able to protect the encapsulated reactive or sensitive compounds from degradation by shielding and stabilizing against environmental and chemical stresses. Thanks to this function, liposomes are of great interest in pharmaceutical, cosmetic, food and biomedical industries [3,4]. Their size, surface properties, hydrophobicity, and functionality can be tailored by tuning the chemistry and composition of phospholipids and by altering environmental conditions, such as solvent type, ionic strength, pH, and temperature [5–7]. Most of all, the abilities to deliver active and labile molecules into cells or tissues either by vesicle fusion or control of membrane permeability have provided them with more practical applications [8–11].

Despite liposomes have such extensive applicability in academy as well as in industries, they have several critical set-backs, which are currently hampering much wider uses. The carriers made with lipid molecules readily fuse into the membrane bilayers. This makes them fade away quickly upon administrating through organs [12]. Also, liposomes have tendency toward being taken away rapidly

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by immune cells in the blood. In order to resolve these problems, a number of studies have been performed. In typical, they have tailored the periphery of liposomes to physically shield the inner part from external stresses: for instance, the structural stability of liposomes can be enhanced by co-assembly of amphiphilic graft copolymers, so that they can afford to stay in the blood much longer than non-modified ones [13]. One more critical issue that limits the application of liposomes in complex formulations is that the liposomal structure is susceptible to the presence of surfactants with low packing parameters. On adding a very small amount of these surfactants, the liposome-to-micelle transition readily occurs [14,15].

To overcome the above-mentioned drawbacks, in this study, we introduce a facile and trouble-shooting approach in which liposomes were incorporated into microgel microparticles and physically immobilized in the hydrogel network. Hydrogels are able to load and release drug molecules through their web-like molecular network. In principle, the diffusion of drug molecules is mainly ruled by the viscosity of the medium phase, which can be understood by the Stokes-Einstein equation, $D = k_B T/6\pi\eta a$, where D is the diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the medium, and a is the hydrodynamic radius of the particle. This means that the release kinetics is controllable via regulation of matrix viscosity. To provide a rationale for this, we immobilize the liposomes in uniform microgel particles. This particle system is named as liposomes-in-microgel (L-i-M). For this, we produce a uniform-sized liposomes-in-water-in-oil (L/W/O) emulsion by using a capillary-based microfluidic technique and the L/W drops containing the liposomes are solidified by photo-polymerization [16,17]. Basically, the hydrogel phase is made of poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC) having an excellent biocompatibility [18]. Finally, we try to demonstrate that our approach to immobilize the liposomes in the hydrogel microparticles enables a matrix-mediated controlled release.

2. Experimental

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was mercifully offered from Doosan Co. (Korea). 2-Methacryloyloxyethyl phosphorylcholine (MPC) was kindly supplied from KCI Co. (Korea). Cetyl PEG/PPG-10/1 dimethicone (Abil EM 90, Evonik, Germany) and hexyltrimethoxysilane (TCI, Japan) were used as received. Chloroform was purchased from Deajung (Korea). Isopropanol was purchased from Samcheon (Korea). Paraffin oil, glycerin, *N*,*N*′-methylenebisacrylamide (BIS), 2-hydroxy-2-methylpropiophenone (Darocure 1173), fluorescein isothiocyanate (FITC), and fluorescein isothiocyanate dextran (FITC-dextran, 70 kDa) were all purchased from Sigma–Aldrich (USA). Texas Red-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red-DHPE) was bought from Invitrogen (USA). All other chemicals were reagent grades and used without further purification. For all experiments, deionized double distilled water was used.

2.2. Synthesis of DPPC liposomes

DPPC liposomes were prepared using the modified thin-film rehydration method [19,20]. First, DPPC was completely dissolved in chloroform and evaporated with a rotary evaporate at 52 °C for 2 h to remove all the traces of the organic solvent. Then, the thin DPPC film appeared on the wall of the round flask was rehydrated with water and applied mild sonication for 2 h at 65 °C in order to come off the film into water and to form micron-sized multi-lamellar structured liposomes. The concentration of DPPC in the suspension was set to 1 wt%. To further decrease the particle size and fabricate uni-lamellar liposomes, strong probe-sonication was carried out pulsing every 1 s for 10 min with a power of 130 W at room temperature (VCX130, Sonics & materials Inc.). The size of liposomes was characterized with a dynamic light scattering (ELS-Z, Otsuka electronics, Japan). The morphology of liposomes in an aqueous phase was observed with a TEM (Energy-Filtering Transmission Electron Microscope, LIPRA120, Carl Zeiss, Germany). The test samples were negatively stained with 1 wt% of uranyl acetate in the aqueous solution. Then, they were dried in air before TEM observation.

2.3. Fabrication of micro-capillary microfluidic devices

To fabricate a capillary-based microfluidic devices, first, a round capillary was tapered by heating and pulling a cylindrical glass capillary (outer diameter = 1.0 mm, World Precision Instruments, USA) with a pipette puller (Model P-97, Sutter Instruments, USA). The end tip of the tapered glass capillaries was cut to the designated diameter using a microforge station (Micro Forge MF 830, Narishige, Japan). To prevent any wetting of the trimmed round capillary by the aqueous inner fluid, hydrophobic coating of the round capillary was conducted with 1 wt% of hexyltrimethoxysilane in toluene. For generation of uniform emulsions, a tapered cylindrical capillary was inserted into a square capillary (Atlantic International Technology, USA). The inner diameter of the square capillary was the same as the outer diameter of the round capillary by 1 mm. Each end of the square capillary was fit into with a needle tip and completely glued with epoxy resin.

2.4. Synthesis of L-i-M microparticles

To generate emulsions, each fluid was loaded into a glass syringe (Hamilton Gastight, USA) connected with a polyethylene tube (PE-5, Scientific Commodities, USA). Then, the tube was connected with the needle installed at each end of the square capillary. The dispersion fluid (DF) was an aqueous monomer solution containing the liposome suspension (0–15 wt%), MPC (15 wt%), BIS (a crosslinker, 0.5–1.5 wt%), Darocure 1173 (a photo-initiator, 0.5 wt%), and glycerin (30 wt%). The outer fluid (OF) consisted of paraffin oil and 2 wt% Abil EM 90. The flow rate of each fluid was precisely controlled with a syringe pump (Pump 11 Elite, Harvard Apparatus, USA). The emulsion drops were collected through the round capillary and observed with a bright-field microscope (Samwon, NSI-100, Korea). Then, the emulsion drops were solidified by photo-polymerization under UV 365 nm for 1 min. The paraffin oil, remnant monomers, and other additives were thoroughly removed with a large amount of isopropanol by repeated centrifugation at 4000 rpm. Finally, the particles were re-dispersed in water. The volumes of the microgel particles in a confined state, a swollen state, and a collapsed state were determined by measuring their diameters after drying, before swelling, and after swelling with a bright-field microscope. To demonstrate that the liposomes were locked in the microgel particles, we encapsulated 50 μL of FITC-dextran in the core and co-assembled a small amount of Texas Red-DHPE with the DPPC layers, respectively. The distribution of fluorescence probe molecules in the particles was detected with an inverted fluorescence microscope (IX-81, Olympus, Japan).

2.5. Investigation of releasing profiles

Release studies were carried out by using pyrene (λ_{ex} 336/λ_{em} 393) as a model drug. For this, a pyrene-loaded liposomal suspension was produced using the same preparation procedure.