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Drug release from nanoparticles embedded in four different nanofibrillar cellulose aerogels

Hanna Valo [a], Suvi Arola [b], Päivi Laaksonen [b], Mika Torkkeli [c], Leena Peltonen [a], Markus B. Linder [b], Ritva Serimaa [c], Shigenori Kuga [d], Jouni Hirvonen [a], Timo Laaksonen [a,*]

[a] Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FIN-00014 Helsinki, Finland
[b] VTT Bio and Chemical Processes, VTT Technical Research Center of Finland, Tietotie 2, Espoo, P.O. Box 1000, FIN-02044 VTT, Finland
[c] Department of Physical Sciences, University of Helsinki, P.O. Box 64, FIN-00014 Helsinki, Finland
[d] Department of Biomaterials Science, Graduate School of Agriculture, University of Tokyo, Japan

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ABSTRACT

Highly porous nanocellulose aerogels prepared by freeze-drying from various nanofibrillar cellulose (NFC) hydrogels are introduced as nanoparticle reservoirs for oral drug delivery systems. Here we show that beclomethasone dipropionate (BDP) nanoparticles coated with amphiphilic hydrophobin proteins can be well integrated into the NFC aerogels. NFCs from four different origins are introduced and compared to microcrystalline cellulose (MCC). The nanocellulose aerogel scaffolds made from red pepper (RC) and MCC release the drug immediately, while bacterial cellulose (BC), quince seed (QC) and TEMPO-oxidized birch cellulose-based (TC) aerogels show sustained drug release. Since the release of the drug is controlled by the structure and interactions between the nanoparticles and the cellulose matrix, modulation of the matrix formers enable a control of the drug release rate. These nanocomposite structures can be very useful in many pharmaceutical nanoparticle applications and open up new possibilities as carriers for controlled drug delivery.

1. Introduction

Insoluble drug compounds within nanoparticle formulations have shown major advantages in bioavailability when compared to bulk drug materials (Fakes et al., 2009; Jinno et al., 2006; Valo et al., 2011). Since the nanoparticles have a larger surface area and enhanced surface interactions compared to commonly used microparticles, they have an extremely high tendency to aggregate. Thus, it is necessary to develop techniques and materials to improve the stability and manufacturing aspects of nanoparticles in the liquid state, as well as in the dry form, in order to apply them into actual drug formulations. Incorporation of drug nanoparticles into nanofibrous cellulose hydrogels and formation of dry aerogels could produce an efficient protection against the particle aggregation. Furthermore, the structured nanofibrillar cellulose (NFC) aerogels could improve the control of the drug release due to the varying characteristics of the celluloses with varying origin and modifications.

Recently, freeze-drying has been successfully used to form flexible and highly porous nanofiber networks, aerogels, consisting of long and entangled cellulose I nanofibrils (Aulin et al., 2010; Paakko et al., 2008). Freeze-drying has also been used as a sensitive method in order to remove water from the nanoparticle formulations and to improve their stability and manageability (Abdelwahed et al., 2006; Van Eerdenbrugh et al., 2008). NFC have been used to make aerogels either by itself (Aaltonen and Jauhiainen, 2009) or in composite formulations wherein porous NFC templates have formed magnetic (Olsson et al., 2010) or electrically conductive flexible aerogels (Paakko et al., 2008), or aerogels with tunable oleophobicity (Aulin et al., 2010). Porous structures of the aerogels with large surface areas can interact and prevent the aggregation of the nanoparticles more efficiently compared to the conventional microcrystalline cellulose. Overall, individual cellulose elementary

Abbreviations: BC, Bacterial cellulose; BDP, Beclomethasone dipropionate; DCBD, Double cellulose-binding domain; F0, Freeze-dried formulation of BDP:HFBI-QC physical mixture; F1, Freeze-dried formulation of BDP microparticles (without HFBI); F2, Freeze-dried formulation of BDP nanoparticles coated with HFBI(-DCBD); F3-BC, Freeze-dried formulation of BDP nanoparticles coated with HFBI(-DCBD) and embedded in BC; F4-TCF, reeze-dried formulation of BDP nanoparticles coated with HFBI(-DCBD) and embedded in TC; F5-QC, Freeze-dried formulation of BDP nanoparticles coated with HFBI(-DCBD) and embedded in QC, F6, MCCFreeze-dried formulation of BDP nanoparticles coated with HFBI(-DCBD) and embedded in MCC; F7-RC, Freeze-dried formulation of BDP nanoparticles coated with HFBI(-DCBD) and embedded in RC; HFBI, Hydrophobin protein HFBI; HFBI-DCBD, Fusion protein of HFBI and DCBD; MCC, Microcrystalline cellulose; QC, Nanofibrillar cellulose from quince seeds; RC, Nanofibrillar cellulose from red pepper; TC, TEMPO-oxidized nanofibrillar cellulose from birch wood.

* Corresponding author. Tel.: +358 9 191 59674; fax: +358 9 191 59144.
E-mail address: timo.laaksonen@helsinki.fi (T. Laaksonen).

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fibrils and crystals with nanometer widths, extracted from plant sources or produced by bacterial cultures have gained increasing attention and may act as promising candidates for biobased-nanocomposite products (Eichhorn et al., 2010; Klemm et al., 2011; Siró and Plackett, 2010).

The cellulose fiber dimensions, the proportions of amorphous and crystalline regions, as well as its surface chemistry may vary depending on the origin and chemical or mechanical nanofibrillation processing. Nanofibrillar celluloses from plant materials may have accompanying substances on the surfaces of the fibrils, such as disoriented polymers (mainly hemicelluloses and lignin) (Iwamoto et al., 2008; Silva et al., 2011). Partial removal of the substances from the carbohydrate matrix can be usually performed, but impurities still usually exist. Some of the hemicelluloses, such as xylans, carry carboxyl groups, which are potential sites for binding through ionic charge (Fall et al., 2011). They also make aqueous dispersion of the otherwise non-dispersable cellulose fibers possible. Apart from the native charged sites, the broad possibilities for chemical modification of the cellulose allows covalent attachment of functionalities onto the surfaces for specific applications. For example carboxylate groups can be introduced on cellulose fibril surfaces by oxidation with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) (Isogai et al., 2011; Saito et al., 2006a, 2007). In addition to plant cellulose, cellulose can also be produced by the species of various genera of bacteria (*Gluconacetobacters*). Bacterial cellulose (BC) is a good candidate for the fabrication of cellulose-based hydrogels, since it is produced as pure cellulose (Chang and Zhang, 2011). Unlike native celluloses obtained from plant sources, BC does not contain compounds like lignin, pectin and hemicellulose (Gelin et al., 2007; Helenius et al., 2006). When water is removed from the cellulose fibers during drying, hydrogen bonds are formed between neighboring fibers creating a tight fiber network. This process is irreversible and is known as "hornification" (Hult et al., 2001; Diniz et al., 2004). This property makes the aerogel structures created during freeze-drying of nanofibrillar cellulose materials quite durable (Paakko et al., 2008).

Hydrophobins are amphiphilic proteins of fungal origin that have the ability to form self-assembled monolayers on either hydrophobic or hydrophilic materials and thus change their surface properties (Linder, 2009). The property of some hydrophobins to turn the wettability of solid surfaces into the opposite has been widely shown (Lumsdon et al., 2005; Opwis and Gutmann, 2011; Vejnovic et al., 2010; Wang et al., 2010). Hydrophobins (here refers to the HFBI protein) have been used to form hydrophilic drug nanoparticles from a hydrophobic drug substance (Valo et al., 2010, 2011). Furthermore, hydrophobins can be genetically modified to gain benefits such as specific adhesion onto the target surfaces (Varjonen et al., 2011). To achieve sufficient compatibility between the drug nanoparticles and the NFCs, the cellulose-binding function of hydrophobin can be obtained by fusing two cellulose binding domains (CBDs) to the hydrophobin by genetic engineering (Valo et al., 2011). CBDs are non-catalytic individual parts of the cellulose degrading enzymes that have been isolated from various cellulases (Linder and Teeri, 1997). We used fungal CBDs that interact with cellulose via hydrophobic interactions by aromatic amino acids organized on the surface of the protein (Varjonen et al., 2011).

Aerogels based on different nanofibrillar celluloses (NFCs); bacterial cellulose, cellulose extracted from red pepper (RC) and quince seeds (QC) as well as TEMPO-oxidized nanofibrillar birch cellulose (TC), were studied as templates for drug nanoparticles with and without specific binding properties created by the amphiphilic hydrophobins. Beclomethasone dipropionate was used as a model poorly soluble compound and nanoparticles of the drug were prepared with a simple precipitation method (Galindo-Rodriguez et al., 2004; Matteucci et al., 2006; Valo et al., 2010; Wang et al., 2007). The role of the cellulose matrix is evaluated in order to see whether the origin of this interesting new class of biomaterials can influence the release rate from bound drug nanoparticles. The choice of these different sources of nanofibrillar cellulose highlights the most common differences that the material might have for drug formulations. The surface charge of the fibers is an important parameter for interactions with drug nanoparticles. Thus, non-charged cellulose (BC) and fibers with either native hemicellulose (RC, QC) or chemically modified carboxyl groups (TC) are expected to behave differently due to their charge. Furthermore, the source can be either from wood (TC), fruits (QC, RC) or bacteria (BC), all of which have slightly different fiber morphologies. Furthermore, the two fruit-based celluloses differ in their processing history and solid form. QC was a hydrogel, whereas RC had been dried prior to use. In this paper, the origins and properties of cellulosic fibrils are outlined in terms of aerogel formation with a drug delivery point of view in mind.

2. Materials and methods

2.1. Materials

Quince cellulose mucilage was extracted from quince seeds as described earlier (Vignon and Gey, 1998). Seeds were incubated in fresh milliQ water and the resulting mucilage was filtered through cotton cloth to remove the large impurities. Soluble impurities were removed by successive ethanol extractions and centrifugations. Dry weight 0.12% was determined after freeze-drying. Bacterial cellulose (0.3%) was purified from commercial Nata-de-Coco cubes (Fitrite Incorporated, Philippines). Purification was done by repeated water washes and neutralization with 50 mM sodium acetate of a slightly ground mass. The suspension was then further homogenized with a disperser (ultra Turrax, IKA, Germany) as described earlier (Boisset et al., 2000). TEMPO-oxidized cellulose was obtained from UPM (UPM Kymmene, Finland) (0.8%) and was produced as described elsewhere (Saito et al., 2006a, 2006b, 2007). Cellulose nanofiber from red pepper (pimiento) was prepared as follows: A first-sized pimiento fruit was cut into several pieces and seeds were removed. The skin was immersed in boiling water for making ready peeling-off of the outer skin, which was removed. The remaining skin was immersed in 4% aq. NaOH for 2 days at room temperature. The skin was washed with water repeatedly to remove alkali, and then immersed in acetone for one day for removing lipid matter. After removing acetone with water, the solid was immersed in 1% aq. $NaClO_2$ containing 0.4% acetic acid and heated for 2 h. After thorough washing with water the material was freeze-dried. Commercial microcrystalline cellulose (MCC) was used as a reference (Avicel PH101, FMC International, Ireland).

The model drug was beclomethasone dipropionate (Sigma, St. Louis, MO, USA). HFBI (Linder et al., 2001) and HFBI-DCBD (HFBI-double cellulose binding domain) (Linder et al., 2004) were expressed and purified as described elsewhere. The theoretical isoelectric points were determined based on their sequences (http://web.expasy.org/protparam/). Methanol (Riedel-de Haën, Seelze, Germany) was used as a solvent in the precipitation process and Sodium dodecyl sulfate (SDS) (Sigma–Aldrich, USA) in the dissolution media. The water used was ultrapurified (Millipore, Molsheim, France). The stability studies were performed in buffer at pH 8. All other chemicals were of analytical grade obtained from standard sources and used without further purification.

2.2. Methods

2.2.1. Precipitation of the BDP nanoparticles

34.8 mM beclomethasone dipropionate (BDP, MW 521.1 g/mol) solution was prepared by dissolving BDP in methanol. 0.25 ml of