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Synthesis and *in vitro* assessment of anticancer hydrogels composed by carboxymethylcellulose-doxorubicin as potential transdermal delivery systems for treatment of skin cancer

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ABSTRACT

Malignant melanoma is the most lethal form of skin cancer in humans which is difficult to treat by conventional surgery and chemotherapeutics. Despite unquestionable progresses in recent years demonstrated by anticancer drug carriers to target tumor local microenvironment, it is growing at a rate of one million new cases being reported annually because overcoming the skin physiological barriers and the side effects associated with chemotherapy still remain threatening challenges. Herein, we designed and developed a novel polysaccharide-based prodrug composed of carboxymethylcellulose (CMC) polymer with anticancer drug doxorubicin (DOX) forming electrostatic nanocomplexes in aqueous solution. The results evidenced the effect of degree of substitution (DS = 0.77 and 1.22) of CMC on the physicochemical properties of the CMC-DOX complexes associated with the formation of supramolecular colloidal nanostructures. They were stabilized by electrostatic interactions between anionic carboxylate groups from CMC and cationic amino groups of DOX while the polysaccharide polymer chain encapsulated the hydrophobic drug in the aqueous medium. Moreover, these polymer-drug nanoparticulate systems were crosslinked with citric acid for producing advanced tuned drug delivery hydrogels. The results demonstrated the effect of DS of CMC and the addition of DOX on the physicochemical properties of the hydrogel network structures produced including the swelling behavior and gel fraction. Moreover, the distinct DS of CMC tailored the DOX release kinetics *in vitro* showing activity for killing melanoma cancer cells while less cytotoxicity towards normal cells. To this end, an innovative platform was developed based on colloidal polysaccharide-drug nanocomplexes producing anticancer hydrogels offering promising perspectives for skin cancer applications using transdermal drug delivery chemotherapy.

1. Introduction

Skin cancer represents one of the most commonly occurring carcinoma in human and it is growing at a fast rate of approximately one million new cases being reported worldwide annually. Malignant melanoma is the most lethal type of skin cancer and it is associated with unfortunate prognosis usually leading to the death of patient by metastasis. For that reason, melanoma continues to remain a very important life-threating disease. Although there are several possibilities for anti-melanoma therapy, in many cases it can be resistant to some treatments. In addition, when detected at the early stages, the primary cutaneous melanoma can be commonly managed by conventional surgery procedure. However, the advanced metastatic melanoma cannot be appropriately treated by surgery alone, requiring supplementary therapeutic procedures such as chemotherapy, immunotherapy, biochemotherapy, and adoptive cell therapy [1–3]. To reduce or circumventing the common side effects caused by systemic administration of anticancer drugs (*i.e.*, oral or intravenous injection), topical (*i.e.*, local) transdermal drug delivery systems offer an encouraging strategy for the effective therapy of skin cancer [1–3].

Drug delivery systems (DDS) based on polymers are the utmost interesting vehicles in anti-cancer therapy because they present several advantages as (nano)carriers for antineoplastic agents, including increased drug solubility, better bioavailability, high stability, controlled drug release, selective organs or tissue distribution, and reduction of the total dose required. Polymers are very versatile macromolecules that can be engineered to meet numerous properties required for

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developing innovative and sophisticated applications in biology and medicine. The intense research at the interface of polymer chemistry and biomedical sciences has given rise to the polymer-based pharmaceuticals, known as 'polymer therapeutics', which emerged as a new promising field of research. Essentially, it encompasses rationally engineered macromolecular systems associated with active drugs, such as polymer–protein conjugates, polymer-drug conjugates, polymer-drug complexes, polyplexes for encapsulating nucleotides, and supramolecular drug-delivery systems [1, 4, 5].

Among different types of biocompatible polymers, carbohydrate-based polymers or polysaccharides (*e.g.*, hyaluronic acid, chitosan, and cellulose) are the most common natural polymers with chemical structures consisting of long chains of monosaccharide (or disaccharide) units bound by glycosidic linkages. Carboxymethylcellulose (CMC), as a broadly available derivative of cellulose polysaccharide, finds widespread use in biology, medicine, nutrition and pharmaceutical formulations including development of nanocarriers for delivery of anticancer therapeutic agents. It presents excellent properties such as biocompatibility, biodegradability, non-toxicity, suitable reactivity for facile chemical modification combined with availability and low cost. Moreover, they possess highly reactive chemical groups including hydroxyl and carboxyl groups, which can allow chemical biofunctionalization and the formation of reversible and covalent crosslinked polymer networks and conjugates with tailored structures. In addition, CMC presents biocompatibility to the skin and mucous membranes, which has been approved by the United States Food and Drug Administration (FDA) for parenteral use in drug products [4, 6–10].

Therefore, the association of polysaccharides with anticancer drugs is an interesting approach to overcoming the most challenging factor in cancer therapy related to the poor solubility and toxicity of anticancer therapeutic agents for normal cells and healthy tissues. Novel polymer-drug systems have been developed to target delivery of these drugs to the specific site of action minimizing the adverse side-effects and with improved dose efficiency [3–6, 9]. For instance, doxorubicin (DOX) has been chosen as "model anticancer drug" successfully applied in treating a wide range of cancer tumors. However, as an anthracycline drug, besides poor water solubility, it presents serious side effects, such as cardiotoxicity and the development of multi-drug resistance. For that reason, studies focused on the encapsulation and controlled/targeted delivery of DOX by different polymer-based nanocarriers have been reported as they can overcome these problems [11].

To this end, polymeric nanoparticulate systems are of paramount relevance for developing innovative polymer-drug with anticancer activity with tunable physicochemical properties combined with specificity and targeting characteristics. Supramolecular polymer structures (SPS) are very versatile entities as they are composed by reversible macromolecular interactions involving the chemical functional groups, such as hydrogen bonding, electrostatic, hydrophilic and hydrophobic interactions. Supramolecular nanosized materials show interesting and useful properties resulting from their dynamic nature, such as stimuli-responsiveness, adaptability and water-dispersity for creating drug delivery carriers and hydrogel networks. Recent progresses in polymer-based supramolecular networks for the formation of hydrogels and novel materials for a broad range of applications have been reported in the literature [12]. Specially designed polymer-drug systems aim to mimicking the ability of nature to create functional and dynamic superstructures of macromolecules with tunable conformation and size by the combination of hydrophobic and hydrophilic domains and complex balance of charges [13]. For that reason, the study of intermolecular interactions in solution is of central importance to most chemical and biochemical processes. The solution behavior of molecules, their stabilization or aggregation, complex formation, and/or interaction with the solvent, is a consequence of the balance of intermolecular interactions and crucial when considering their biological activity. Physicochemical characteristics such as molecular mass, particle size, chemical functionalities, zeta potential, and hydrophilicity-hydrophobicity are key aspects that contribute to the overall performance of the system in pharmaceutical formulations, biochemical and biomedical applications. Amphiphilic macromolecules such as polymers offer a robust way to regulate the size, shape, and surface chemistry of the resulting water-dispersible nanostructures. Furthermore, by mixing oppositely charged amphiphilic molecules they can produce morphologies different from those formed by individual molecules. According to the literature, polymer-based complexes and conjugates (*e.g.*, CMC with drugs, proteins, *etc.*) can undergo to the formation of supramolecular structures referring to the structural organization of the carbohydrate-based polymer molecules beyond the individual molecule owing to their strong intra- and intermolecular interactions associated with oppositely charged drugs [14, 15].

One additional option of rational design of innovative polymer-drug systems for topical drug delivery systems is based on chemically crosslinked hydrogels. Hydrogels are three-dimensional, hydrophilic polymeric networks that are capable of absorbing large amounts of water, biological fluids, or molecules. The unique physical properties of chemical hydrogels have sparked particular interest in their use in drug delivery applications to improve the efficacy of the therapeutic agents and minimize undesirable side effects. Their highly flexible structure can easily be tuned by controlling the density of covalent crosslinks in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Hydrogels serve as an *in situ* vehicle for localized delivery of antineoplastic agents by topical application, allowing minimally or noninvasive delivery, avoiding side effects of systemic chemotherapeutics and while reducing infection risk associated with surgical procedures [3, 16].

Carbohydrate-based polymeric hydrogels have been studied as anticancer drug carriers which are poorly soluble in water and highly cytotoxic for chemotherapeutic applications. Besides molar mass, the degree of substitution (or carboxymethylation, DS) of carboxymethylcellulose plays a pivotal role on all properties, including water solubility, pH-sensitivity, chemical reactivity and stability, rheology and biodegradability, which can be tuned for a myriad of applications in biomedical, food, and pharmaceutical fields [17]. Interestingly, despite intensive research in the field of biopolymer hydrogels and drug carriers systems for biomedical applications including polysaccharides and cellulose derivatives [7,8,10,18,19], no published report was found in the consulted literature of systems composed of CMC-DOX nanoparticulate polymer-drug complexes embedded in chemically crosslinked hydrogel networks for potential therapy of skin cancer.

Thus, in this research it is presented for the first time the development of carboxymethylcellulose-doxorubicin (CMC-DOX) nanosized complexes with two degree of substitutions *via* the formation of supramolecular structures in aqueous media. These CMC-DOX nanoparticulate complexes were chemically crosslinked with citric acid forming dual-responsive polymer-drug hydrogel networks with tunable drug release profile *in vitro* against melanoma cancer cells.

2. Material and methods

2.1. Materials and cell cultures

Sodium carboxymethylcellulose with two degree of substitution DS = 0.77 (CMC-0.77, average molar mass M_w = 250 kDa and, viscosity 735 cps, 2% in H_2O at 25 °C) and DS = 1.22 (CMC-1.22, M_w = 250 kDa, viscosity 660 cps, 2% in H_2O at 25 °C), 2-(N-Morpholino) ethanesulfonic acid (MES, >99%, low moisture content), doxorubicin hydrochloride (DOX, ≥98.0%), citric acid (CA, ≥99.5%,), 3-(4,5-dimethylthiazol-2yl) 2,5-diphenyltetrazolium bromide (MTT, >98%), Triton™ X-100, sodium dodecyl sulfate (SDS, ≥99.0%), paraformaldehyde (95%) and hydrochloric acid (HCl, 37%) were purchased from Sigma-Aldrich (USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), penicillin G sodium, streptomycin sulfate and amphotericin-b were supplied by Gibco BRL