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Nanofibrillar cellulose hydrogels and reconstructed hydrogels as matrices for controlled drug release

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ABSTRACT

Concentrated 3% and 6.5% anionic nanofibrillar cellulose (ANFC) hydrogels were introduced as matrix reservoirs for controlled delivery applications of small molecules and proteins. A further aim was to study how the freeze-drying and subsequent rehydration of ANFC hydrogel affects the rheological properties and drug release of selected model compounds from the reconstructed hydrogels. It was demonstrated that the 3% and 6.5% ANFC hydrogels can be freeze-dried with suitable excipients into highly porous aerogel structures and redispersed back into the hydrogel form without significant change in the rheological properties. Freeze-drying did not affect the drug release properties from redispersed ANFC hydrogels, indicating that these systems could be stored in the dry form and only redispersed when needed. For large molecules, the diffusion coefficients were significantly smaller when higher ANFC fiber content was used, indicating that the amount of ANFC fibers in the hydrogel can be used to control the release rate. The release of small molecules was controlled with the ANFC fiber content only to a moderate extent. The results indicate that ANFC hydrogel can be used for controlled delivery of several types of molecules and that the hydrogel can be successfully freeze-dried and redispersed.

1. Introduction

Hydrogels have various uses in biomedical applications and are well suited for regenerative medicine (Peppas et al., 2006; Slaughter et al., 2009) and controlled drug delivery (Gupta et al., 2002). Nanofibrillated cellulose (NFC) hydrogel has been widely investigated in pharmaceutical and biomedical fields, such as in drug delivery (Laurén et al., 2014), scaffold synthesis (Borges et al., 2011), and as cell and drug carriers (Thérien-Aubin et al., 2016; Valo et al., 2013). NFC hydrogel is also applicable as a cell culture scaffold for three dimensional cell culturing (Bhattacharya et al., 2012; Lou et al., 2014, 2015; Malinen et al., 2014). It is produced from the natural biopolymer cellulose, and wood pulp is a commonly used starting material in the production (Klemm et al., 2011). The surface of native NFC fibers can be further chemically modified with e.g. TEMPO [(2,2,6,6-tetramethylpiperidin-1-yl)oxyl] oxidation to contain negatively charged carboxyl groups directly on the fiber surface (Saito et al., 2006, 2007). This way, it is possible to produce anionic NFC (ANFC) fibers. Further, native as well as anionic TEMPO oxidized NFC grades have been shown to be biocompatible and nontoxic in various in vitro cell models (Alexandrescu et al., 2013; Bhattacharya et al., 2012; Hannukainen et al., 2012; Hua et al., 2014; Vartiainen et al., 2011), making them appealing for pharmaceutical applications.

Since cellulose retains moisture and is both biocompatible (in humans) and biodegradable (in nature), NFC has been investigated in wound treatment applications facing the need for faster and more effective wound healing (Chinga-Carrasco and Syverud, 2014; Lin and Dufresne 2014; Powell et al., 2016; Zhang et al., 2013). Previously, we have indicated the suitability of native NFC wound dressing in clinical use in treatment of skin graft donor sites of burn patients (Hakkarainen et al., 2016). NFC hydrogels are also of special interest in the wound healing applications due to high moisture retaining ability and biocompatibility (Lin and Dufresne 2014; Zhang et al., 2013). Furthermore, NFC hydrogel can be freeze-dried into solid aerogel structure, which may be utilized in drug delivery (Pääkkö et al., 2008; Valo et al., 2013). However, it is generally acknowledged that the drying of NFC

Abbreviations: ANFC, anionic nanofibrillar cellulose; BSA, bovine serum albumin; *D*, diffusion coefficient; DSC, differential scanning calorimetry; exp, excipients trehalose and polyethylene glycol; FITC-DEX, fluorescein isothiocyanate–dextran; KETO, ketoprofen; LZ, lysozyme; MZ, metronidazole; NAD, nadolol; NFC, nanofibrillar cellulose; PEG, polyethylene glycol; TEMPO, [(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl]; tre, trehalose

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promotes irreversible hydrogen bonding between neighboring NFC nanofibrils, known as hornification (Diniz et al., 2004; Wan et al., 2010). So far it has been a major manufacturing challenge to produce dry NFC while maintaining the nano-scale dimensions, and several techniques such as oven drying, freeze-drying, supercritical drying and spray-drying have been investigated (Kolakovic et al., 2012; Peng et al., 2012, 2013). Freeze-drying produces NFC aerogels with lamellar structures and significant lamellar aggregation (Peng et al., 2012).

In addition to the preservation of NFC nano-scale dimensions throughout drying process, the preservation of rheological properties of the NFC hydrogels is important for drug delivery applications. These should stay reasonably similar during processing in order to have reliable, controlled and predictable drug release formulations. It has been shown that once-dried TEMPO-oxidized ANFC fibers can be re-dispersed, reversing the hornification effect introduced by the drying, by involving higher energy consumption (Kekäläinen et al., 2014). This is probably due to the high surface charge density present in the TEMPO-oxidized ANFC fibers, which is much lower in the absence of carboxyl groups on the native NFC fiber surface. Furthermore, the electrostatic repulsion forces working between the anionically-charged fibers stabilizes the aqueous suspension (Isogai et al., 2011). The high surface density of negative charges has been used to electrostatically immobilize biomolecules (Weishaupt et al., 2015). Therefore, the high aspect ratio as well as anionic charge of ANFC fibers can be utilized as rate-controlling parameters in controlled drug release applications. Rheological properties and redispersability of highly concentrated 3–6.5% ANFC hydrogels after drying and consequent rehydration and re-gelling have not been studied extensively in the literature, even though these might be especially useful in e.g. wound healing applications. Previous studies have mainly focused on the evaluation of redispersibility and rheological properties of dilute 0.5–1% NFC suspensions after spray drying or freeze-drying (Missoum et al., 2012; Žepič et al., 2014).

Freeze-drying is often used in the manufacturing of solid dosage forms of sensitive protein pharmaceuticals to improve the storage stability (Carpenter et al., 1997; Tang and Pikal, 2004). However, cryo- and lyoprotectants are usually required to prevent structural damage of protein pharmaceuticals induced by freezing and drying steps in the freeze-drying process (Carpenter et al., 1997; Franks, 1998; Hubálek 2003). We studied the prospect that these excipients could retain the structure of ANFC to a certain extent during freeze-drying. Furthermore, based on the literature (Kekäläinen et al., 2014), ANFC seems to be a more suitable grade than native NFC for the preparation of freeze-dried formulations intended for consequent reconstruction and rehydration, and was chosen as the hydrogel forming material.

We propose that with regard to preservation of ANFC structure it may be beneficial to use trehalose to replace the hydrogens bonds that are lost due to the sublimation of water in the freeze drying of ANFC. The suitability of trehalose as a lyo- and cryoprotectant has already been confirmed in the freeze-drying of protein pharmaceuticals (Chang et al., 2005; Jovanović et al., 2006), red blood cells (Han et al., 2005), platelets (Crowe et al., 2003; Wolkers et al., 2001) and liposomes (Christensen et al., 2007). It has been proposed that the molecular mechanism of protein as well as cell membrane stabilization with trehalose is achieved through water-trehalose hydrogen-bond replacement, coating by a trapped water layer and/or mechanical inhibition of the conformational fluctuations (Lins et al., 2004). Low trehalose concentration of 5–100 mM does not provide cryoprotection for proteins, but in combination with 1% polyethylene glycol (PEG) stabilization can be achieved (Carpenter et al., 1993; Prestrelski et al., 1993). Furthermore, PEG as hygroscopic polymer improves the water uptake (Jeon et al., 1991) which can be beneficial for reconstitution and rehydration of ANFC aerogels.

The aim of the current work was to characterize rheological as well as drug release properties of 3% and 6.5% ANFC hydrogels prior to and after freeze-drying and reconstruction. Here, a combination of trehalose and PEG 6000 was chosen as the cryo- and lyoprotectants in the freeze-drying of ANFC hydrogels into aerogels in order to minimize the hornification of ANFC fibers and to preserve the nano-scale structure of ANFC fibers. The freeze-dried aerogel formulations were rehydrated and re-gelled into their original concentrations in order to reform the hydrogel structure prior to the release testing and rheological measurements. Model compounds in the release studies included small molecules metronidazole (MZ), nadolol (NAD) and ketoprofen (KETO) with molecular weight below 500 M. 4 kDa FITC-dextran (FITC-DEX), lysozyme (LZ) and bovine serum albumin (BSA) represented high molecular weight compounds. The model compounds were selected based on their size, weight and different charges at pH 7.

2. Materials and methods

2.1. Materials

3.2% (11804-3) and 6.8% (11815-3) anionic NFC (ANFC) hydrogels (FibDex™) were kindly provided by UPM-Kymmene Corporation, Finland. Cellulose kraft pulp was chemically modified and fibrillated to form ANFC hydrogels (Saito et al., 2006, 2007). A Carboxylic acid content 1,06 mmol/g pulp was determined by conductometric titration according to the standard SCAN-CM 65:02. The diameter of most of the fibrils is in the range of 4–10 nm and length 500–10 000 nm measured using electron microscopies. All model compounds and reagents were of analytical grade. Bio-Rad Protein Assay reagent was purchased from Bio-Rad, USA. 4 and 10 kDa FITC-dextrans were purchased from Sigma-Aldrich, Sweden. D-(+)-trehalose dihydrate was purchased from Sigma-Aldrich, USA. Metronidazole was purchased from Sigma-Aldrich, China. Nadolol was purchased from Sigma-Aldrich, Finland. Ketoprofen was purchased from Orion Pharma, Finland. Lysozyme from hen egg white was purchased from Roche, Germany. Polyethylene glycol 6000 was purchased from Fluka, Switzerland. Dulbecco's Phosphate Buffered Saline (10×) concentrate without calcium and magnesium was purchased from Gibco, UK. Acetonitrile was of analytical grade, Sigma-Aldrich, Germany.

2.2. Methods

2.2.1. Preparation of the ANFC hydrogel formulations

ANFC hydrogel formulations were prepared in 10 ml syringes. The hydrogels were homogenized with model compounds by mixing for 10 min inside two attached syringes (200 times through the syringe nozzle). Table 1 contains the formulation compositions of the physical mixtures of ANFC hydrogels and model compounds. The ANFC amount in the final formulations was 3% or 6.5% (m/m). The measured pH for pure ANFC hydrogels was 7. For MZ, NAD and KETO, an excess amount of each drug was used in relation to their solubility in pH 7. Therefore, the drug containing ANFC hydrogel formulations were monolithic dispersions. MZ, NAD and KETO were added as dry powders into the ANFC hydrogels with final concentrations of 2% for MZ, 1.7% for NAD and 3.4% for KETO. The amount of 4 kDa FITC-DEX, BSA and LZ in the formulations did not exceed the solubility limit at pH 7. Therefore, these formulations were considered to be monolithic solutions. 4 kDa FITC-DEX was added into the ANFC hydrogel from 1 mg/ml stock solution with a 1% final concentration of FITC-DEX. BSA and LZ were added as dry powders into the ANFC hydrogels with final concentrations of 1% for BSA and 0.5% for LZ. The 3% and 6.5% ANFC hydrogel formulations with MZ, NAD, KETO and BSA were also prepared with 1% of PEG6000 and 0.3% of trehalose for freeze-drying. Dilute 1.1% ANFC hydrogels were crosslinked with cations calcium (Ca^{2+}), aluminum (Al^{3+}) and iron (Fe^{3+}) and were used to study the effect of crosslinking on rheology and drug release properties with 10 kDa FITC-DEX and MZ (detailed information in supplement).

1.1% (w/v) anionic ANFC hydrogel (UPM Oyj, Finland) was used with crosslinking cations aluminum sulfate hydrate (Sigma-Aldrich,