Colloids and Surfaces B: Biointerfaces xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

5-Fluorouracil delivery from metal-ion mediated molecularly imprinted cryogel discs

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form
19 December 2014
Accepted 22 December 2014
Available online xxx

Keywords:
Coordinate bonding
Cryogels
5-Fluorouracil delivery
Molecular imprinting

ABSTRACT

The objective of this study is to prepare imprinted cryogel discs for delivery of 5-fluorouracil. The coordinate bond interactions are utilized to accomplish a coordination complex between metal-chelate monomer N-methacryloyl-L-histidine and 5-FU with the assistance of Cu^{2+} ion. The complex is copolymerized with hydroxyethyl methacrylate to produce poly(hydroxyethyl methacrylate-N-methacryloyl-(L)-histidine methyl ester) cryogel discs. The cryogel discs are characterized thoroughly by performing swelling tests, scanning electron microscopy, differential scanning calorimetry and X-ray diffraction studies. In vitro delivery studies are performed to investigate the effects of cross-linker ratio, medium pH and drug concentration. 5-FU imprinted cryogel discs have highly macroporous structures. Drug molecules are homogeneously dispersed in the 5-FU imprinted cryogel matrix. The cumulative release of 5-FU decreased by increasing the cross-linker density in the polymer matrix. Delivery rate of 5-FU varied with different pH values in a coordination complex since metal ion acts as a Lewis acid, and the ligand, i.e. 5-FU acts as a Lewis base. The cumulative release of 5-FU increased with increasing drug concentration in polymer matrix. The nature of the 5-FU transport mechanism is non-Fickian.

1. Introduction

5-Fluorouracil (5-FU) is an antineoplastic agent used in the chemotherapeutic treatment of a range of solid tumors causing carcinomas in the gastrointestinal tract, liver, breast, brain and so on [1–3]. The metabolism of 5-FU is very fast in the human body. 5-FU is metabolized intracellularly to fluorouridine triphosphate, fluorodeoxyuridine monophosphate and fluorodeoxyuridine triphosphate [4]. These active metabolites lead both DNA-directed and RNA-directed cytotoxicities [4,5]. However, more than 80% of administered 5-FU is catabolized to inactive metabolites by dihydropyrimidine dehydrogenase in the liver [6–8]. Thus, to enhance its therapeutic activity very high dose of 5-FU is employed [9–11]. However, the elevated levels of 5-FU in serum can cause adverse effects to the human body [3,11,12]. Lately, a number of controlled drug delivery approaches have been explored to reduce toxicity of 5-FU and enhance its therapeutic index [13,14]. Devising smart polymers with the assistance of molecular imprinting technology (MIT) for the controlled drug delivery is one of the most efficient approaches [12,15,16].

Molecular recognition plays a vital role in nature. For instance, biological processes such as antigen-antibody recognition, enzymatic catalysis, signal transduction and nucleic acid interactions are based on molecular recognition mechanism [17]. These incomparable and brilliant systems inspired scientists to invent molecularly imprinted polymers (MIPs) as their synthetic counter parts with outstanding robustness and stability [18]. MIPs are smart polymers which have tailor-made specific binding sites to interact with the template molecule [19,20]. The interactions between template and functional monomer may occur through covalent bonding [21], non-covalent interactions [22], covalent [23] or metal-ion mediated imprinting approach [24,25]. Moreover, drug delivery via MIT based smart polymers is related to the nature of binding interactions of template–monomer complex [26,27]. A number of molecularly imprinted drug delivery studies have investigated non-covalent approach including hydrogen bond formation, hydrophobic interactions, charge transfer and van der Waals forces [28,29]. However, non-covalent approach of imprinting can be insufficient for drug delivery systems because of relatively weak interactions between the template and functional monomer [30]. An alternative approach is metal ion-mediated imprinting based on formation of coordinate bond between template and functional monomer. The specificity, strength and directionality of coordinate bonds make them better candidate than non-covalent interactions and as eligible as covalent bonds [31]. Furthermore, non-covalent

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http://dx.doi.org/10.1016/j.colsurfb.2014.12.038
0927-7765/

interactions can be weakened or damaged by strongly polar solvents whereas metal coordination bonds have strength against a wide range of solvent environment. Additionally, the ability of coordinate bonds to form and cleave in response to variations in external pH can be helpful in the development of pH-responsive delivery systems [32,33]. Therefore, metal ion mediated imprinting approach is an efficient alternative for drug delivery systems over traditional imprinting approaches [31].

Cryogel is a kind of hydrogel formed as a result of cryogenic treatment. In cryogelation, while aqueous phase produces ice crystals under frozen conditions, the monomeric or polymeric precursors form cross-linked gel in unfrozen or moderately frozen phases [34,35]. Cryogelation is followed by thawing of these ice crystals which act as porogen. Gelation at subzero temperature limits the motion of molecules and leads to easier and more specific molecular imprinting [24]. Since, cryogels combine some unique features including mechanical and chemical robustness, biocompatibility, low cost and easy preparation, they have a great potential to be used in various biomedical applications and tissue engineering studies [36]. Due to the macroporous and tissue-like structure, cryogels offer unique 3D scaffolds as implantable biomaterials for local delivery of therapeutic molecules.

The implantable systems can deliver large dose of drugs close to the tumor for a sustained drug delivery. Therefore, they can efficiently increase therapeutic effect of chemotherapy and decrease the toxic effects of drug greatly [37]. Poly(hydroxyethyl methacrylate) [PHEMA] is one of the most widely used biomaterials due to useful properties like high water content, low toxicity and good tissue compatibility [38]. It has also high blood-compatibility and shows high resistance to degradation [39,40]. Cryogels can be implanted subcutaneously or intra-peritoneally [41,42]. Because PHEMA is not biodegradable [43], after the drug has been released, minor surgery is necessary for the removal of the delivery system from the body.

In the present study we report implantable cryogel discs for a local administration of drug molecules. The novelty of this study is based on the introduction of metal coordinate bond in molecularly imprinted cryogel disc. To accomplish effective imprinting we prepared metal-chelate complex of N-methacryloyl-L-histidine (MAH), a polymerizable derivative of L-histidine, and 5-FU via Cu^{2+} ion coordination. Then, the complex is polymerized using hydroxyethyl methacrylate (HEMA) as the main monomer. The cryogel discs are characterized by swelling test, scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and X-ray diffraction (XRD) studies. The in vitro delivery studies are carried to evaluate the effects of cross-linker ratio, pH, and 5-FU content on delivery rate of 5-FU in buffer medium.

2. Experimental

2.1. Materials

5-FU is obtained from Koçak Farma (Tekirdağ, Turkey). L-Histidine methylester and methacryloyl chloride are supplied by Sigma (St Louis, USA). HEMA and N,N′-methylene-bisacrylamide (MBAAm) are obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. N,N,N′,N′-tetramethyl ethylene diamine (TEMED) and ammonium persulfate (APS) are obtained from Fluka (Buchs, Switzerland). All other chemicals are of reagent grade and are purchased from Merck AG (Darmstadt, Germany). Water used in all the experiments is purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed-bed system. Buffer and sample solutions are filtered before use through 0.2 μm membrane (Sartorius, Göttingen, Germany). All glassware are extensively washed with dilute nitric acid before use.

2.2. Preorganization of MAH–Cu^{2+} complex with 5-FU

Preparation and characterization of MAH are reported in detail elsewhere [44]. The following procedure is applied for the preorganization of MAH–Cu^{2+} complex with 5-FU: Firstly, MAH (0.223 g, 1.0 mmol) is added slowly into 15 mL of ethanol and then treated with copper(II) nitrate hemi(pentahydrate) [$Cu(NO_3)_2{\cdot}2.5H_2O$] (0.232 g, 1.0 mmol) at room temperature. The solution is magnetically stirred for 3 h until the color of solution turned clear blue. Ethanol is then removed on a rotary evaporator to yield a blue solid, i.e. MAH–Cu^{2+} complex which is recrystallized using ethanol/acetonitrile. Then, the MAH–Cu^{2+} complex (30 mg, 0.1 mmol) and 5-FU (13 mg, 0.1 mmol) are put into eppendorf tubes containing 1.0 mL of 3-(N-morpholino) propanesulfonic acid (MOPS) buffer of pH 7.4. The mixture is stirred for 1.5 h to allow the preorganization of the 5-FU and MAH–Cu^{2+} complex.

2.3. Preparation of 5-FU-imprinted cryogel discs

5-FU imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-(L)-histidine methyl ester) [PHEMAH] cryogel discs are prepared in purified water with the following molar ratios of the HEMA and MBAAm (n_{HEMA}/n_{MBAAm}): 4:1 (1.006 g/0.310 g), 8:1 (1.136 g/0.175 g), 16:1 (1.214 g/0.094 g) with the abbreviated names as PHEMAH-4, PHEMAH-8 and PHEMAH-16, respectively. Precisely, MBAAm is dissolved in 12.0 mL of purified water. MAH–Cu^{2+}–(5-FU) complex and HEMA are dissolved in 1.8 mL of purified water. This solution is mixed with the previous solution and 1.0 mg of MAH–Cu^{2+}–(5-FU) complex is added into this solution. Then, the mixture is degassed and kept in an ice bath for 10 min. The cryogel is then synthesized by free radical polymerization initiated by APS and TEMED. After adding APS (16 mg), the solution is cooled in an ice bath for 5 min. TEMED (20 μL) is added and the reaction mixture is stirred for 1 min. Then, the reaction mixture is poured between two glass plates separated with 1.5 mm thick spacers. The polymerization mixture is frozen at −16 °C for 24 h and then thawed at room temperature. After melting of ice crystals, the cryogel is washed immediately with 20 mL of water, the cryogel is cut into circular discs (0.7 cm in diameter).

The percent loading of 5-FU in the cryogel discs is determined spectrophotometrically at 266 nm. The percent of 5-FU loading are calculated as follows:

$$\text{5-FU loading}\ (\%) = \frac{\text{total amount of loaded 5-FU during polymerization} - \text{amount of 5-FU washed away}}{\text{total amount of loaded 5-FU during polymerization}} \times 100 \quad (1)$$

2.4. Characterization of cryogel discs

The synthesized cryogel discs are characterized by the following techniques.

The specific surface area of cryogel discs in dry state is determined by using multipoint Brunauer–Emmett–Teller (BET) apparatus (Quantachrome, NOVA 2000, USA). Firstly, cryogel discs are weighed and placed in a sample holder and degassed using N_2-gas at 150 °C for 1 h. Then, the cryogels are weighed again. Adsorption of the gas is applied at −210 °C and desorption is

Please cite this article in press as: K. Çetin, A. Denizli, 5-Fluorouracil delivery from metal-ion mediated molecularly imprinted cryogel discs, Colloids Surf. B: Biointerfaces (2015), http://dx.doi.org/10.1016/j.colsurfb.2014.12.038