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Linseed oil based nanocapsules as delivery system for hydrophobic quantum dots

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ABSTRACT

In the present work, the CdSe/ZnS hydrophobic quantum dots were embedded within the polyelectrolyte nanocapsules. The core of the capsules, which consists of a mixture of the linseed oil with chloroform, was prepared using the spontaneous emulsification technique. The obtained emulsions were stabilized with lecithin and encapsulated using the layer-by-layer (LbL) adsorption of polyelectrolytes. The pair of biocompatible polyelectrolytes was used: the cationic poly-L-lysine hydrobromide (PLL) together with the anionic poly-D-glutamic acid sodium salt. The saturation LbL method, which is based on the stepwise formation of consecutive layers on the initial emulsion without the intermediate rinsing step, was applied to form the capsule shells. Their growth was evidenced by the capsule size and electrophoretic mobility measurements. The emulsion and the capsules were deposited on a mica surface and the deposit topology was examined by the means of atomic force microscopy (AFM). The presence of quantum dots within the oil cores was confirmed by recording the fluorescent spectra of the samples containing CdSe/ZnS. In order to evaluate cytotoxicity of the capsules, their influence on the viability of mouse embryonic fibroblasts was examined using the MTT test, followed by optical-microscope observation of morphology of the cells after hematoxylin–eosin staining.

1. Introduction

A development of the noninvasive imaging techniques is of a great importance in biomedical applications [1,2]. Detection and tracking of abnormalities, such as cancer or inflammation, would be difficult without using proper contrast agents. To this end, both the conventional organic dyes and the fluorescent quantum dots are being used [3]. The quantum dots are small nanocrystals made of semiconductors, such as CdTe, CdSe, CdS or the carbon-based dots, which are often coated with an insulating outer shell of a different material (e.g., ZnS). They offer unique fluorescent properties, such as wide excitation spectra and intensive, narrow emission, tunable by varying their size or composition. The quantum dots are more stable and resistant to photobleaching than traditional fluorescent markers [4–9]. A major weakness of such probes lies in their cadmium-related toxicity, the necessity of a surface modification in order to render them water-soluble, as well as a lack of the ability of selective accumulation in the area of interest. Therefore, they require an appropriately designed carrier, which would deliver a probe to the desired site without causing any damage to the surrounding tissues [10–13]. The advantage of the nanocarriers over microvehicles relies on a more efficient uptake of the smaller objects by the cells [14]. On average, the pore sizes in the cell membrane of tumor cells are in the range of 380–780 nm, which makes the nanosized carriers easier for targeting. Apart from the proper size, the nanocarriers should be stable, biocompatible, easy to manufacture and to use. They should also be able to improve the solubility of hydrophobic compounds and protect the active agent from harsh biological environment [15,16].

The list of colloidal carriers used for drug delivery and imaging purposes includes simple emulsions, liposomes, different nanoparticles and nanocapsules. A convenient procedure of fabricating nanocapsules with promising therapeutic benefits is the so-called layer-by-layer (LbL) assembly of polyelectrolytes, which has been extensively studied over last years [17–20]. This procedure is based on a sequential adsorption of various polyanions and polycations on the charged surfaces and interfaces. Therefore, it can be considered as the electrostatically driven process leading to the formation of a stable film assembly with desired thickness and properties. Initially applied on flat microscopic surfaces, the layer-by-layer adsorption technique has become a versatile tool for the formation of polyelectrolyte nanocapsules [21–25]. The shell can be functionalized to reduce toxicity and to regulate the release rate of active agent, as well as to minimize the response of a host immune system or to

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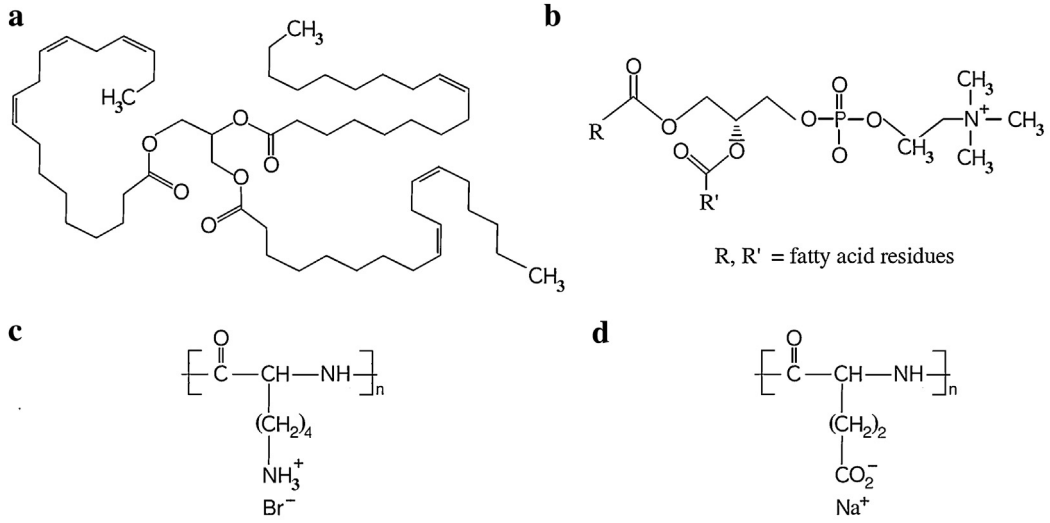

Fig. 1. Chemical structures of the studied compounds: (a) linseed oil, (b) lecithin, (c) poly-L-lysine hydrobromide and (d) poly-D-glutamic acid sodium salt.

immobilize specific antibodies for targeted delivery [26–30]. The core of a capsule can be either solid (e.g., charged colloidal particles, hydrophilic nanoobjects) or liquid (emulsion droplets). In the latter case, the hydrophobic components can be solubilized in the swollen micelles or (nano-) microemulsion droplets and directly encapsulated within the polyelectrolyte shell [31–34]. An important prerequisite for the use of liquid cores in the layer-by-layer encapsulation is a stable surface charge of the applied template. Thus, the use of ionic surfactants in the emulsification process helps to meet that requirement. Additionally, these surfactants should be preferably soluble in the oil phase, because the water-soluble surfactants tend to desorb from the oil–water interface and preferentially form complexes with polyelectrolytes [35]. In order to minimize the toxicity of obtained carriers, a use of biological-origin compounds with surface-active properties is worth consideration.

The nanoemulsion templates for the LbL encapsulation can be prepared using either high-energy (mechanical) or low-energy methods [36–38]. In our previous work, we reported the preparation and characterization of the natural-oil nanoemulsions using the spontaneous emulsification technique (the "Ouzo effect") [39]. At the beginning of this two-step process, the oil is mixed with a water-miscible solvent. Upon the addition of aqueous phase, a dispersion of oil droplets in water is formed due to interdiffusion of the water and water-soluble organic phase and oil supersaturation. The size of obtained emulsion is easy to control by changing basic parameters of the process, such as the oil-to-ethanol ratio, temperature or a choice of surfactant [40–43]. Particularly, the emulsions of linseed oil stabilized with lecithin were characterized by a small size, a low polydispersity and a stable surface charge, which makes them good candidates for further encapsulation within polyelectrolytes. Additionally, both the components are natural and biocompatible products. In this work, we used the linseed oil-chloroform Ouzo emulsion with lecithin as a core for encapsulation of the CdSe/ZnS hydrophobic quantum dots, which were enclosed within the two (PLL/PGA) bilayers. We compared the cytotoxicity of the resulting capsules with that for the bare emulsion containing quantum dots.

2. Materials and methods

2.1. Materials

The linseed oil, phosphatidylcholine (lecithin from egg yolk, type VI-E, ≥99%) and both the polyelectrolytes [poly-L-lysine hydrobromide (PLL), $M_W \sim 15{,}000–30{,}000$ and poly-D-glutamic acid sodium salt (PGA), $M_W \sim 15{,}000–50{,}000$] were purchased from Sigma–Aldrich (for their chemical structures, see Fig. 1). The hydrophobic CdSe/ZnS quantum dots, coated with long-chain alkyl-thiol and soluble in chloroform were ordered from PlasmaChem GmBH, Berlin. The average size of quantum dots is ca. 7 nm. The characteristic emission wavelength of the quantum dots used in this study is equal to 650 nm. The sodium chloride, ethanol (99.8% pure) and chloroform were obtained from POCH Gliwice. All the materials were used without further purification. The water used in all experiments was prepared in the three-stage Millipore Direct-Q 3UV purification system.

2.2. Preparation of the emulsions

The emulsions were prepared using the spontaneous emulsification technique, also known as the "Ouzo effect". In the first step of this process, an organic phase consisting of selected oil and a water-miscible solvent is prepared. The resulting homogeneous solution is mixed with water. A rapid diffusion of the water-soluble solvent into the aqueous phase causes the supersaturation of oil and liquid–liquid nucleation of emulsion droplets. In our previous work, the emulsions of linseed oil were prepared upon mixing of the oil phase with ethanol and water [39]. Here, in order to increase the solubility of hydrophobic quantum dots in the emulsion core, a part of the linseed oil was replaced with chloroform containing 1–5 mg/ml of CdSe/ZnS. The volume ratio between the linseed oil and chloroform was equal to 1:1. Later on, 0.1 ml of the oil phase was injected into 2 ml of ethanol, which contained 5×10^{-2} M lecithin. A solution of the oil with ethanol was mixed with a magnetic stirrer at 350 rpm for a few hours, until a clear single-phase solution was obtained. In the second step of the process, 0.1 ml of the previously prepared organic phase was added to 10 ml of water during continuous stirring at 350 rpm. Due to the rapid diffusion of ethanol into the aqueous phase, an O/W emulsion containing 0.05 vol% of oil phase was formed instantaneously. During this process, the solution becomes cloudy, which indicates a presence of oil droplets big enough to scatter the light.

2.3. Preparation of the capsules

The nanocapsules were obtained by a direct encapsulation of the oil cores within the polyelectrolyte shells using the LbL method. The idea of the preparation process of nanocontainers is schematically