Colloids and Surfaces B: Biointerfaces 143 (2016) 131–138

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Intracellular interactions of electrostatically mediated layer-by-layer assembled polyelectrolytes based sorafenib nanoparticles in oral cancer cells

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ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form 7 March 2016
Accepted 8 March 2016
Available online 10 March 2016

Keywords:
Sorafenib
Kinase inhibitor
Layer-by-layer assembled polyelectrolytes
Calcium carbonate nanoparticles
Oral cancer cells

ABSTRACT

In this paper, we report the preparation of LbL-nanoSraf (100–300 nm) comprising of layer-by-layer (LbL) assembled polyelectrolytes dextran-sulfate/poly-L-arginine, with a multikinase inhibitor sorafenib (Sraf) encapsulated calcium carbonate ($CaCO_3$) nanoparticles for oral cancer therapy in vitro. The zeta potential of LbL-nanoSraf exhibited a negative charge of the polyanionic dextran sulfate, which alternated with a positive charge of polycationic poly-L-arginine indicating a successful LbL assembly of the two polyelectrolyte bilayers on the $CaCO_3$ nanoparticles. The LbL-nanoSraf exhibited an encapsulation efficiency of 61 ± 4%. The LbL-nanoSraf was characterized using field-emission gun scanning electron microscopy, X-ray powder diffraction, atomic force microscopy and confocal laser scanning microscopy. Confocal laser scanning microscopy, flow cytometry and transmission electron microscopic investigations showed the internalization of LbL-nanoSraf in human oral cancer (KB) cells. The LbL-nanoSraf exhibited more potent antiproliferative, apoptotic and antimigratory activities in KB cells than the free drug Sraf. The findings could promote the application of nano-sized LbL assembled polyelectrolytes for the delivery of Raf-kinase inhibitors and provide mechanistic insights for oral cancer therapy.

1. Introduction

Oral cancer has emerged as one of the deadliest cancers and the incidence of oral cancer is increasing with an alarming rate [1,2]. The major causes of oral cancer include the consumption of alcohol and tobacco and exposure to human papillomavirus (HPV). Despite continued advancement in diagnostic techniques and treatment modalities such as surgery and chemoradiotherapy, the treatment of oral cancer is still a major challenge [1–3]. Therefore, a new therapeutic modality for oral cancer remains a priority. Small molecule inhibitors of tyrosine kinases (TKIs) such as sorafenib represent an attractive therapeutic strategy for cancer treatment [4]. Sorafenib inhibits tumor cell proliferation, tumor angiogenesis, metastasis and invasion [4,5]. Sorafenib has been shown to inhibit tyrosine kinases, RAF/MEK/ERK pathways and c-Raf-1 and B-Raf proteins [4,5]. The therapeutic effects of sorafenib have been validated in a number of preclinical and clinical studies including advanced hepatocellular carcinoma, renal cancer, breast cancer, colon cancer, melanoma, non-small cell lung cancer and drug resistant cancers [6,7]. Recently, the combination of sorafenib and radiation has been found to produce synergistic effects in oral carcinoma [8,9]. However, poor oral bioavailability, low solubility, non-specific targets, rapid metabolism and clearance, repeated high dose medication, adverse side-effects such as gastrointestinal bleeding, hypertension, hepatotoxicity, dermatological toxicity (hand-foot syndrome), dysgeusia and diarrhoea severely limit the therapeutic effectiveness of sorafenib in cancer patients [10,11]. However, an efficient delivery system can circumvent the side effects of sorafenib, which in turn will increase the efficacy of sorafenib for oral cancer therapy.

Several types of delivery systems have been designed to increase the oral bioavailability and targeting ability of sorafenib [12–18]. Sorafenib nanoformulations [12], solid lipid nanoparticles [13], folate-polymeric polyethylene glycol-block-poly(ε-caprolactone) micelles with superparamagnetic iron oxide [14], transferrin-albumin nanoparticles [15], peptide arginine-glycine-aspartic acid (RGD)-porous silicon nanoparticles [16], multiblock polymer poly(lactic acid)-poly(ethylene glycol)-poly(L-lysine)-diethylenetriamine pentaacetic acid, the pH-sensitive material poly(L-histidine)-poly(ethylene glycol)-biotin [17], and lipid coated nanodiamonds [18] have been reported so far using

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http://dx.doi.org/10.1016/j.colsurfb.2016.03.024
0927-7765/

sorafenib. Herein, we employed a layer-by-layer (LbL) deposition technique of electrostatically interacting polymers pairs of opposite charges onto a core template followed by the dissolution of the core template. This versatile technique allows an efficient encapsulation of a small molecule inhibitor under mild conditions [19].

Small molecule inhibitors face drawbacks such as instability, inability to cross the blood-brain barriers, pH sensitivity and rapid degradation. Nonetheless, an efficient loading of small molecule inhibitors in a polyelectrolyte shell could overcome some of these disadvantages. A modification of the particle surface by coating with ionic polyelectrolytes layers could make the small molecule inhibitors more effective in therapeutic regimens. The surface chemistry retains the activity in an intact state and allows a sustained release of the entrapped molecules from the system [20,21]. Earlier studies reported calcium carbonate ($CaCO_3$) microparticles using LbL assembly for the delivery of bioactive compounds [22,23]. Nanocolloids of paclitaxel and lapatinib prepared by the sonication-assisted LbL method were evaluated in multidrug resistant ovarian cancer cells [24]. Further, the polymeric multilayer dextran-sulfate/poly-L-arginine microcapsules as vaccine-delivery vehicles in dendritic cells displayed well-toleration in the mucosal tissue and induced potent cellular immune responses [25]. $CaCO_3$ nanoparticles were earlier reported for the sustained release of granulocyte colony-stimulating factor and betamethasone phosphate [26]. Recently, Vergaro et al., have shown $CaCO_3$ nanoparticles synthesized by an atomization process with silane modification and polyelectrolyte nanocapsules-based cisplatin delivery system [27]. Inspired by these findings, we used $CaCO_3$ as the core template and dextran-sulfate/poly-L-arginine as the polyelectrolytes (PE).

Herein, we synthesized LbL-nanoSraf comprising of LbL assembled polyelectrolytes dextran-sulfate/poly-L-arginine, with sorafenib (Sraf) encapsulated $CaCO_3$ nanoparticles (NP). The physicochemical characterization of the LbL-nanoSraf such as size, charge, surface morphology and the uptake efficiency in KB human oral cancer cells were undertaken. Further, the intracellular interactions of LbL-nanoSraf in KB cells were analyzed. LbL-nanoSraf potently inhibited the proliferation of KB cells and induced apoptosis in these cells. Interestingly, LbL-nanoSraf strongly retarded the migration of KB cells than Sraf. The present study revealed the superior anticancer potential of LbL-nanoSraf system in comparison with free drug Sraf against oral cancer cells.

2. Experimental

2.1. Materials

Sorafenib tosylate (Sraf) was a kind gift given by Cipla Ltd., India. Calcium chloride, sodium carbonate, dextran sulfate (10 kDa), poly-L-arginine HCl (70 kDa), rhodamine B (RhB), sulforhodamine B (SRB), propidium iodide (PI), fluorescein isothiocyanate (FITC) and phosphate buffered saline (PBS) pellets were purchased from Sigma-Aldrich, India. Ethylenediaminetetraacetic acid disodium salt (EDTA), dimethyl sulfoxide (DMSO) and other chemicals were of analytical grade. Human oral cancer KB cells were procured from National Centre for Cell Science (NCCS), Pune, India. Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution was procured from HiMedia, Mumbai, India and used as the cell culture medium. Cells were grown in a humidified environment at 37 °C with 5% CO_2.

2.2. Preparation of LbL-nanoSraf

The LbL-nanoSraf was prepared using $CaCO_3$ as a biocompatible core template. $CaCO_3$ NPs were synthesized as described previously with some modifications [19,26]. To encapsulate the drug Sraf or dye RhB, 200 μL of a 2 mg/mL stock solution of drug or dye was added to 0.1 M $CaCl_2$ and mixed for 10 min. This solution was then added dropwise to 0.1 M Na_2CO_3 solution under vigorous stirring at 2000 rpm for 15 min. A stirring speed at 2000 rpm led to the formation of small-sized NPs. The $CaCO_3$ NPs were washed with distilled water and subsequently coated by dispersing in 5 mL of polyanionic dextran sulfate solution (2 mg/mL) containing 0.5 M NaCl. The $CaCO_3$ NPs were collected by centrifugation and the excess dextran sulfate was removed by washing two times with sterile distilled water. The $CaCO_3$ NPs were stirred in 5 mL of polycationic poly-L-arginine solution (1 mg/mL) in 0.5 M NaCl, centrifuged, and washed with sterile distilled water to remove excess poly-L-arginine. The layering process was repeated till the polyelectrolytes dextran sulfate/poly-L-arginine was deposited forming two bilayers. Then, the LbL NPs were incubated in 0.2 M EDTA solution (pH 5.2) for 10 min for the $CaCO_3$ core dissolution, centrifuged and the pellet was washed carefully. The washing steps were repeated three times to remove the dissolved ions. The NPs were finally resuspended in 1 mL of PBS (pH 7.4) and the concentration was determined by hemocytometry. The bare LbL NPs were prepared similarly in the absence of the drug Sraf or dye RhB.

2.3. Physico-chemical characterization of LbL-nanoSraf

2.3.1. Dynamic light scattering and zeta-potential analysis

The size and size distribution of bare LbL NP (1 mg/mL) and LbL-nanoSraf (1 mg/mL) suspension were measured by dynamic light scattering (DLS, 90 Plus, Brookhaven Instruments Corporation, USA). The surface charge of bare LbL NPs and LbL-nanoSraf was determined by ZetaPlus Zeta-potential analyzer (Brookhaven Instruments Corporation, USA) at room temperature in double deionised water. The zeta potential was determined at each adsorption step during the LbL synthesis procedure. The experiments were performed three times.

2.3.2. Optical microscopy

A drop of LbL-nanoSraf suspension was placed on a glass slide and the number of particles was counted using a haemocytometer with the help of an Olympus light microscope.

2.3.3. Field-emission gun scanning electron microscopy analysis

For field-emission gun scanning electron microscopy analysis (FEG-SEM), the dried Sraf-$CaCO_3$ NP and LbL-nanoSraf samples before and after the core dissolution with EDTA were placed on a stub, and coated with gold using a sputter gold coater Auto Fine Coater (JEOL, Tokyo, Japan). The images were captured using a FEG-SEM (JSM-7600F, Japan).

2.3.4. Confocal laser scanning microscopic analysis

A drop of RhB-$CaCO_3$ NP and LbL-nanoRhB suspensions before and after the core dissolution with EDTA was placed on a cover glass-slip. Images were taken using FV500 Confocal laser scanning microscope (CLSM, Olympus, Tokyo, Japan). The line scan fluorescence intensity of RhB was recorded.

2.3.5. X-ray diffraction analysis

To analyze the degree of crystallinity, X-ray diffraction (XRD) identification of Sraf-$CaCO_3$ NP vs LbL-nanoSraf was performed by powder XRD using a Philips powder diffractometer PW3040/60 with Cu Kα radiation (λ = 1.54 Å) at an accelerating voltage of 40 kV, current 30 mA, scanning rate 4°/min and 2θ range from 20° to 80°.

2.3.6. Atomic force microscopic analysis

To analyze the surface topography, a drop of Sraf-$CaCO_3$ NP and LbL-nanoSraf suspensions were applied onto a freshly cleaved mica