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# Silicone-stabilized liposomes as a possible novel nanostructural drug carrier

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ABSTRACT

Development of silicone stabilized liposomes which can serve as novel drug nanocarriers is presented. Silicone precursor 1,3,5,7-tetramethylcyclotetrasiloxane ($D_4^H$) was introduced into the bilayer of the cationic liposomes prepared from egg yolk phosphatidylocholine (PC) and double-tailed dimethyldioctadecylammonium bromide (DODAB). The silicone material was created inside of the liposomal bilayer in the base-catalyzed polycondensation process of the $D_4^H$ what was confirmed employing $^{29}$Si solid-state MAS NMR and FTIR measurements. Surfactant lysis experiments revealed that resulted systems can be effectively stabilized. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements demonstrated that the silicone-stabilized liposomes have typical lipid vesicle's morphology and mean hydrodynamic diameters in the range of about 110 nm. They have considerably lower tendency for aggregation than the pristine liposomes. The permeability of vesicles can be tuned by introducing various amounts of silicone precursor into the liposome bilayer, as confirmed in calcein-release studies. The effect of fetal bovine serum (FBS) on the stability of liposomes was also tested in *in vitro* studies. Biological studies revealed that resulted liposomes can be considered as possible drug nanocarriers because they are not toxic to human skin fibroblasts (HSFs) and mouse embryonic fibroblasts (MEFs).



## 1. Introduction

Recently, there has been a growing interest in development of novel advanced drug delivery systems (DDS). In general, DDS are expected to increase therapeutical efficacy while reducing toxicity of the biologically active molecule. Liposomes—artificially constructed phospholipid vesicles are probably the most frequently studied DDS. That is due to their unique properties such as non-toxicity, biodegradability, as well as simplicity of preparation and control over the composition and size. Liposomes are capable of encapsulating hydrophilic drugs in their inner aqueous compartments, as well as, hydrophobic compounds in the bilayer lipid membranes [1]. Thus, they are considered as possible vehicles for the delivery of therapeutic compounds, such as drugs or proteins. Unfortunately, the major problem with liposomes is their low stability during storage which is related to their strong tendency for aggregation, degradation, and fusion leading to uncontrolled leakage of the entrapped compounds [2].

Various strategies have been proposed to overcome those problems [3,4]. The main approaches involve optimization of the lipid composition in the membrane [5], adsorption of polymeric material at the liposomal surfaces [6–8] especially using the layer-by-layer method [9,10] and covalent grafting of hydrophilic polymers onto the head groups of phospholipids [11]. A widely applied approach to stabilize liposomes is to coat their surface with a stealth material such as poly(ethylene glycol) (PEG) [12,13]. PEG coating provides the steric stabilization of liposomes thus reducing their tendency to aggregate and fuse with each other. Additionally, coating limits nonspecific interactions of liposomes with blood components (opsonization) which increases the period of their presence in blood circulating in the body [14]. As a result, PEGylated liposomes have found applications in systemic drug delivery [15]. Despite the undisputable advantages and the clinical success of PEGylated formulations, some toxic effects were observed due to the presence of PEG [16]. The PEGylated liposomes are linked with skin reaction known as "Hand-Foot syndrome" which results in skin

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eruptions/ulcers on the palms of the hands and soles of the feet. Another drawback of PEGylated liposomes is the presence of large molecules of PEG on the liposomal surface which may reduce their interactions with cells and consequently may hinder entry of liposomes into the tumor tissue thus reducing the accumulation of therapeutical amount of drug in the cells [17,18].

Liposomes have been also covered with inorganic silica shell [19,20] or trapped into sol–gel silica materials [21]. Crosslinked polymer shell may be also created on the surfaces of liposomes to achieve their stabilization [22,23]. Nonetheless the material selection for the crosslinking process is severely limited due to the short list of polymers approved for their use in drug delivery [24]. In response to this challenge, an organic-inorganic hybridization strategy has been developed for the engineering of the liposomal cerasomes. That interesting concept of vesicle stabilization by applying the sol–gel process for an organo-alkoxysilane with a lipid-like structure was presented by Katagiri et al. [25,26].

In our previous paper we have proposed the new method of stabilization of liposomes by covering their surface with a thin silicone layer [27]. Silicones belong to the group of biomaterials used in prosthetic devices such as implant for breast enlargement, tissue augmentation and for treatment of hydrocephalus [28]. They exhibit interesting properties, including low toxicity, good corrosion resistance, low surface tension, and excellent thermal stability in the broad range of temperatures [29]. Moreover, due to the longer bonds compared to typical organic polymers, silicones reveal excellent gas permeability [30]. Thus the silicone layer ensures high mechanical, thermal and chemical stability for the fragile liposome vesicles while not compromising biocompatibility. On the other hand, the presence of liposomal bilayer structure in which the silicone layer is created reduces the overall rigidity characteristic for typical silica nanoparticles. Previously we have described preparation of silicone-coated liposomes using base catalyzed polycondensation/polymerization processes of tetra(methyldimethoxysilylethyl)tetramethyl-cyclotetrasiloxane at the lipid vesicle surface [27]. The process was initialized at two pH values, namely at 8.5 and 10.2 and carried out at 70 °C. The surfactant- lysis experiment confirmed high stability of resulted silicone-coated liposomes. However the cryo-TEM micrographs demonstrated that procedure with the higher value of pH (10.2) leads to partial disintegration of liposomes.

Here we present the novel approach allowing to obtain more stable liposomal systems under considerably milder conditions. Thin silicone layer was produced inside the liposomal bilayer preserving the positive zeta potential values of the liposomal nanoparticles that can ensure attractive interactions with negatively charged living cell membranes. By employing the commercially available silicone precursor—1,3,5,7-tetramethylcyclotetrasiloxane ($D_4^H$) with Si-H being prone to the base-catalyzed polycondensation processes even at room temperature definitely milder conditions can be applied to achieve effective stabilization. The obtained nanostructures were characterized with various methods: dynamic light scattering, zeta potential measurements, transmission electron microscopy, surfactant-lysis. As our main goal was to obtain the stable nanocarriers with the tunable permeability characteristics, various amounts of silicone precursor ($D_4^H$) was used to stabilize liposomes. The permeability of vesicles was evaluated in calcein-release studies. The effect of the silicone precursor on the liposomes' stability was also tested in *in vitro* studies applying fetal bovine serum (FBS) conditions.

Finally considering the potential biomedical applications of the fabricated silicone-stabilized liposomes (ss-liposomes) *in vitro* studies on human skin fibroblasts and mouse embryonic fibroblasts were also carried out.

## 2. Experimental

### 2.1. Materials

1,3,5,7-Tetramethylcyclotetrasiloxane ($D_4^H$, ABCR), L-α-phosphatidylcholine from frozen egg yolk type XI-E (PC lipid, Sigma, 100 mg/ml solution in ethanol), dimethyldioctadecylammonium bromide (DODAB, Fluka), sephadex (Sigma), Triton X–100 (POCh), calcein (Sigma), Minimum Essential Medium Eagle (MEM, Sigma–Aldrich), Dulbecco's Modified Eagle's Medium – high glucose with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture (DMEM/High, Sigma), fetal bovine serum (FBS; HyClone; collected in South America; triple 0.1 lm sterile filtered), MEM Non-essential Amino Acid Solution (Sigma–Aldrich), sodium pyruvate 1 mM (Sigma–Aldrich), 100 I.U./ml penicillin and 100 lg/ml streptomycin (HyClone; 0.1 lm sterile filtered), XTT *In Vitro* Toxicology Assay Kit (Sigma) were used as received.

### 2.2. Preparation of cationic liposomes

The cationic liposomes were obtained using the procedure developed in our laboratories and described earlier [27]. Briefly, 2.1 mg (10 mol-% of PC lipid) of DODAB was dissolved in ethanol and added to the ethanolic solution of the lipid (250 μl of the solution). Next the mixture was vortexed for about 5 min, ethanol was evaporated under the gentle stream of nitrogen and the resulted film was hydrated and vortexed. The obtained multilamellar liposomal dispersion was subjected to five freeze-thaw cycles from liquid nitrogen temperature to 60 °C and then extruded 5 times through the membrane filters with 100-nm pores using a gas-pressurized extruder.

### 2.3. Preparation of silicone-stabilized liposomes

Silicone-stabilized liposomes named ss-lip-60% and ss-lip-100% were fabricated using $D_4^H$ in the amount constituted 60 and 100% of total molar content of lipids used. An appropriate amount of $D_4^H$ was added to the ethanolic solutions of lipids and then the procedure was the same as for cationic liposomes. The only difference was that in order to initiate the polycondensation processes of the precursor the resulting film was hydrated with water at pH adjusted to 8.5 or with PBS (pH = 8.5) After extrusion the dispersion was stirred for 24 h at room temperature. For characterization of the silicone material created inside the liposomal bilayer, the lipids were removed by precipitation in methanol. The precipitant was washed with methanol followed by centrifugation and this procedure was repeated four times. Finally, the product was dried for 24 h in a vacuum and a white powder was obtained.

### 2.4. Preparation of calcein-loaded nanocarriers

Calcein-loaded nanocarriers were prepared according to the similar procedure as that described for cationic liposomes with the only difference being that the film was hydrated with calcein solution (dissolved in PBS at pH = 8.5) at the concentration causing the self-quenching (20 mM). For extrusion of nanocarriers-loaded with calcein the PPH Marker manual extruder was used. Untrapped calcein molecules were separated employing size-exclusion chromatography on a Sephadex G-50 column using PBS buffer as an eluent.

### 2.5. Preparation of model silicone material from $D_4^H$

To confirm the structure of silicone materials fabricated inside the liposomal bilayer using the solid-state $^{29}$Si Magic-Angle Spin-