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Short communication

Stable vesicle assemblies on surfaces of hydrogel nanoparticles formed from a polysaccharide modified with lipid moieties

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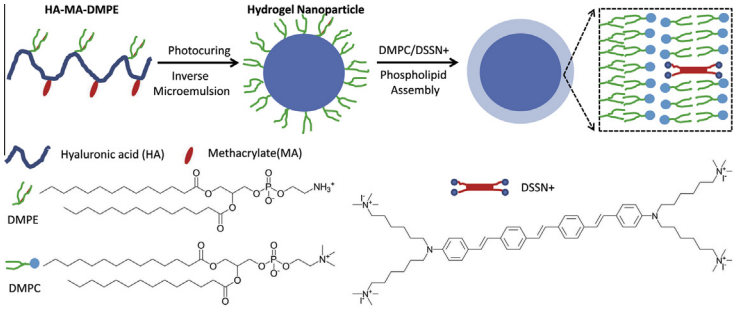
HIGHLIGHTS

- We synthesize hyaluronic acids attached with methacrylates and phospholipids.
- Modified hyaluronic acids form nanohydrogels via a surfactant free route.
- Lipids attached to hyaluronic acids anchor foreign lipids for assembling peripheral vesicles.
- Nanohydrogels swell with pH variations for disassembling peripheral vesicles.

GRAPHICAL ABSTRACT



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ABSTRACT

Micro- and nanoparticle-supported lipid assemblies have significant potential for being used in biology and medicine for sensing, mimicking cellular membranes, and delivering drugs or cosmetic agents. Here, we introduce a new type of nanohydrogels based on the modification of a polysaccharide with lipid moieties, followed by the formation of nanoparticles and assembling lipid bilayers on the particle surfaces. The lipophilic compound 1,2-ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) and the UV-crosslinkable methacrylic anhydride were covalently attached to hyaluronic acid (HA) and the formation of hydrogel nanoparticles via a surfactant-free inverse emulsion mechanism was demonstrated. As an anchoring group, the lipophilic DMPE moiety enables the formation of hydrogel nanoparticles with a spherical morphology in nonpolar media and allows for the stable assembly of lipid bilayers bearing amphiphiles on HA nanohydrogel surfaces. The conjugated oligoelectrolyte, 4,4′-bis[4′-(*N*,*N*-bis(6″-(*N*,*N*,*N*-trimethylammonium)hexyl)amino)styryl] stilbene tetraiodide (DSSN+), was incorporated into the nanohydrogel-supported lipid bilayers, resulting in the formation of stable multilamellar peripheral vesicular structures due to the similarity in chemical structure of DMPE and the assembled lipid molecules.

1. Introduction

Lipid bilayers self-assembled on microspheres of silica [1], polymers such as polystyrene [2] and polyvinyl alcohols [3], and hydrogels such as agarose–gelatin [4] and poly(N-isopropylacrylamide) [5] have received considerable attention for over the last two decades due to their potential in biomedical applications. Pharmaceutical agents or biofunctional proteins could be embedded inside such microsphere-liposome assemblies within the microsphere or supported lipid membranes on the microsphere while maintaining mechanical stability. The so-called lipobeads

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or lipogels [6,7] have been used as artificial biological cells to study membrane biophysics [8], as biosensors coupled with microfluidics [9], in drug delivery [10,11], and in dermatologic applications [12–14]. Hydrogel-liposome assemblies have been fabricated using electrostatic attraction between lipids and hydrogel surfaces [15–17], by transferring aqueous gel beads in an oil medium onto a saturated lipid monolayer at a planar oil–water interface [18], and by forming liposomes followed by gelation of their interior [19]. Other methods of preparation of hydrogel–liposome assemblies include coating microgels with layer-by-layer self-assembled polyelectrolyte multilayers and subsequent formation of lipid shells by vesicle adsorption and disruption [20], and modification of microgel surfaces with hydrophobic fatty acids [3,8,21] after gel formation to anchor lipid molecules and to induce their self-assembly.

However, further decreases in bead or gel size to the nanometer scale are required for future pharmaceutical and cosmetic applications [10,15,17]. When the size of a colloidal system decreases, it is expected that the circulation time in the blood would increase or that the response time for swelling or shrinking under stimulus would decrease, which could improve targeting, transmembrane delivery, and controlled release of drugs [13–17,12,22]. Nanohydrogels are also useful for transdermal delivery and cosmetics. The stratum corneum, the outermost layer of skin, has a morphology that can be represented by a brick (corneocyte) and mortar (intercellular lipid layer) model [14]. Smaller-sized colloids show enhanced permeation into these microstructures and allow for transport of hydrophobic drugs or cosmetic components through the intercellular lipid layers [12,23].

Herein, we demonstrate a new type of hydrogel–liposome assemblies with a nanogel core formed by the hydrophobic anchoring group of a phospholipid. The novel aspects of our work include the formation of nanogels via a surfactant-free inverse emulsion route and the demonstration of stable peripheral lipid vesicular structures bearing an amphiphilic agent. We modified hyaluronic acid (HA) with a phospholipid moiety, 1,2-ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), and with UV-crosslinkable methacrylic anhydride (MA). It is anticipated that the modified HA (HA–MA–DMPE) can form nanospheres in an inverse emulsion media without the use of surfactants, generating nanogel particles upon UV irradiation with phospholipid moieties displayed on the nanogel surfaces. Thus, the phospholipid bilayers, which have a similar chemical structure to that of the surface-anchoring moieties and can accommodate hydrophobic or amphiphilic agents, can self-assemble on the surface of HA nanogels, as illustrated in Scheme 1. As a proof of concept, we demonstrate the assembly of the DMPC lipid bilayers bearing the amphiphilic conjugated oligoelectrolyte 4,4′-bis[4′-(*N*,*N*-bis(6″-(*N*,*N*,*N*-trimethylammonium)hexyl)amino)styryl] stilbene tetraiodide (DSSN+) on a nanogel surface [24,25]. Using cryogenic transmission electron microscopy (cryo-TEM) and the polarity-dependent photoluminescence of the conjugated oligoelectrolyte, we demonstrate the successful assembly of the lipid bilayers on the HA nanogel surface.

2. Experimental

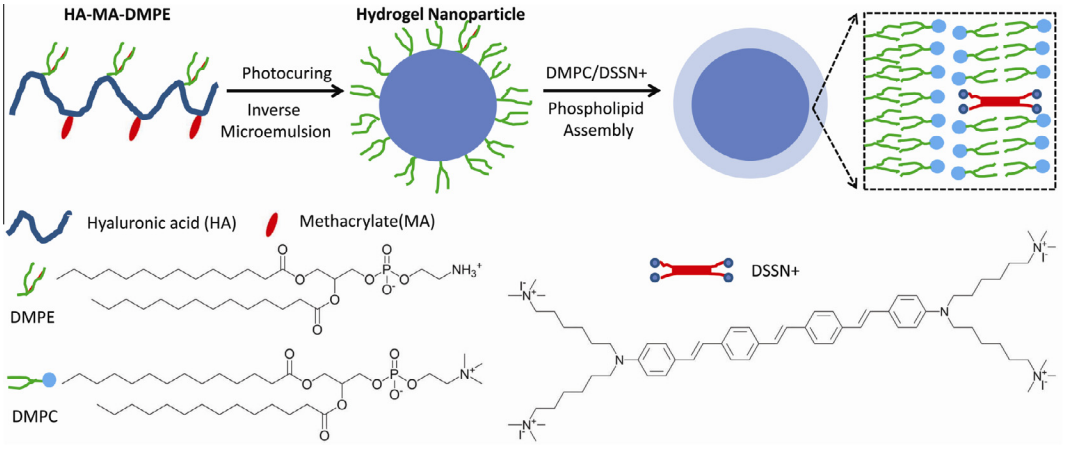
2.1. Materials

Sodium hyaluronate (MW = 10,000–20,000 g/mol, Cat. No. HA10K) was obtained from Lifecore Biomedical, Inc. (Minnesota, USA). Methacrylic anhydride (MA), 2-morpholinoethanesulfonic acid (MES), N-hydroxy succinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), and a photo initiator (Irgacure D-2959) were purchased from Sigma–Aldrich Co. (Montana, USA). A cellulose membrane (MWCO = 3500 g/mol) from Spectrum Laboratories Inc. (California, USA) was used for dialysis. 1,2-Ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) were purchased from Avanti Polar Lipids, Inc. (Alabama, USA). A MES buffer solution (pH 6.4) was prepared from 0.5 M sodium chloride and 50 mM MES.

2.2. Synthesis of methacrylated hyaluronic acid (HA–MA)

To prepare methacrylated HA (HA–MA), a synthetic route similar to that described in the literature [26] was used. In brief, HA (0.5 g, 1.25 mmol on a repeat unit basis, $[(C_{14}H_{20}NO_{11}Na)_n]$ = 401 g/mol) was dissolved in deionized water (25 mL), followed by the addition of a 20-fold excess of methacrylic anhydride (3.7 mL, 25 mmol) relative to the primary hydroxyl groups in HA. The reaction mixture was then stirred for 24 h in an ice bath after adjusting the solution pH to 8.0 using a 5 M NaOH solution. The solution of HA–MA was purified by dialysis for 3 days against distilled water, filtered using 0.2 μm Whatman filter paper, and freeze-dried.

Degree of substitution: 15.0%. ^{1}H NMR (300 MHz, D_2O): δ 6.06–5.61 (d, J = 135 Hz, 2H, C = C-H, H_{aa1}), 2.57 (s, 2H; H_c), 1.89 (s, 3H; H_{b1}), 1.82 (s, 3H; H_b); IR (KBr): ν = 3427 (s), 2922 (w), 1739 (m), 1652 cm^{-1} (s).



Scheme 1. Assembly of the DMPC lipid bilayers bearing DSSN+ conjugated oligoelectrolytes on the surface of a hyaluronic acid hydrogel nanoparticle formed via the inverse microemulsion mechanism under UV irradiation after modification with methacrylates and DMPE.