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Design and evaluation of clickable gelatin-oleic nanoparticles using fattigation-platform for cancer therapy

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ABSTRACT

The principles of bioorthogonal click chemistry and metabolic glycoengineering were applied to produce targeted anti-cancer drug delivery via fattigation-platform-based gelatin-oleic nanoparticles. A sialic acid precursor (Ac_4ManNAz) was introduced to the cell surface. Gelatin and oleic acid were conjugated by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) chemistry with the subsequent covalent attachment of dibenzocyclooctyne (DBCO) in a click reaction on the cell surface. The physicochemical properties, drug release, in vitro cytotoxicity, and cellular uptake of DBCO-conjugated gelatin oleic nanoparticles (GON-DBCO; particle size, ~240 nm; zeta potential, 6 mV) were evaluated. Doxorubicin (DOX) was used as a model drug and compared with the reference product, Caelyx®. A549 and MCF-7 cell lines were used for the in vitro studies. GON-DBCO showed high DOX loading and encapsulation efficiencies. In A549 cells, the IC50 value for GON-DBCO-DOX (1.29 μg/ml) was six times lower than that of Caelyx® (10.54 μg/ml); in MCF-7 cells, the IC50 values were 1.78 μg/ml and 2.84 μg/ml, respectively. Confocal microscopy confirmed the click reaction between GON-DBCO and Ac4ManNAz on the cell surface. Flow cytometry data revealed that the intracellular uptake of GON-DBCO-DOX was approximately two times greater than that of GON-DOX and Caelyx®. Thus, the newly designed GON-DBCO-DOX provided a safe and efficient drug delivery system to actively target the anticancer agents.

1. Introduction

Self-assembled nanoparticles composed of biodegradable and biocompatible polymers are promising drug delivery carriers owing to their tunable physicochemical and biopharmaceutical properties. Nano drug delivery systems can be tailored to target the disease site, improve the bioavailability of poorly water-soluble drugs, and shield the drug from the host immune system, thereby prolonging its circulation time (Kamaly et al., 2016; Park et al., 2016). Several disease-related drugs and/or biological molecules have been encapsulated in different types of biodegradable systems (Cheng et al., 2015; Ma and Williams, 2018).

Recently, we designed a novel fattigation-platform to prepare self-assembled gelatin-oleic nanoparticles (GON) with a combination of naturally biocompatible protein, gelatin, and a fatty acid, oleic acid. Owing to the biodegradable nature of the drug delivery system and the abundance of functional groups such −COOH and $-NH_2$ for modification, the fattigation-platform-based nanoparticles are extremely versatile and have already shown the potential to deliver poorly water-soluble compounds and anticancer agents by increasing their water solubility, enhancing their bioavailability, and targeting cancer cells (Tran et al., 2014; Tran et al., 2013a; Tran et al., 2013b). Furthermore, we demonstrated the advanced theranostic applications of this fattigation-platform through a combination of GON with magnetic nanoparticles (MNP) (Nguyen and Lee, 2017; Tran et al., 2017).

In cancer treatment, the disease site-specificity via active targeting is essential, as it leads to increased internalization and tumor cytotoxicity of the drug in addition to a reduction in side effects (Kudgus et al., 2014; Tran et al., 2014; Xiong et al., 2005). Therefore, several nanoparticles have been investigated over several decades with the use of biological targeting moieties such as peptides, antibodies, and aptamers. These “active targeting” nanoparticles bind to the target cell surface receptors (Bazak et al., 2015; Choi et al., 2016). Other active targeting moieties, such as the folate receptor, transferrin receptor, human epidermal growth factor receptor 2, and G protein-coupled

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receptor, are particularly advantageous for cancer treatment as they reduce the distribution of potentially toxic drugs to healthy tissues, which avoids undesirable side effects (Accardo et al., 2014; Liechty and Peppas, 2012). However, for better cellular targeting and minimized side effects, advanced functional biomaterials that allow precise control and flexibility for successful drug delivery systems is highly encouraging as a proof of concept. The adoption of bioorthogonal click chemistry (copper-free click chemistry) and metabolic glycoengineering for the design of new biomaterial architecture and function to the fattigation-platform can provide an aggressive pathway for active targeting anticancer agents.

In the previous decade, click chemistry has paved the way for novel synthesis reactions via more precise, more efficient, and faster approaches (Such et al., 2012). A typical click reaction is characterized by high efficiency under mild conditions, limited side reactions, high yield, and reduced byproduct formation (Bock et al., 2006). Several studies have demonstrated the application of bioorthogonal click chemistry in the field of biomedicine for various purposes, including the site-specific tagging of proteins or nucleotides, analysis of metabolic pathways, monitoring of zebrafish growth, and surface-modification of nanocarriers (Laughlin et al., 2008; Ngo et al., 2009). Bioorthogonal chemistry, which enables the specific, covalent attachment of a probe molecule to the biological molecule of interest, has powerful applications in the biological field when used in combination with metabolic glycoengineering. Metabolic engineering techniques have enabled the introduction of unnatural glycans on the cell surface through the feeding of specific precursors depending on their intrinsic metabolism. This technique, which was mainly pioneered by the research group of Bertozzi, has various applications, such as the exploitation of metabolic pathways, the analysis of cellular glycans, and 3D cellular assembly (Du et al., 2009; Prescher et al., 2004).

Previous attempts to target cancer by fattigation-platform-based drug delivery systems have been mainly based on the leveraging of the enhanced permeable and retentive vasculature of the disease, which may result in undesirable side effects, rather than the active and site-specific targeting of cancer. In this study, we reported the proof of concept of a novel and facile approach for the preparation of clickable GON, which can enhance the tumor targetability via an improvement in the drug accumulation at tumor sites. This was partially achieved by the enhanced permeability and retention (EPR) effect and mainly by enhanced internalization from the covalent binding of the nanoparticles on the cell surface via metabolic glycoengineering (Hapuarachchige et al., 2014; Lee et al., 2014b). Dibenzocyclooctyne (DBCO) was chosen as the clickable material and doxorubicin (DOX) was used as the model drug. We hypothesized that our dibenzocyclooctyne (DBCO)-functionalized GON could effectively deliver DOX by targeting the azide-modified sialic acid precursors on the cancer cell surface, which were generated by bioorthogonal click chemistry and metabolic glycoengineering. The physicochemical properties and cell targetability of clickable GON were then comprehensively characterized.

2. Materials and methods

2.1. Materials

Gelatin (Type A; bloom strength, 300 gm), glutaraldehyde, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), L-lysine, 2,4,6-trinitrobenzenesulfonic acid solution (TNBS) and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Oleic acid (OA) was purchased from Samchun Pure Chemicals Co. Ltd., Korea. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was purchased from Alfa Aesar (UK) and N-hydroxysuccinimide (NHS) was purchased from Wako Pure Chemicals Industry Ltd., Japan. Click-IT® ManNAz Metabolic Glycoprotein Labeling Reagent (tetraacetylated N-azidoacetyl-D-mannosamine, Ac_4ManNAz, Cat. No. C33366) and Click-IT® tetramethylrhodamine (TAMRA)-DIBO-alkyne (Cat. No. C10410) were purchased from Invitrogen (Carlsbad, CA, USA). DBCO-sulfo-NHS ester was purchased from Click Chemistry Tools (Scottsdale, AZ, USA). DOX hydrochloride was received from Dong-A Pharmaceutical Co. Ltd., Korea. The reference compound, Caelyx® (liposomal DOX hydrochloride), was purchased from Janssen, Korea (Cat. No. EBBS700), and 4% paraformaldehyde was purchased from Electron Microscopy Sciences – EMS (USA). Cell culture media and supplements were purchased from Gibco (Grand Island, NY, USA). High-performance liquid chromatography (HPLC)-grade solvents were obtained from Fisher (Seoul, Korea). All other chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Synthesis of DBCO-modified gelatin-oleic conjugates (GOC-DBCO)

2.2.1.1. Activation of OA. OA (200 µl) was dissolved in 10 ml of absolute ethanol and 1 N NaOH (40 µl) was added to increase the pH of the solution. EDC (310 mg) and NHS (200 mg) were added to the above solution, which was stirred at 300 rpm for 15 min at room temperature.

2.2.1.2. Preparation of gelatin-oleic conjugates (GOC). Gelatin (250 mg) was dissolved completely in distilled water (20 ml) by heating at 37 °C and stirring at 250 rpm in a water bath. This solution was slowly added into the activated OA solution. The mixture was incubated in a shaker (Biofree, Korea) at 200 rpm for 12 h at 37 °C. At the end of the reaction, acetone (25 ml) was added to precipitate the GOC, which were then collected after centrifugation at 15,000 rpm. The supernatant was removed and the GOC were washed three times with distilled water to remove excess alcohol, unconjugated OA, and gelatin. The final product was dried in a vacuum drier (Jeio Tech, VDR-30G, Korea) for 2 days.

2.2.1.3. Synthesis of GOC-DBCO. GOC (15 mg) were dissolved in 50% ethanol (3 ml) by stirring at 300 rpm for 2 h at 37 °C. DBCO-sulfo-NHS (28 mg) was added to the solution, which was then stirred at 200 rpm for 8 h at room temperature. After the reaction, the solution was dialyzed through a cellulose membrane (molecular weight cut-off [MWCO] 1000, Sigma-Aldrich Corporation, St. Louis, MO, USA) against distilled water for 24 h at room temperature to remove excess DBCO-sulfo-NHS and GOC; subsequently, the solution was vacuum-dried for 12 h to obtain the GOC-DBCO.

2.2.2. Preparation of DBCO-modified GON (GON-DBCO)

GOC-DBCO (10 mg) was dissolved in 50% ethanol (3 ml) by stirring at 300 rpm in a water bath at 37 °C. The solution was then stirred at 1500 rpm, with ethanol (4 ml) added dropwise at 1 ml/min by using a peristaltic pump at 37 °C. As the solution turned into a colloidal solution, 100 µl of 25% glutaraldehyde was added for crosslinking, after which the solution was stirred for 3 h. The colloidal dispersion was centrifuged at 12,000 rpm for 25 min and the supernatant was discarded to obtain the GON-DBCO, which were purified by three washes with distilled water (Puris Expe-CB water system, Model: Expe-CB Ele 10, Korea) and centrifuged at 12,000 rpm for 15 min. The resulting nanoparticles were vacuum dried for 2 days. The GON without DBCO were prepared by a similar method.

2.2.3. Preparation of DOX-loaded GON-DBCO (GON-DBCO-DOX)

DOX was loaded into the GON-DBCO via the incubation method. Briefly, GON-DBCO (10 mg) was dispersed in 50% ethanol (1 ml), and water (9 ml) was added. DOX (2 mg) was added to the above solution with stirring at 250 rpm for 12 h in a 37 °C water bath. After 12 h, the solution was centrifuged at 12,000 rpm for 25 min and purified by three washes in distilled water. The GON-DBCO-DOX were freeze-dried until further use.