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Cellulose-based molecularly imprinted red-blood-cell-like microparticles for the selective capture of cortisol

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ABSTRACT

Magnetite-nanoparticle-containing red-blood-cell-like-microparticles (M-RBC-MPs) with a selective ability for trapping cortisol (COR) were synthesized by an electrospray technique of a molecularly imprinted ethyl(hydroxyethyl) cellulose (EHEC)-based precursor. The as-synthezied M-RBC-MPs were ~3-μm-disks with a dent. M-RBC-MPs contained magnetite nanoparticles below 15 nm in diameter, which exhibited magnetization and no room-temperature coercivity. The molecularly imprinted M-RBC-MPs (MI-M-RBC-MPs) passed through pores less than their diameter. The MI-M-RBC-MPs selectively trapped COR from a solution containing molecules similar to COR, whereas non-imprinted M-RBC-MPs did not trap COR. Furthermore, magnets were used to capture the water-dispersed MI-M-RBC-MPs flowing in a tube. Based on the above results, MI-M-RBC-MPs may selectively trap COR while simultaneously circulating in the blood, followed by their removal from the blood using magnets.

1. Introduction

Cortisol (COR) is a steroid hormone produced by the adrenal glands, which is mainly released because of stress; it exhibits several important functions in the body (McEwen, 1998). COR is crucial for maintaining an appropriate balance for human health. Excess COR is produced in case of diseases such as Cushing's syndrome, leading to depression (Holsboer, 2000), weight gain (Torres, Diet, & Nowson, 2007), acne (Karadag, Ertugrul, Tutal, & Akin, 2011), as well as retardation of height in children (Daley-Yates & Richards, 2004).

Meanwhile, molecular imprinting (MI) involves the creation of template-shaped cavities in polymer matrices that retain the memory of the shape, size and functional groups of the template molecules (Lofgreen & Ozin, 2014). MI has been developed to recognize various specific molecules, and it has been utilized for a broad range of applications, including solid-phase separation, assays, sensors, membranes, catalysis, and synthesis (Whitcombe, Kirsch, & Nicholls, 2014). Biologically significant molecules are targets of MI for drug delivery, biosensors, diagnostics and therapeutic devices (Hilt & Byrne, 2004). Column chromatography combined with MI has been reported to be effective for the selective capture of creatinine (CRE) or COR (Baggiani, Giraudi, Trotta, Giovannoli, & Vanni, 2000; Chang, Ko, & Hsu, 2009; Sreenivasan & Sivakumar, 1997). However, this technique is limited as it cannot be applied inside the human body.

Meanwhile, cellulose is a renewable bioresource, and is expected for applications in functional materials, such as biomedical (Jorfi & Foster, 2015; Mondal, 2017; Ullah, Wahid, Santos, & Khan, 2016), magnetic (Biliuta & Coseri, 2016), sensing and device (Mahadeva, Walus, & Stoeber, 2015), and catalytic (Kaushik & Moores, 2016) materials. Nano and micro cellulosic materials (Lavoine, Desloges, Dufresne, & Bras, 2012; Quirós, Boltes & Rosal, 2016) contribute to these attractive applications. Cellulose is a linear polymer of glucose rings linked by acetal groups. Intramolecular and intermolecular hydrogen bonded hydroxyl groups render chain rigidity to cellulose. Cellulose ethers are derivatives of cellulose in which the OH groups are partly substituted by the 2-ethoxyethoxy group ($OCH_2CH_2OCH_2CH_3$) in ethyl(hydroxyethyl)cellulose (EHEC). Thus, EHEC is soluble in ethanol, but weakly soluble in water. The solubility of EHEC in organic solvents is useful for synthesizing multifunctional inorganic-organic hybrid materials.

Previously, our group has prepared red-blood-cell-like microparticles (RBC-MPs) by the electrospray technique (Hayashi et al., 2010a). RBC-MPs are formed by the electrospray technique because of the characteristic intermolecular hydrogen bonds of cellulose. RBC-MPs can pass through microvessels, which exhibit different biodistribution of spherical microparticles. RBC-MPs did not accumulate in the lungs and spleen and these MPs circulated in the blood, whereas a considerable amount of spherical microparticles accumulated in these organs (Hayashi et al., 2018). RBC-MPs loaded with a transforming growth factor-beta receptor inhibitor could treat liver fibrosis without pneumotoxicity (Hayashi et al., 2018).

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In this study, the following hypotheses were made: Molecularly imprinted RBC-MPs containing magnetite nanoparticles (MI-M-RBC-MPs) can selectively capture COR during their circulation in the blood owing to the desirable biodistribution of RBC-MPs; subsequently, the COR-trapped MI-M-RBC-MPs can be collected using magnets. Based on these hypotheses, MI-M-RBC-MPs were prepared. Furthermore, their ability to trap COR was investigated, and the possibility of collecting the COR-trapped MI-M-RBC-MPs using magnets was verified.

2. Experimental section

2.1. Materials

Iron(III) allylacetylacetonate (IAA) was prepared according to a previously reported method (Yogo, Nakamura, Kikuta, Sakamoto, & Hirano, 1996). Ethanol (Kishida Chemical, Osaka) was dried over magnesium ethoxide and then distilled before use. Methylhydrazine, 2-hydroxyethyl methacrylate (HEMA), *N,N'*-methylenebisacrylamide (MBA), 2,2-dimethoxy-2-phenylacetophenone (DMPAP), and ethyl(hydroxyethyl)cellulose (EHEC) were used as received from Tokyo Chemical Industry (Tokyo). COR was purchased from Wako Pure Chemical Industries (Osaka). Cholic acid (COA), tyrosine (TYR), and creatine (CN) were purchased from Kishida Chemical. Creatinine (CRE) was purchased from Sigma-Aldrich (MO). Fig. S1 shows the molecular structures of COR, COA, TYR, CN, and CRE.

2.2. Synthesis of magnetite nanoparticles

Allyl-group-modified magnetite nanoparticles (Allyl-Fe_3O_4 NPs) were synthesized using a method previously reported by our group (Hayashi, Shimizu, Asano, Sakamoto, & Yogo, 2008; Hayashi, Moriya, Sakamoto, & Yogo, 2009; Hayashi et al., 2010b, 2010c, 2013; Hayashi, Sakamoto, & Yogo, 2016). Briefly, IAA was dissolved in ethanol at a concentration of 100 mM. Methylhydrazine (800 mM) and distilled water (DW, 500 mM) were added into the IAA solution. The mixed solution was refluxed for 9 h, and the products collected by centrifugation at 22,140 × *g* for 20 min were re-dispersed in ethanol. This process was repeated at least three times to wash the products.

2.3. Preparation of MI-M-RBC-MPs

First, COR (13 μM), MBA (78 μM), HEMA (260 μM), EHEC (230 μM), and DMPAP (860 nM) as the initiator for photopolymerization were added to an ethanol solution containing Allyl-Fe_3O_4 NPs (500 nM). The mixed solution was subjected to electrospray in a chemical hood at a flow rate of 1 mL/h using a needle (inner diameter = 0.6 mm, and length = 60 mm). The electric field was set at 12 kV, and the distance between nozzle and collector was 20 cm. Then, to polymerize MBA, HEMA, and allyl groups of Allyl-Fe_3O_4 NPs, the particles obtained by electrospray were exposed to a UV light of 222 nm for up to 30 min (E500-222P, Excimer Inc., Japan). Finally, COR within the particles was extracted with ethanol, thus affording MI-M-RBC-MPs.

2.4. Preparation of non-imprinted RBC-MPs containing magnetite nanoparticles (NI-M-RBC-MPs)

NI-M-RBC-MPs were prepared by electrospraying the ethanol solution containing MBA (78 μM), HEMA (260 μM), EHEC (230 μM), DMPAP (860 nM), and Allyl-Fe_3O_4 NPs (500 nM), followed by the polymerization of the MBA, HEMA, and allyl groups of Allyl-Fe_3O_4 NPs within the particles obtained by electrospray. The electrospray and polymerization conditions were the same as those utilized for preparing MI-M-RBC-MPs.

2.5. Structural analysis and magnetic properties

Transmission electron microscopy (TEM, H-800; Hitachi, Tokyo) was employed to examine the sizes and shapes of the products. The crystalline phase of MI-M-RBC-MPs was analyzed by X-ray diffraction (XRD; Rigaku SmartLab; Rigaku, Tokyo). The amounts of inorganic and organic phases of the MI-M-RBC-MPs were measured by thermogravimetry (TG)-differential thermal analysis (TG 8120; Rigaku, Tokyo) at a heating rate of 10 °C/min from ambient temperature to 800 °C in an oxygen flow of 50 cm^3/min. Fourier transform infrared (FTIR) spectra of MI-M-RBC-MPs were recorded on an FTIR spectrometer (Nexus 470; Nicolet, Madison, WI). The magnetic properties of MI-M-RBC-MPs were measured with a vibrating sample magnetometer (VSM-C7-10A; Toei Kogyo, Tokyo) at room temperature.

2.6. Abilities of MI-M-RBC-MPs to pass through pores less than their diameter

Dynamic light scattering (DLS) measurements (DelsaMax PRO equipped with DelsaMax ASSIST; Beckman Coulter, CA) were carried out to measure the hydrodynamic diameter of MI-M-RBC-MPs, before and after filtration through a membrane filter with 1-μm pores.

2.7. Abilities of MI-M-RBC-MPs and NI (Non-imprinted)-M-RBC-MPs to selectively trap Cor

MI-M-RBC-MPs and NI-M-RBC-MPs (20 mg) were immersed in 4 mL of the solution containing COR, COA, TYR, CRE, or CN at 50 μM. At 3, 6, 12, 24, 36, and 48 h after immersion, the supernatants were collected by centrifugation at 22,140 × *g* for 20 min. The absorbances of COR at 248 nm, COA at 190 nm, TYR at 224 nm, CRE at 223 nm, CN at 193 nm in the supernatants were measured by UV–vis spectroscopy. The concentrations of the residual COR, COA, TYR, CRE, or CN in each supernatant were estimated from the absorbance using calibration curves.

2.8. Magnetic capture of flowing MI-M-RBC-MPs

MI-M-RBC-MPs dispersed in water were flowing at 2 mL/s in a polypropylene tube, which was sandwiched between two permanent magnets (400 mT), using a syringe pump.

3. Results and discussion

3.1. Synthesis of MI-M-RBC-MPs

MI-M-RBC-MPs were prepared by the following procedures: (1) preparation of RBC-shaped particles, mainly comprising EHEC, by the electrospray of an ethanol solution containing COR, MBA, HEMA, EHEC, DMPAP, and Allyl-Fe_3O_4 NPs (Fig. 1(a)); (2) polymerization of the MBA, HEMA, and allyl groups of the surface of Allyl-Fe_3O_4 NPs using DMPAP as the polymerization initiator (Fig. 1(b)); and (3) removal of COR from the RBC-shaped particles with ethanol (Fig. 1(b)). COR is a template molecule, while HEMA and MBA are functional monomers in the MI process (Chen, Xu, & Li, 2011). A self-assembly is formed around COR coordinated by HEMA and MBA through non-covalent interaction, such as hydrogen bonding (Chen et al., 2011). The self-assembled complex is mixed in an ethanol solution of EHEC at the nanometer level. The formation of RBC-like shape is attributed to the β-glucose structure of EHEC, which is a key matrix of the self-assembled complex. After the formation of RBC-like microparticles by electrospray, the C═C bonds of HEMA and MBA of the complex are copolymerized *via* photo-induced radical polymerization (Fig. 1(b)). Concurrently, allyl-Fe_3O_4 is copolymerized with HEMA or MBA by using its C═C bonds. HEMA, MBA, and allyl-Fe_3O_4 are bound together through the covalent carbon-carbon bonds. However, EHEC is intact during copolymerization of the functional monomers because EHEC has no