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A novel approach for fabricating nanocomposite materials by embedding stabilized core-shell micelles into polysaccharide cryogel matrix

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ABSTRACT

We report a novel approach for fabricating nanocomposite polysaccharide-based carriers for sustained delivery of poorly-water-soluble drugs by embedding stabilized core-shell micelles (SPM) possessing hydrophobic cores into super-macroporous hydroxypropyl cellulose (HPC) cryogels. Firstly, nano-sized SPM were synthesized by loading and photochemical crosslinking of pentaerythritoltetraacrylate (PETA) in poly(ethylene oxide)-*b*-poly (propylene oxide)-*b*-poly(ethylene oxide) ($PEO_{19}PPO_{29}PEO_{19}$) core-shell micelles. Next, HPC cryogels containing different amount of SPM were fabricated by combination of cryogenic treatment and photo-crosslinking. A crosslinking agent, *N,N′*-methylenebisacrylamide, was used to enhance the density of polymer network. The effect of SPM concentration on gel fraction yield, swelling degree, cryogel morphology and mechanical properties were studied. Nanocomposite cryogels were loaded with curcumin and their encapsulation efficiency and drug release profile as a function of SPM content were investigated. The cytotoxic effect of blank and curcumin loaded nanocomposite cryogels was assessed as well.

1. Introduction

Research and development of biodegradable polymer materials have significantly contributed to various biotechnological advancements in drug delivery, tissue engineering, and medical device improvement (Kamaly, Yameen, Wu, & Farokhzad, 2016). In particular, the successful clinical translation of the earlier macroscale drug delivery (MDD) systems has led to the evolution of controlled-release drug delivery platforms that are capable of overcoming pharmacological limitations with substantial advantages over conventional dosage forms. Different types of polymers in varying physical forms have been used to fabricate MDD devices for delivery of small-molecule drugs, proteins, oligonucleotides, silencing RNA, plasmid DNA and antibodies (Kearney & Mooney, 2013). Macroscale systems allow effective use of different controlled release mechanisms (diffusion, drug–carrier affinity or degradation of the material) to achieve a desired time interval of drug delivery to target organs, tissues, cells, etc. In diffusion-based systems, drugs are typically encapsulated into an internal reservoir or mixed homogeneously in the material. The release rates from diffusion or reservoir devices can be tuned by adjusting the drug solubility in the polymer, the drug diffusivity through the polymer matrix and the partition coefficient (ratio of drug solubility in matrix to drug solubility in surrounding media).

Hydrogels are key class of polymeric materials widely exploited in medicine as tissue engineering scaffolds, diagnostics tools, cell immobilization matrices, drug delivery systems, etc. (Hoffman, 2012). Currently, there is an increasing interest in the so called cryogels which are super-macroporous hydrogels characterized with a heterogeneous open-porous structure (Okay & Lozinsky, 2014). Such materials offer new perspectives for development of innovative systems for biomedical and pharmaceutical applications (Henderson, Ladewig, Haylock, McLean, & O'Connor, 2013). Recently, cryogels based on temperature responsive poly(ethoxytriethyleneglycol acrylate) (PETEGA) and poly (N-isopropylacrylamide) (PNIPAAm) were exploited for sustained release of the water-soluble drugs verapamil hydrochloride (Kostova et al., 2011). As far as PETEGA and PNIPAA are in a hydrophobic state at physiological temperature (37 °C), the relatively slow rate of drug release was attributed to the low degree of swelling of the polymer network. In the case of highly hydrophilic gels like 2-hydroxyethylcellulose/ chitosan cryogel, however, the increased density of

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polymer network was critical to slow down the diffusion and to achieve a prolong release of the water-soluble drug metronidazole (Stoyneva, Momekova, Kostova, & Petrov, 2014). On the other hand, the information about application of polymer cryogels for controlled delivery of hydrophobic drugs is rather limited. This fact might be associated with specific difficulties to load and disperse homogeneously water-insoluble molecules in a hydrophilic material. Therefore, a strategy based on incorporation of lipophilic emulsion micro-droplets in polymeric cryogels was employed to develop cryogel materials capable to solubilize and release in controlled manner hydrophobic drugs (Komarova, Starodubtsev, Lozinsky, Nasimova, & Khokhlov, 2013; Sowasod, Nakagawa, Charinpanitkul, & Tanthapanichakoon, 2013). The drug release from such composite materials, however, was accompanied by release of emulsion phase.

Polymeric core-shell micelles have received increasing attention due to their ability to load and deliver poorly water-soluble drugs (Kataoka, Harada, & Nagasaki, 2012). Polymeric micelles are nanosized aggregates, formed via self-assembly of amphiphilic copolymers in aqueous media, characterized with a hydrophobic core segregated from the aqueous exterior by a hydrated shell. Due to their unique architecture, small size, good biocompatibility, high *in vivo* stability, etc., core-shell micelles have been mainly considered for developing nanoformulations intended for parenteral administration. To the best of our knowledge, the idea to embed core-shell micelles into cryogel matrix in order to achieve a sustained release of poorly water-soluble drugs from macroscopic hydrophilic carriers has not been exploited yet. Although, the synthesis and characterization of some composite cryogel materials with embedded different polymer microparticles has been reported by others (Kirsebom, Mattiasson, & Galaev, 2010; Podorozhko, Dyakonova, Kolosova, Klabukova, & Lozinsky, 2012; Podorozhko, Lunev, Ryabev, Kildeeva, & Lozinsky, 2015), we are the first to introduce a strategy for fabrication of macroscale carriers based on cryogels and stabilized core-shell micelles for sustained delivery of hydrophobic active substances like curcumin.

Curcumin (diferuloylmethane) is a natural polyphenolic compound and main active component of turmeric, derived from the rhizome of *Curcuma longa* (Zingiberaceae) (Singh & Khar, 2006). This medicinal plant has been used for centuries in traditional eastern medicine as a hepatoprotective, anti-infectious and anti-inflammatory remedy (Shehzad, Wahid, & Lee, 2010). A convincing number of recent studies has corroborated the ethnopharmacological use and has shown that curcumin is endowed by pleiotropic pharmacological activities, mainly due to modulation of NFκB and other cell signaling pathways, implicated in cell survival, apoptosis and angiogenesis (Bansal, Goel, Aqil, Vadhanam, & Gupta, 2011; Sou, 2012). However, the clinical translation of curcumin's therapeutic potential is still an elusive goal due to its unfavorable biopharmaceutical and pharmacokinetic properties. Curcumin is characterized with a remarkably low aqueous solubility (11 ng/ml) and high chemical instability ($t_{1/2}$ at pH 7.4 and 37 °C is less than 10 min) (Zhongfa et al., 2012; Priyadarsini, 2009). In addition curcumin is promptly cleared from the circulation and extensively metabolized and thus is characterized with tremendously low bioavailability, even when applied at high oral doses of several grams daily (Bansal et al., 2011; Sou, 2012). Considering the above mentioned issues and especially the prominent lipophilicity, curcumin is a good candidate for incorporation into novel nanocomposite cryogel systems for topical application. Moreover, curcumin has been proven to selectively inhibit the overactivity of JAK-3 kinase, which is a characteristic feature of the life-threatening orphan disease cutaneous T-cell lymphoma (CTCL), and hence a suitable candidate for topical therapeutic interventions in this condition (Krejsgaard et al., 2010).

The present work describes a novel approach for fabricating functional nanocomposite carriers via embedding SPM in polysaccharide cryogels, applicable for topical delivery of curcumin. The effect of SPM concentration on the gel fraction yield, swelling properties and elastic modulus of cryogels was investigated. Next, nanocomposite cryogels were tested as matrices for loading and sustained delivery of the hydrophobic drug curcumin. In addition, the cytotoxicity potential of non-loaded and curcumin loaded cryogels was tested on non-malignant (HEK-293) and malignant cutaneous T-cell lymphoma (HUT-78) cell lines.

2. Materials and methods

2.1. Materials

$PEO_{19}PPO_{29}PEO_{19}$ (Pluronic P65, donated by BASF, Germany), hydroxypropyl cellulose (molar mass 1150 KDa, donated by Hercules Inc. Aqualon Division, USA), pentaerythritoltetraacrylate (containing 350 ppm monomethyl ether hydroquinone as inhibitor, Sigma-Aldrich), (4-benzoylbenzyl)trimethylammonium chloride (BBTMAC, 95%, Sigma-Aldrich), *N,N′*-methylenebisacrylamide (BAAm, 99%, Sigma-Aldrich) and curcumin (analytical standard grade, ≥98%, Sigma-Aldrich) were used as received.

2.2. Synthesis of stabilized polymeric micelles

$PEO_{19}PPO_{29}PEO_{19}$ (2 g) was dissolved in 100 mL of distilled water and temperature was adjusted at 40 °C. PETA (0.3 g), dissolved in 2 mL of acetone, was added drop-wise to the micellar solution under stirring and argon was bubbled through the solution for 45 min. Then, irradiation with a full spectrum UV–vis light (Dymax 5000-EC UV curing equipment with a 400 W metal halide flood lamp; dose rate = 5.7 J cm^{-2} min^{-1}) for 20 min was conducted. SPM were purified by dialysis against water (cellulose membrane, cutoff 10 KDa) and recovered by freeze drying. Cross-linking efficiency of 62% was determined gravimetrically as follows:

$$\text{Crosslinking efficiency (\%)} = \frac{\text{SPM weight after dialysis}}{\text{SPM weight before dialysis}} \times 100$$

2.3. Synthesis of nanocomposite cryogels

Given amounts of SPM (10, 30 and 50 wt.% with respect to HPC) were dispersed in 8 mL of distilled water and HPC (0.2 g) was added under stirring to obtain a homogeneous mixture. BBTMAC (0.01 g, 5% wt.% to HPC) and BAAm (0.02 g, 10 wt.% to HPC) were dissolved in 2 mL distilled water and added under stirring to polymer solution at room temperature. The mixture obtained was poured into 8 Teflon dishes (2 cm in diameter) forming a 2.5 mm thick layer. Then, the solution was frozen at −20 °C for 2 h. The frozen system was irradiated with full spectrum UV–vis light for 2 min using a Dymax 5000-EC UV equipment with a 400 W metal halide flood lamp (dose rate = 5.7 J cm^{-2} min^{-1}). Finally, cryogels were immersed in distilled water (1 L) and extracted for 6 days at room temperature. Water was exchanged 4 times. Gel fraction (GF) yield and swelling degree (SD) were determined gravimetrically by the following equations:

$$\text{GF yield (\%)} = \frac{\text{weight of freeze dried cryogel}}{\text{initial weights of HPC, SPM and BAAm}} \times 100$$

$$\text{SD} = \frac{\text{weight of swollen criogel}}{\text{weight of freeze dried cryogel}}$$

2.4. Instrumentation

Dynamic light scattering (DLS) measurements were carried out on a ZetasizerNanoBrook 90Plus Zeta (Brookhaven, USA), equipped with a 35 mW red diode laser, (λ = 640 nm) at a scattering angle of 90°. The ζ potential was calculated from the obtained electrophoretic mobility by the Smoluchowski equation: