In Vitro Delivery of Docetaxel to Cancer Cells by Hybrid PLGA@Organosilica Nanoparticles with Redox-Sensitive Molecular Gates

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ABSTRACT

A novel type of nanomedicine based on a PLGA nanoparticle core and a redox-responsive amorphous organosilica shell has been successfully developed. The outer layer is obtained by self-assembly of silicate ions with a disulfide bridge containing silsesquioxane. These organic linkers work as molecular gates that can be selectively cleaved by reducing agents. This system is particularly suitable for storage and release of hydrophobic drugs, as docetaxel (DTX), as the treatment with reducing compounds leaves open doors that allow for the discharge of DTX in the organic matrix. These nanohybrids impose a better control and slower release of encapsulated molecules than bare PLGA nanoparticles, are reasonably stable in physiological medium and show higher cytotoxic activity over HeLa cells than the free drug.

Keywords: cancer therapy, docetaxel, PLGA-silica, redox-responsive, controlled release

1 INTRODUCTION

One of the most successful current nanoplatforms for the delivery of docetaxel (DTX) and other hydrophobic drugs to cancer cells are those based on biodegradable nanoparticles of poly(lactic-*co*-glycolic acid) (PLGA) [1]. Unfortunately, in most cases they suffer from burst release [2], as the accumulation of the drug on the surface of the particle causes the premature discharge of more than 20-30% of the cargo, leading to increased toxicity and decreased therapeutic activity [3]. Therefore, it is compulsory to implement accurate control over the release behavior of PLGA nanoparticles [4-12]. To address this issue, recently, we have developed a novel hybrid material based in spherical PLGA nanoparticles containing hydrophobic molecules which have been covered by a thin layer (6-10 nm) of a redox-responsive amorphous organosilica shell (PLGA@SiOS) [13]. We illustrate this concept by the self-assembly of tetraethyl orthosilicate and a silsesquioxane containing a disulfide bridge. As a consequence, the outer layer incorporates a number of disulfide bonds working as chemical doors that can be selectively cleaved by intracellular reducing compounds (e.g., glutathione, GSH), leading to disassembly of the silica wall. Herein, we present the *in vitro* evaluation in HeLa cervix cancer cell line of this novel hybrid organic-inorganic nanoplatform loaded with the antitumor drug DTX (PLGA-DTX@SiOS).

2 EXPERIMENTAL

PLGA PURASORB® 5004 (lactide:glycolide = 53:47, Mw ~ 20000) was provided by Purac. Other reagents were purchased from Aldrich except HPLC solvents (HPLC grade from Scharlab). HeLa cells were originally obtained from the American Type Culture Collection (Rockville, MD) maintained in RPMI media supplemented with 10% fetal bovine serum (FBS, from Lonza, Verviers, Belgium) at 37 ºC under a humidified atmosphere of 95% air and 5-10% CO_2.

2.1 Synthesis of DTX-loaded PLGA-organosilica nanoparticles (PLGA-DTX@SiOS)

Initially, DTX loaded PLGA nanoparticles coated with a cationic shield of cetyltrimethylammonium bromide (CTAB) were prepared by a modified oil-in-water (o/w) emulsion procedure [14]. Afterwards, PLGA-DTX@CTAB nanoparticles were covered with a thin layer of a redox-responsive amorphous organosilica shell, containing intercalated disulfides bridges, by self-assembly of tetraethyl orthosilicate (TEOS) and Bis[3-(triethoxysilyl)propyl] disulfide (TESPDS). The initial gel molar composition was 1:0.20:0.15:58:2232 SiO_2/TESPDS/NH_4OH/iPrOH/H_2O. The solution was left stirring for 96 h. Particles were recovered by centrifugation (9600 g, 30 min), washed with H_2O and ethanol (EtOH) and freeze dried. 100 nm average diameter particles were obtained. For the sake of comparison, DTX-loaded PLGA nanoparticles covered with a thin layer of amorphous silica (PLGA-DTX@SiO_2), were prepared by polymerization of TEOS over PLGA-DTX@CTAB spheres. All materials were characterized by elemental and thermogravimetric analysis, TEM, FESEM, Z-potential, ^{29}Si-MAS-NMR, and FTIR.

2.2 Redox-Responsive Release of Docetaxel

0.5 mg of the hybrid PLGA@organosilica material weighted in a μg scale for each data point were suspended in 0.5 mL of PBS and placed in a Slide-A-Lyzer Mini Dialysis Device (10K molecular weight cutoff). Each

microtube was dialyzed to 14 mL of PBS at 37 ºC while gently shaking. Then, GSH was added after 2 h up to 10 mM. At the corresponding time the suspension was diluted with 0.5 mL of acetonitrile (ACN) and ultrasonicated for 30 minutes to ensure all the particles were dissolved and the remaining DTX was totally released. DTX concentration was determined by RP-HPLC and ESI-MS. A control experiment was done with PLGA-DTX@SiO_2 material following the same procedure, but in the absence of GSH. Initial DTX loading in the materials was calculated by promoting complete release with ultrasonication (30 min) in ACN of a non-dialyzed sample. Triplicate samples were run for every experiment.

2.3 *In vitro* study

HeLa cells (2000 cells/well, 96-well plates) were treated with DTX loaded nanocarriers, or DTX (in DMSO), with final doses ranging from 0.0001 to 1 μg mL^{-1} (in DTX equivalents) during 24 hours. At the end of the incubation period, MTT solution in PBS was added at a final concentration of 0,2 mg mL^{-1} to the wells and 4 h later formazan crystals were dissolved in DMSO and spectrophotometrically measured at 550 nm. Half maximal inhibitory concentration (IC_{50}) data were evaluated by variable slope curve-fitting using Prism 5.0 software (GraphPad, San Diego, CA). Three to five independent experiments were performed for the different samples.

3 RESULTS AND DISCUSSION

Monodispersed nanoparticles were obtained with average diameter in the range 40-155 nm and typical core-shell outline, with an organic core made of PLGA@CTAB and an inorganic shell built with amorphous silica of 6-10 nm thick (Table 1 and Figure 1a). In the case of PLGA-DTX@SiOS material the coating also intercalates a number of disulfide bridges, building an organosilica corona. Zeta potential determination confirmed stable colloids in aqueous medium with negative charge on the surface due to partially ionized silanol groups. Samples were highly homogeneous, presenting less than 5% of considerably bigger particles that tend to collapse. Additional characterization of these materials may be found elsewhere [13].

Sample	SiOS/SiO_2 (M)	D (nm)	Z (mV)
PLGA-DTX@SiO_2[a]	0	92±42	-20
PLGA-DTX@SiOS[a]	0.20	76±27	-12

Table 1: Compositional and structural characteristics of as-synthesized materials.

In this work we have incorporated a hydrophobic drug, DTX, within the PLGA core. After the self-assembly of silica and the silsesquioxane the amount of DTX loaded in the organic matrix reaches 1 wt%. Then, in order to check the redox-responsive character of these organic-inorganic composites, we have carried out a release experiment of PLGA-DTX@SiOS sample in PBS solution containing GSH (10 mM), monitoring the DTX concentration by HPLC-UV analysis. DTX loaded PLGA@SiO_2 material (with no disulfide gates in the silica coating) was used as control. DTX nanomedicine design and release mechanism driven by reducing compounds are tentatively depicted in Figure 2.

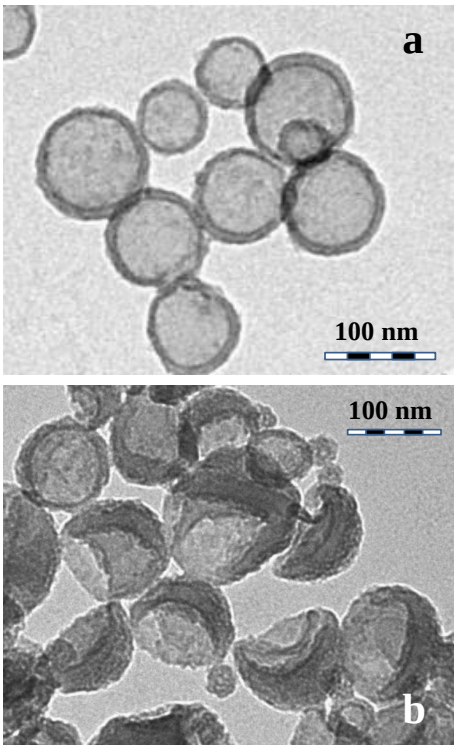

Figure 1: (a) TEM image of as-synthesized hybrid PLGA-DTX@SiOS nanoparticles. (b) TEM image of PLGA-DTX@SiOS nanoparticles after drug release.

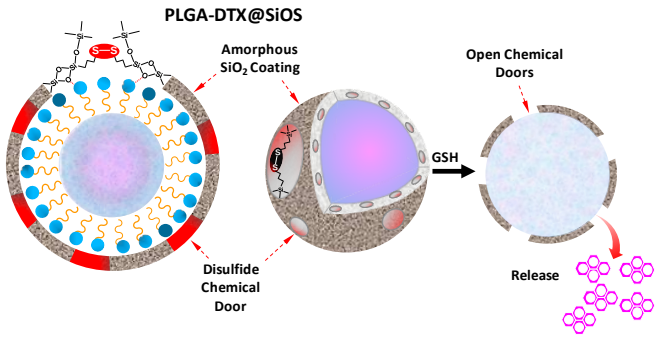

Figure 2: Design of the novel DTX nanoplatform and the release mechanism driven by reducing compounds.

PLGA-DTX@SiOS is quiet stable in PBS and only releases DTX after GSH addition. Drug discharge takes place by disulfide bridges reduction and water diffusion through the pores created in the organic-inorganic wall, degrading the organosilica shell. Here, PLGA-DTX@SiOS exhibits a sustained release of DTX that achieves about 80% after one week. Conversely, PLGA-DTX@SiO_2, with pure silica external coating, presents a very different release pattern. In this case, the thin inorganic layer suffers a slow degradation in PBS [15], and after 48 h the carried molecule slowly diffuses outside the hybrid structure. Both PLGA-DTX@SiOS and PLGA-DTX@SiO_2 materials show collapse of most nanoparticles after exposure to reducing agents (Figure 1b). This is a consequence of outer shell partial breaking, due to erosion caused by disulfide bridges cleavage, which leads to severe particle destabilization. As regards in biological applications of these materials, and especially DTX systemic administration, the silica sealed structure with intercalated disulfide molecular- bridges is not a definitive locking system, but it is able to keep safe the therapeutic charge enough time before reaching the target cells.

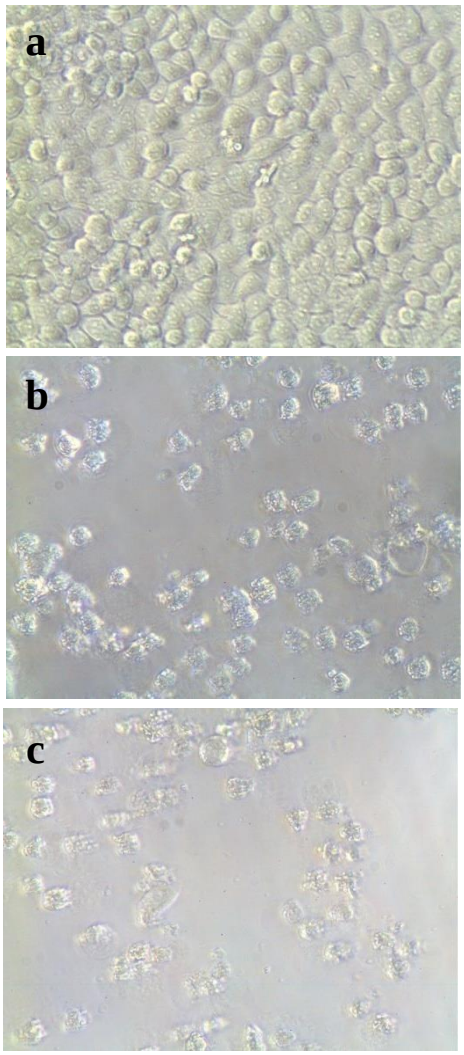

Figure 3: Morphology of HeLa cells after incubation with PLGA@SiOS (a), DTX (b) or PLGA-DTX@SiOS (c).

The observation under the inverted microscope revealed that cytotoxicity was due only to DTX, as cells treated with free DTX and PLGA-DTX@SiOS were dying and did not proliferate, whereas cells treated with DTX-free PLGA@SiOS showed a normal morphology and proliferation rate (Figure 3).

MTT cytotoxicity experiments were conducted by incubating during 24 h HeLa cancer cells with DTX or the nanomedicine (0.0001 to 1 µg mL^{-1} in DTX equivalents) and IC_{50} values were determined (Table 2 and Figure 4). PLGA-DTX@SiOS sample presented a cell survival value clearly lower than the free drug. This is due to the extremely low solubility of DTX in cell culture medium, which complicates its availability in the cell culture. Moreover, it is noticeable that DTX-free PLGA@SiOS nanoparticles show non-significant cytotoxic activity in this experimental conditions.

Sample	IC_{50}	n [b]
DTX	0.013±0.003	5
PLGA-DTX@SiOS[a]	0.004±0.002	3

Table 2: IC_{50} values (mean ± SEM, in µg mL^{-1}) for free DTX and PLGA-DTX@SiOS in HeLa cells. [a] DTX loading: 1 wt%; [b] n = number of experiments.

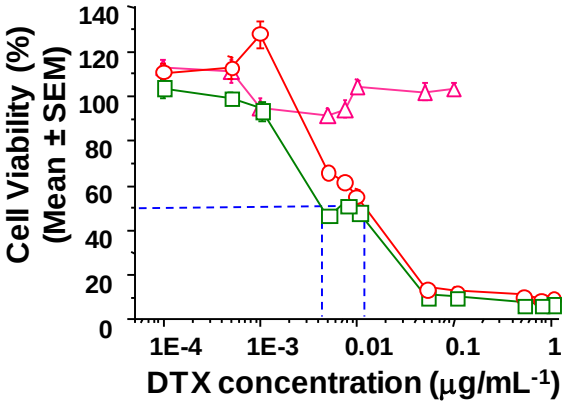

Figure 4: Cytotoxic activity of DTX (○), PLGA@SiOS (△) and PLGA-DTX@SiOS (□) in HeLa cells. Concentration corresponds to DTX equivalents. Number of experiments (n) = see Table 2.

4 CONCLUSION

Novel nanomedicines based in a PLGA nanoparticle core containing docetaxel and a redox-responsive amorphous organosilica shell have been successfully synthesized. The outer layer incorporates a number of disulfide bridges working as molecular gates that can be selectively cleaved by intracellular glutathione, allowing

the discharge of stored molecules in the organic matrix. These nanohybrids impose a better control and slower release of encapsulated molecules than bare PLGA nanoparticles, are reasonably stable in physiological medium and potentially sensitive to redox mechanisms, also improving cytotoxicity and availability of free DTX in *in vitro* studies.

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