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Nanostructured polysaccharidic microcapsules for intracellular release of cisplatin

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ABSTRACT

Carbohydrate polimeric microcapsules were assembled using a LbL approach onto a $CaCO_3$ core. The microcapsules were used to delivery the anticancer drug *cisplatin* into HeLa and MCF-7 cancer cell lines. Drug encapsulation, measured by ICP spectroscopy, was around 50% of the charging solution. Fluorimetric measurements showed an efficient cellular uptake of polysacchardic microcapsules in both cell lines. The drug-loaded capsules demonstrated a better efficiency against cell viability than the free drug. Specifically, the amount of platinum reaching genomic DNA was measured, showing that encapsulation improves the nuclear delivery of the drug for both cell lines.

1. Introduction

In the last few years biopolymeric nanoengineered 2D and 3D structures have gained an increasing attention due to their high potentialities in the biomedical field, as drug delivery carriers and as scaffolds for tissue engineering [1,2]. In this respect, natural occurring polysaccharides are the most popular biopolymeric materials due to their biocompatibility, biodegradability, high availability and reduced costs in their processing [3–5]. Moreover, polysaccharides, due to their chemical structure, display bioadhesive properties, such as high affinity for mucosal surfaces, making them ideal drug carriers able to prolong drug residence time and therefore to increase drug bioavailability. Among the various polysaccharide based drug carriers, layer-by-layer self assembled (LbL) polysaccharide capsules are particularly attractive since they can be loaded with active molecules, degraded by proteolytic processes and engineered for specific cell targeting [6–11]. The LbL technique is based on the alternate deposition of oppositely charged polyelectrolytes onto the surface of a support. In the case of the fabrication of hollow capsules, micro/nano-particles are used as sacrificial supports, which are dissolved after completion of the LbL assembly of the polyelectrolyte nanostructured shell [12–16]. Stimuli responsive polymeric capsules and nanoshells formed via layer-by-layer (LbL) were extensively presented in literature [17,18]. Shells with a controlled architecture, and thus with finely tuned functionality, can be obtained by controlling the assembly conditions and depending on the intrinsic properties of the selected polysaccharides. Both the hollow cavity and the polyelectrolyte shell can be loaded with active molecules and nanoparticles [19–25]. Studies reported in literature and also in our previous work demonstrated that LbL capsules, obtained by using $CaCO_3$ microparticles as sacrificial supports, can be considered as ideal drug carriers due to their excellent biocompatibility and ability to readily penetrate cancer cells [26–31]. In the present work novel LbL capsules, obtained by the self assembly of two polysaccharides onto calcium carbonate ($CaCO_3$) microparticles were fabricated, characterized and used as delivery system for the anticancer drug cisplatin. Namely, cationic chitosan and anionic pectin were used for the LbL deposition of the polysaccharide

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shell. Chitosan is a cationic copolymer of glucosamine and *N*-acetyl glucosamine obtained by the deacetylation of chitin [32]. Due to the presence of reactive amino groups, to its biocompatibility, biodegradability and bioadhesive properties, chitosan was deeply investigated for its use in pharmaceutical formulations, tissue engineering and food technology [33]. Pectin is an anionic polysaccharide extracted from citrus peels and apple pomace consisting mainly of linear chains of α-(1-4)-D-galacturonic acid. The carboxylic acids are partially methoxy-esterified and the degree of esterification affect to the anionic charge of pectins and their interaction with cations, such as Ca^{2+}, and polycations, such as chitosans [34,35]. Pectin can also interact with poorly soluble calcium salts, such as $CaCO_3$. Interestingly, it has been demonstrated that pectins could target tumor cells by binding to the galectin-3 receptor to inhibit cancer progression and metastasis [36–38]. Although chitosan and pectin are very promising polysaccharides for the fabrication of drug delivery systems, chitosan-pectin LbL complexes have been scarcely reported [34,39,40].

Herein, the chitosan and LM pectin LbL assembly onto $CaCO_3$ microparticles was characterized by ζ-potential measurements. Hollow capsules were then obtained by dissolution of the $CaCO_3$ microparticles and they were characterized by scanning electron microscopy and confocal microscopy. The hollow capsules were then used as carrier system for the most widely used platinum-based anti-neoplastic agent, *cis*-dichloro diammineplatinum(II) (*cis*-$[PtCl_2(NH_3)_2]$, cisplatin) [41]. Cisplatin is usually administered intravenously for treatment of solid malignancies [42]. However, a major obstacle to more widespread use of cisplatin is the persistence of severe toxic side effects [43]. Other disadvantages associated with cisplatin clinical use include short circulation period [44], intrinsic or acquired resistance of some tumors and its limited aqueous solubility (1.0 mg/mL) [45]. The use of a carrier system to deliver platinum drugs into cancer cells has been emerging as a promising strategy to enhance their anticancer activity and to mitigate negative side-effects. In this respect, anionic cisplatin was loaded into the spacing between chitosan and pectin layers [46], and thus protected, by its interaction with cationic chitosan [47]. The drug loading, release profiles, as well as cytotoxicity against HeLa and MCF-7 cells were evaluated. The results obtained by using chitosan and pectin pair were compared to those obtained by using two well known synthetic polyelectrolytes, namely cationic poly(allylamine) hydrochloride (PAH) and anionic polystyrene sulfonate sodium salt (PSS).

2. Materials and methods

2.1. Materials

Cationic poly(allylamine hydrochloride) (PAH, Mw 70 kDa), anionic poly(styrene sulfonate) (PSS, Mw 70 kDa) and Chitosan (CHI, medium molecular weight, 448877) were purchased from Sigma-Aldrich. Low methoxyl pectin (PEC LM 38% degree of esterification, CU701, lot 968, from citrus fruits) was kindly provided by Herbstreith & Fox (Nuremberg, Germany). $CaCO_3$ microparticles were kindly provided by PlasmaChem GmbH (Germany). All reagents were of analytical reagent grade and double distilled water was used. Commercial products were purchased from Sigma-Aldrich.

2.2. Capsules fabrication

Polyelectrolyte microcapsules were prepared by alternating incubation of $CaCO_3$ microparticles in CHI and PEC aqueous solutions (0.5 mg mL^{-1}). The pH of the polymer solutions was adjusted to 5.0 by addition of acetic acid or NaOH/HCl, respectively. Capsules were fabricated in a two-step procedure. In a first step, the $CaCO_3$ microparticles were coated by using a LbL technique. 1.0 mg of powder of $CaCO_3$ were dispersed in a solution containing the polycation (chitosan, CHI). The dispersion was continuously shaken for 20 min. The excess polycation was removed by three centrifugation/washing steps with deionized water. Thereafter, 1.0 mL of solution containing the polyanion (pectin, PEC) was added and the dispersion was continuously shaken for 20 min, followed again by three centrifugation/washing steps. This procedure was repeated 3 times for each polyelectrolyte resulting in the deposition of six polyelectrolyte layers on the $CaCO_3$ particles. Coated particles were treated with 0.5% glutaraldehyde (GA) at room temperature (20–25 °C) for 30 min followed by three centrifugation/washing steps. In a second step, the $CaCO_3$ core was removed by complexation with EDTA: coated particles were shaken for 2 min with 1.0 mL of 0.2 M EDTA solution at pH 5.5 (adjusted by HCl), followed by centrifugation and redispersion in 1.0 mL of a fresh EDTA solution pH 7.5. The samples were then washed four times with deionized water and stored at 4 °C in water, as previously suggested by Volodkin et al. [48,49]. To fabricate capsules with fluorescent-labeled polyelectrolytes, the same procedure was used resorting to use polymer FITC-conjugated during coating procedure. In order to study the localization and internalization of these microparticles, polymeric colloids of micro-$CaCO_3$ microparticles were prepared using two synthetic polyelectrolytes, PSS and PAH-FITC.

2.3. Scanning electron microscopy (SEM)

For SEM analysis, samples were prepared by applying a drop of the particle suspension to a SiO_2 wafer and then dried overnight. The samples were then sputtered with gold and measurements were conducted using a Carl Zeiss Merlin Microscope.

2.4. Cell culture

MCF-7 (human breast cancer), HeLa (human cervical cancer), cell lines were used. Cancer cell lines were maintained in DMEM medium supplemented with FBS (10%), penicillin (100 U/mL culture medium), streptomycin (100 μg/mL culture medium), glutamine (5%). Cells were grown in a humidified incubator at 37 °C, 5% CO_2, and 95% relative humidity. Cell lines were serum-starved for 24 h before any test.

2.5. Cytotoxicity test

Cytotoxicity was tested towards MCF-7 and HeLa cell lines and evaluated by MTT, measuring the activity of living cells via mitochondrial dehydrogenase activity. The key component is 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or MTT. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals, insoluble in aqueous solutions. Cells (10^5 cells/mL) were added to 24-well culture plates at 1000 μL/well, serum-starved for 24 h, and incubated at 37 °C in 5% CO_2, 95% relative humidity for different time point (24, 48, 72 h) with the microcapsules, counted with a bright line hematocytometer (cell counting chamber, Sigma), suspended in complete medium (ratio cell:capsules = 1:10) or, as a control, with complete culture medium alone. After the incubation period (24, 48, 72 h), a MTT solution in an amount equal to 10% of the culture volume was aseptically added to the cultures. Cultures were returned to incubator for 3 h. Then, the resulting MTT formazan crystals were dissolved with dimethysulfoxide within 15 min. Absorbance at wavelength of 570 nm was spectrophotometrically measured using the ELISA plate reader. Results were expressed as mean ± standard deviation of three separate samples.