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Regular Article

Light-sensitive dextran-covered PNBA nanoparticles as triggered drug delivery systems: Formulation, characteristics and cytotoxicity

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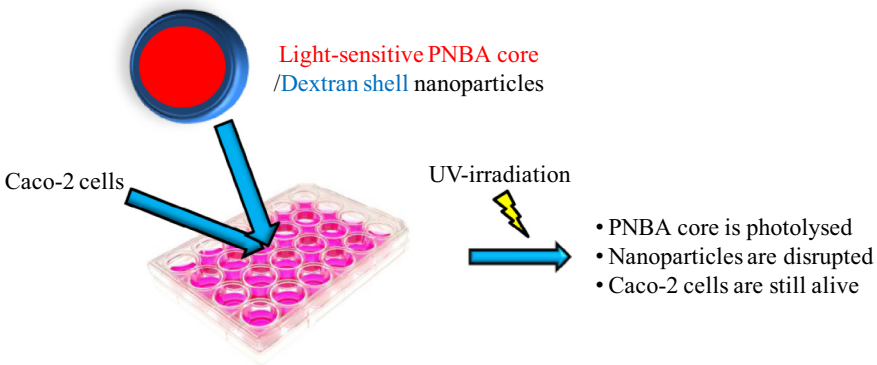
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: For some years, smart nano-objects are one of the main focuses of current research. In the framework of polymeric nanomedicine, *o*-nitrobenzyl alcohol derivatives lead to light-responsive polymeric materials. At this day, nanomedicine based on polysaccharide/poly(*o*-nitrobenzyl acrylate) (PNBA) copolymers have never been reported.
Experiments: For the first time, PNBA core/dextran shell nanoparticles (NPs) were formulated by evaluating two different processes: (i) nanoprecipitation of preformed Dextran-g-PNBA glycopolymers, (ii) emulsion/evaporation using azido-functionalized PNBA and alkynated dextran, carrying out (or not) an interfacial click chemistry reaction. NPs' characterization, colloidal stability in the presence of salts and of an anionic competitive surfactant (SDS) and light-induced disruption were assessed. Finally, the potential use of these NPs as photo-responsive drug delivery systems was investigated by a preliminary *in vitro* cytotoxicity study using Caco-2 cells.
Findings: Whatever the process, the photosensitive property and the colloidal stability of NPs in the presence of salts were proved. However, triazole rings between the dextran shell and the PNBA core avoid the dextran shell desorption in the presence of SDS. NPs' biocompatibility towards Caco-2 was proved and 100% cell viability was still observed after exposure to NPs following by 60 s UV-irradiation.

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1. Introduction

For more than a decade, polymeric nanomedicine that includes micelles, nanoparticles (NPs) or nanocapsules received increasing attention, and many reviews dealing with this subject were published [1–5]. Drug may be loaded into such nano-objects to reduce dosage, minimize side-effects, protect drug from degradation and thus to enhance its efficiency. Nevertheless, a long period in the bloodstream is often expected (case of stealthy nano-objects). To prevent the adsorption of opsonins (circulating proteins) on the nano-object's surface and its subsequent trapping by the reticuloendothelial system therefore its removing from blood flow [6,7], polyethylene oxide (PEO, also called polyethylene glycol - PEG) is commonly used to cover the nano-object and to provide it stealthiness. However, although PEO is approved by Food and Drug Administration (FDA) [8] and after close to nearly half a century of clinical uses, it was shown that PEO induces hypersensitivity reaction, complement activation and anti-PEO antibody formation reactions [8,9]. Several neutral hydrophilic polysaccharides were already reported as promising alternatives of PEO due to their inherent biodegradability, immunogenicity, and bioactivity [10]. In fact, some polysaccharides as dextran allow the colloidal stability of such nano-objects [11–13], prevent interactions with cells and proteins, thus extending the nano-objects' circulation half-life [9], and ensuring their stealthiness. After functionalization of such hydrophilic shell (PEG or dextran for instance) by adequate ligands as antibodies, carbohydrates or peptides, nano-objects can target specific cells to be treated. These nano-objects were called drug delivery system (DDS) [14–18] and some of them, based on biodegradable materials, were approved by FDA and are already commercially available [19].

After encapsulation of drug into DDS, the drug release occurs by DDS degradation or swelling, and/or by diffusion outside the nanocarrier. Commonly, biodegradable hydrophobic (co)polyesters as poly(lactic acid) (PLA), poly(glycol acid) (PGA) and poly(lactide-*co*-glycolide) (PLGA) were used to formulate DDS. In these cases, drug release occurs by diffusion and degradation of the DDS. For some years, scientists took interest to introduce sensitive parts in polymeric materials to reach DDS that are sensitive to internal or external stimuli. Different types of smart or stimuli–responsive DDS are reported as thermosensitive DDS, pH-sensitive DDS, light-sensitive DDS, molecule-responsive DDS, ... [20–24]. After DDS formulation and according to adequate stimulation, the loaded drug is released when and where physicians and clinicians want.

Among all the light-responsive polymeric materials described in literature [25–30], *o*-nitrobenzyl alcohol derivatives are today the most studied in polymer science. *o*-Nitrobenzyl ester bonds are known to be cleft by UV or two photons adsorptions, yielding to carboxylic acid functions [31]. Such bonds were already assessed to light-induce the disassembly of block copolymer micelles [32–36]. Very recently, some of us have reported the first controlled polymerization of *o*-nitrobenzyl acrylate (NBA) [37], then the synthesis of amphiphilic grafted photosensitive glycopolymers called Dex-g-PNBA, composed on dextran (Dex) as hydrophilic backbone and poly(*o*-nitrobenzyl acrylate) (PNBA) as photo-responsive grafts [38]. To the best of our knowledge, the formulation of nano-objects from polysaccharide/PNBA copolymers has never been reported. Indeed, only two papers are dealing with the light-disruption of nano-objects based on diblock copolymers containing one PNBA block and one poly(2-ethyl-2-oxazoline) [39] or polydimethylacrylamide [40] block.

This study aimed to investigate for the first time the formulation of PNBA core/dextran shell NPs that can be used as DDS for anticancer treatments. Two different formulation processes were compared (nanoprecipitation and emulsion/organic solvent evaporation). More precisely, within the emulsion/organic solvent evaporation process, an *in situ* Huisgen-type Copper(I)-catalyzed Azide–Alkyne Cycloaddition (CuAAC) click-chemistry was carried out in some experiments. Then, NPs batches were characterized in term of size, zeta potential, dextran amount and colloidal stabilities in the presence of salts and of an anionic competitive surfactant. The photosensitive character of these PNBA-based NPs was evaluated by varying different irradiation parameters. Finally, and because the colorectal cancer is known to be the third most common cancer worldwide [41–43], preliminary study on the *in vitro* cytotoxicity of these PNBA-based NPs (before and upon UV-irradiation) towards human epithelial colorectal adenocarcinoma (Caco-2) cells was reported.

2. Experimental

2.1. Materials

Amphiphilic alkynated dextran was derived from dextran [38]. The yield of substitution (δ) of such alkynated dextran was estimated equal to 15% (15 pending alkynyl groups per 100 glucopyranosic units), and its surface tension property was checked (Fig. S1). Azido-functionalized poly(*o*-nitrobenzyl acrylate) $PNBA_M$-N_3, where M is the number average molecular weight, was derived from $PNBA_M$-Br obtained *via* a controlled Single Electron Transfer-Living Radical Polymerization (SET-LRP) of *o*-nitrobenzyl acrylate (NBA) [37]. Dex-g-PNBA glycopolymers were obtained by carrying out CuAAC in DMSO [38]. For more information, such glycopolymers are called Dex(δ)-g-α$PNBA_M$, where α is the number of $PNBA_M$ grafts per 100 glucopyranosic units. Weight fractions of PNBA (F_{PNBA}) into such Dex-g-PNBA are varying from 40 to 85% as shown in Table 1. Whatever F_{PNBA}, all Dex-g-PNBA are insoluble in water, but soluble in DMSO or in THF/H_2O mixtures.

Tetrahydrofuran (THF), dichloromethane (DCM), copper bromide (CuBr, 99.9%), sodium dodecylsulfate (SDS), ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich and used without further purification.

2.2. Elaboration of nanoparticles

2.2.1. Nanoprecipitation

25 mg of Dex-g-PNBA were dissolved in 5 mL of THF/H_2O mixture (95/5, v/v; except in case of Dex(15)-g-3$PNBA_{3,700}$ where 75/25, v/v was used) for 24 h, then added drop wise (0.1 mL per min) into 10 mL of distilled water under magnetic stirring. After complete addition, 10 mL of distilled water were added portionwise to freeze the NPs dispersion. Finally, THF was removed by centrifugation (15,000 rpm, 15 °C, 30 min). NPs were washed twice by using distilled water, and then were freeze-dried.

2.2.2. Emulsion/organic solvent evaporation

25 mg of either PNBA-Br, PNBA-N_3 or mixtures (from 0/1 to 1/0) were dissolved in 1 mL of DCM. Meanwhile, 50 mg of alkynated dextran were dissolved in 10 mL of distilled water (DCM-saturated). The organic phase was added under vigorous stirring to the aqueous one, then the mixture was sonicated (pulsed mode, 46 W, 2 min, ice bath) using a Vibracell 75,043 model (Bioblock Scientific). After sonication, DCM was evaporated at 37 °C for 2.5 h under stirring. Suspension was then centrifuged (10,000 rpm, 15 °C, 60 min) and the collected NPs were resuspended in water, centrifuged again in order to remove the non-adsorbed alkynated dextran, and finally freeze-dried.

In some experiments an *in situ* CuAAC was carried out by adding 5 mg of CuBr to the first emulsion under N_2 flow and prior to the