Journal of Colloid and Interface Science 534 (2019) 105–109

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Short Communication

Tunable morphology of lipid/chitosan particle assemblies

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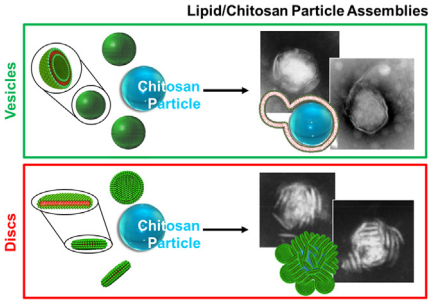
GRAPHICAL ABSTRACT

Influence of the lipid membrane morphology (*i.e.* lipid discs, or lipid vesicles) on the lipid/chitosan particle assemblies.



ARTICLE INFO

Article history:
Received 22 June 2018
Revised 24 August 2018
Accepted 27 August 2018
Available online 6 September 2018

Keywords:
Lipid discs
Lipid vesicles
Chitosan
Lipid/polymer particle assemblies

ABSTRACT

Lipid/chitosan (CS) particle assemblies have recently been developed as new promising carriers for drug delivery applications. The present work reports for the first time the formation of such assemblies by a simple spontaneous adsorption of lipid membranes onto the CS particle surfaces. As shown by dynamic light scattering (DLS) measurements, final non-aggregated assemblies with relatively satisfactory size distributions were obtained by using this process. Furthermore, a particular attention has been paid herein to the effect of the initial morphology of lipid membranes (*i.e.*, vesicular or discoidal) on the resulting characteristics of assemblies. To this end, each one of these membranes was mixed with CS particles, and the obtained assemblies were observed by transmission electron microscopy (TEM). According to these observations, the vesicular lipid membranes seem to wrap mostly CS particles. In contrast, lipid discs are not reorganized onto the particle surface but would rather be stacked onto the CS particle.

1. Introduction

Chitosan particles have been widely investigated in the past decades due to their ability to transport, protect drugs, and target specific sites [1]. Numerous studies have been dedicated to their synthesis *via* an ionic gelation process with the aim of obtaining colloids with controlled sizes and size distributions [2–5]. However, limitations regarding their colloidal physicochemical and biological properties are quickly reached in *in vivo* conditions (*e.g.*, in the presence of relatively high amounts of salts or proteins, pH variation) [6]. To overcome these limitations, their surface modification by a lipid membrane is particularly appealing in the drug delivery area. Indeed, this surface modification provides several interesting advantages as a drug release from assemblies not only controlled by their core (polymer degradation), but also by their lipid shell (diffusional barrier) [7–14]. Furthermore, the resulting biomimetic lipid surface [15] can easily be modified by

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https://doi.org/10.1016/j.jcis.2018.08.098
0021-9797/

hydrophilic poly(ethylene glycol) chains and targeting ligands (leading to long-circulating targeted assemblies). Concerning the polymer core, it brings a structural integrity and stability to this lipid coat (*versus* hollow liposomes). Such core-shell assemblies (named LipoParticles) have already been examined with synthetic polymer cores (*e.g.*, based on poly(lactic acid) [16–18], poly(lactic-*co*-glycolic acid) [19,20], and polystyrene [21,22]).

This core-shell nano-organization (in which each particle is covered with a lipid membrane) has previously been observed by microscopy [16,21,23–25]. However, in the case of CS particle cores, the final structures obtained so far in the literature appear to be much less controlled. Several synthesis methods of these assemblies with CS particles have been developed such as reverse phase emulsion [26,27], lyophilisation (mixture of CS particles with a vesicle suspension followed by freeze drying) [28], or lipid film hydration by a CS particle suspension [29–31]. More recent studies [32–34] focused on the preparation of more individualized and better defined assemblies. Nevertheless, this has required a supplementary extrusion step of assemblies through polycarbonate membranes with pore sizes in the sub-micrometer range after the lipid film hydration step by a CS particle suspension. Moreover, TEM images of resulting assemblies may raise some reservations about their morphology [32–34].

The lipid film hydration method by a suspension of synthetic polymer particles has previously been compared to the spontaneous reorganization of lipid vesicles onto particles to synthesize LipoParticles [21]. This study revealed that the second process was the most suitable to obtain assemblies with satisfactory sizes and size distributions. This explains why this procedure (*i.e.*, "two-step method") [13] was the most reported in the literature. However, no work has been carried out so far on lipid/CS particle assemblies prepared by this way. Moreover, to the best of our knowledge no work about the influence of the lipid membrane morphology on the lipid adsorption process has been reported in the literature despite the understanding aspects about the lipid adsorption, and the opportunity to tune the final morphology of assemblies. Consequently, the aim of this work is to form and to characterize assemblies obtained by adding two types of preformed lipid membranes (classical vesicles or discs) to CS particle suspensions. The influence of each type is examined on the size, size distribution, zeta potential, and morphology of final assemblies.

2. Results and discussion

As previously mentioned, CS particles and lipid membranes were independently prepared before mixing. CS particles were obtained *via* an ionic gelation process by using tripolyphosphate as ionic crosslinking agent. The details of experimental conditions are described in a previous work [2]. Resulting CS particles are spherical, with a reproducible sub-micrometer size, and a narrow size distribution. Concerning the preparation of lipid membranes, it firstly requires the formation of multilamellar lipid vesicles by the well-known Bangham method (lipid film hydration) [35]. The lipid formulation was composed of 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphatidylcholine (DPPC) and 1,2-dipalmitoyl-*sn*-*glycero*-3-phosphatidic acid (DPPA) at a DPPC/DPPA molar ratio of 90/10 or 0/100. At the end of the lipid film hydration step, large vesicles of several micrometers were obtained with a very broad size distribution. An ultrasonication step (for 60 min. at 70 °C) of these multilamellar vesicles was therefore performed to obtain discs [36,37]. On the other hand, unilamellar vesicles were obtained thanks to the extrusion of multilamellar vesicles through a polycarbonate membrane with pore sizes of 100 nm [38]. The characterization of CS particles and 90/10 DPPC/DPPA lipid membranes was achieved by DLS, zeta potential measurements (Table 1), and (cryo)-TEM (Fig. 1). These (cryo)-TEM images reveal satisfactory morphologies, sizes and size distributions for all the elaborated objects. The discoidal shape of lipid membranes was confirmed by electron tomography. This technique retrieves 3D structural information from a tilt series collected from –60° to +60° (with the Saxton scheme [39] on a JEOL JEM-2100F) of 2D projections (75 2D images acquired with a GATAN Ultrascan 1000 CDD

Table 1
Average hydrodynamic diameters (D_Z), size distributions (PDI) measured by DLS (see size distribution profiles in ESI 2), and zeta potentials (ζ) of CS particles, lipid membranes (discs or vesicles, 90/10 and 0/100 mol. DPPC/DPPA), and resulting lipid/CS particle assemblies obtained by spontaneous adsorption of lipid membranes onto CS particles (n = 12). Measurements were performed after a 20-fold dilution in deionized water.

	D_Z (nm)	PDI	ζ (mV)
CS particles	160 ± 25	0.13 ± 0.03	+28 ± 5
90/10 mol. DPPC/DPPA formulation			
Discs	71 ± 25	0.46 ± 0.12	−45 ± 6
Vesicles	106 ± 4	0.07 ± 0.03	−49 ± 9
Assemblies with discs	261 ± 35	0.16 ± 0.06	−32 ± 3
Assemblies with vesicles	260 ± 55	0.24 ± 0.09	−28 ± 3
0/100 mol. DPPC/DPPA formulation			
Discs	81 ± 16	0.21 ± 0.02	−42 ± 1
Vesicles	108 ± 1	0.04 ± 0.01	−42 ± 2
Assemblies with discs	175 ± 18	0.36 ± 0.05	−40 ± 2
Assemblies with vesicles	157 ± 21	0.18 ± 0.08	−39 ± 6

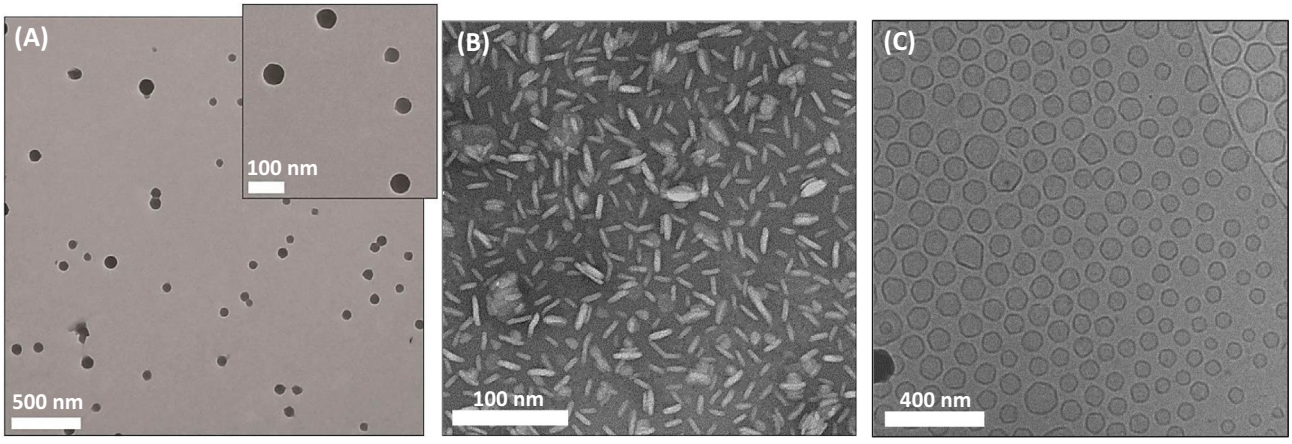

Fig. 1. TEM images of CS particles (A), and 90/10 mol. DPPC/DPPA discs with sodium silicotungstate (2% w/w) as contrast agent (B). Cryo-TEM image of 90/10 mol. DPPC/DPPA vesicles (C).