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Mesoporous silica for drug delivery: Interactions with model fluorescent lipid vesicles and live cells

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ABSTRACT

Formulated mesoporous silica nanoparticle (MSN) systems offer the best possible drug delivery system through the release of drug molecules from the accessible pores. In the present investigation, steady state and time resolved fluorescence techniques along with the fluorescence imaging were applied to investigate the interactions of dye loaded MSN with fluorescent unilamellar vesicles and live cells. Here 1,2-dimyristoyl-*sn*-glycero-3-phospocholine (DMPC) was used to prepare Small Unilamellar Vesicles (SUVs) as the model membrane with fluorescent 1,6-diphenyl-1,3,5-hexatriene (DPH) molecule incorporated inside the lipid bilayer. The interaction of DPH incorporated DMPC membrane with Fluorescein loaded MSN lead to the release of Fluorescein (Fl) dye from the interior pores of MSN systems. The extent of release of Fl and spatial distribution of the DPH molecule has been explored by monitoring steady-state fluorescence intensity and fluorescence lifetime at physiological condition. To investigate the fate of drug molecule released from MSN, fluorescence anisotropy has been used. The drug delivery efficiency of the MSN as a carrier for doxorubicin (DOX), a fluorescent chemotherapeutic drug, has also been investigated at physiological conditions. The study gives a definite confirmation for high uptake and steady release of DOX in primary oral mucosal non-keratinized squamous cells in comparison to naked DOX treatment.

1. Introduction

Mesoporous silica systems have been emerged as one of the most promising nano-vehicles due to their biocompatibility, large surface area, highly controllable and monodispersive nature of large accessible pore size, cost-effective easy synthetic routes and capability of easy loading and releasing of drug molecules from their mesopores [1–4]. Mesoporous silica nanoparticles (MSN) are capable of encapsulating functional molecules into their pores to protect them from physiological environment and their known chemical inertness make them suitable Drug Delivery System (DDS) for effective chemotherapy. These positive properties make MSN as suitable candidate for drug and gene delivery, cell imaging, and cancer therapy applications [5–10]. Regarding cytotoxicity large MSN (104–335 nm) shows much lower cytotoxic response compared to small MSN (14–16 nm) [11] and this implies that the surface area of nanoparticles may play an important role in controlling cytotoxicity [11].

Fluorescent dye molecules incorporated inside mesoporous silica shows novel optical characteristics due to their controlled host-guest interactions which find enormous applications in the field of optical and bioscience [12–14], pH responsive opto-mechanical drug delivery systems and host-guest interaction (electrostatic, covalent, or coordination bonding) [15–20]. Now, designing a pH responsive system is quite costly and functionalization of MSN surface is very much needed to increase the adsorption of molecule inside their pores. All these complicated procedures and selective surface functionalization increase the total cost of fabrication which has adverse effect on pharmaceutical industry both from cost as well as reproducibility point of view. A more facile and economic method for fabrication of a suitable non-functionalized MSN system for effective drug delivery system is therefore desirable in the field of nanomedicine. Distinct spectral properties of Fluorescein derivatives find various biological applications [23]. Fluorescein has been used extensively as a diagnostic tool in the field of ophthalmology and optometry and finds increasing applications during brain tumor surgery. The xanthene dye Fluorescein (Fl) which was proved to be a popular fluorophore in biosciences and in medical

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treatment like fluorescein angiography [21,22], therefore, has been chosen in the present investigation.

On the other hand, membranes not only act as a barrier between the internal and external environments of a cell, but also play a very critical role in modulating several important cellular functions [24]. Cellular membranes are composed of a complex combination of various lipid molecules and proteins and each of the components performing a specific set of functions. Lipid vesicles serve as nearly perfect membrane models for studying interactions of various drugs, metal ion, proteins and peptides with cellular membranes [25]. DMPC bilayer membrane vesicle [26] is composed of two 14-C long chain saturated hydrophobic tails and the phosphatidylcholine (PC) head-group is zwitter-ionic at the physiological pH of 7.4.

To date different fluorescent probes [27–29] are used to monitor interactions of membranes with drugs, peptides, nanoparticles, etc. [25,30] Among them 1,6-diphenyl-1,3,5-hexatriene (DPH) is most popular and widely used because of its two fluorescence lifetime components; the shorter lifetime component (around 9%) arises from the DPH population localized near the membrane interface while the longer lifetime component (around 91%) arises from the DPH population embedded in the membrane interior [31]. Out of these two populations of DPH in membrane, the DPH molecules close to the membrane surface are known to remain in parallel orientation to the membrane plane, while, in the interior these molecules are aligned perpendicular to the membrane plane. In addition, DPH is feebly fluorescent in aqueous solution but becomes fluorescent on incorporation into hydrophobic region [32] of DMPC lipid bilayer. The very weak fluorescence spectrum of DPH in water consists of a single broad band with emission maximum at 448 ± 3 nm, which is very distinct from the fluorescence of DPH when embedded into membranes [33].

In the present study the interaction between dye-loaded MSN and DMPC membrane has been investigated in details where the studied DMPC membrane is originally tagged with fluorescent DPH probe. It is well known that silica nanoparticles bind to the surface of membranes with high affinity [34]. Spectroscopic investigation revealed that the embedded dye loaded in MSN releases once it interacts with a dye-tagged simple DMPC vesicle model membrane through host guest interaction. The interaction of the fluorescein loaded MSN with DPH-tagged DMPC vesicles has been monitored through steady-state fluorescence and fluorescence lifetime experiments of DPH-tagged DMPC in presence of increasing concentrations of fluorescent MSN.

The objective of the present study is to observe the release of dye molecules (as a mimicking system to small molecule drugs) from the pores of MSN while interacting with the membrane DMPC. Due to the inherent chemical inertness, delivery by MSN without changing their physical characteristics makes this system more promising to be a potential biocompatible nanoscale carrier. Finally, live cell study by fluorescence microscopy was also conducted to confirm the delivery efficiency of a fluorescent drug, doxorubicin, by using MSN as nanocarriers.

2. Materials and Methods

2.1. Chemicals

Tetraethyl orthosilicate [TEOS, $Si(OC_2H_5)_4$], cetyltrimethylammonium chloride (CTAC, CH_3-$(CH_2)_{14}$-CH_2-$N(CH_3)_3^+Cl^-$), ethanol (EtOH, C_2H_5OH), triethanolamine (TEA, $N(CH_2CH_2OH)_3$), Fluorescein ($C_{20}H_{12}O_5$), dimyristoylphosphatidylcholine (DMPC) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma-Aldrich and used without further purification. TES-NaCl buffer (pH 7.4) and Sodium Acetate-Acetic Acid Buffer (pH 4.9) were purchased from SRL (India).

2.2. Synthesis of Mesoporous Silica Nano-particles

The mesoporous silica nanoparticles (MSN) were prepared directly from tetraethyl orthosilicate accordingly to the procedure [35] with slight modification, where TEOS was added to the aqueous solution containing CTAC, ethanol and triethanolamine. 6.4 mL of deionized double distilled Milli-Q water, 0.9 g of EtOH, 1.04 g of 25 wt% CTAC solution and 0.02 g of TEA were mixed together and stirred in a water bath at 60 °C for 30mins. Followed by 0.73 mL of TEOS was added into this mixture drop wise under constant stirring. The solution became opaque initially which turns into intense white color gradually and stirring continues for additional 2 h by keeping the reaction mixture in the same water bath at 60 °C. Finally the reaction mixture was slowly cooled to room temperature by turning off the heat under constant stirring. The solid reaction product was finally filtered off by centrifugation and washed thoroughly by ethanol, dried in oven for 4 h and then calcined for another 4 h at 773 K.

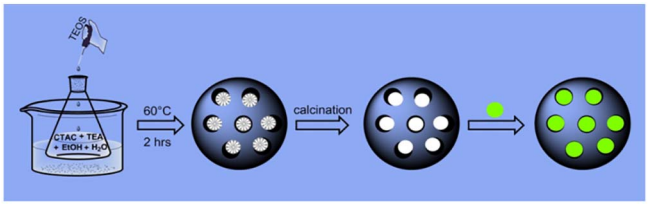
2.3. Dye Encapsulation in MSN

Fluorescein ($C_{20}H_{12}O_5$) dye solution is prepared by dissolving appropriate amount of fluorescein (Fl) dye in 10 mL EtOH to get the resultant 1 mM concentration and then 0.1 mg of the above prepared MSN powder is added to this solution. The solution is then stirred for 24 h at room temperature. After dye incorporation inside nanopores, the dye encapsulated solid MSN were separated by centrifugation and washed them thoroughly by EtOH to remove excess free dye molecules and the dye molecules attached to the external surface of MSN (Scheme 1). The entire process of MSN synthesis followed by dye incorporation is shown in Scheme1.

2.4. Preparation of DPH Labelled SUVs

Pure DMPC lipid was dissolved in a 2:1 $CHCl_3$:MeOH mixture in a glass vial which was purged under argon to completely remove the solvent, yielding a uniform lipid film. This lipid film sample was then dried under vacuum and stored at − 20 °C until use. These lipid films composed of DMPC were then hydrated properly to transform them into SUVs (at room temperature) in TES-NaCl buffer with pH 7.4 and Sodium Acetate-Acetic Acid Buffer (pH 4.9) respectively to get 0.5 mM solution (0.3 mg/mL). After hydration of the respective samples, the mixtures were vortexed to completely disperse the lipids in aqueous solution. The dispersion was then sonicated for about 8 min in three equal time intervals by using dr. Heilscher (Germany) probe sonicator (200 W). The sonicated samples were then allowed to stand for 40 min at 37 °C in a dry-bath incubator followed by centrifugation at 10,000 rpm for 15 min to remove aggregated lipids and titanium particles which has been introduced as an impurity from the sonicator probe during the process of sonication. These SUVs have an average diameter of 30–50 nm, as confirmed from DLS measurements.

0.5 mM DMPC SUVs were then labelled with hydrophobic membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH) maintaining a probe to lipid ratio of 1:500 [36,37]. Stock solution of DPH was made in DMSO and appropriate amount of probe were added to the vesicle solutions. The staining of the vesicles with increased incorporation of



Scheme 1. Schematic representation of stepwise synthesis of MSN followed by dye (Fl) encapsulation inside the pores.