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Technetium-99m-labeled nanofibrillar cellulose hydrogel for *in vivo* drug release

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ABSTRACT

Nanoscale celluloses have recently gained an increasing interest in modern medicine. In this study, we investigated the properties of plant derived nanofibrillar cellulose (NFC) as an injectable drug releasing hydrogel *in vivo*. We demonstrated a reliable and efficient method of technetium-99m-NFC labeling, which enables us to trace the *in vivo* localization of the hydrogel. The release and distribution of study compounds from the NFC hydrogel after subcutaneous injection in the pelvic region of BALB/c mice were examined with a multimodality imaging device SPECT/CT. The drug release profiles were simulated by 1-compartmental models of Phoenix® WinNonlin®. The NFC hydrogel remained intact at the injection site during the study. The study compounds are more concentrated at the injection site when administered with the NFC hydrogel compared with saline solutions. In addition, the NFC hydrogel reduced the elimination rate of a large compound, technetium-99m-labeled human serum albumin by 2 folds, but did not alter the release rate of a small compound ^{123}I-β-CIT (a cocaine analogue). In conclusion, the NFC hydrogels is easily prepared and readily injected, and it has potential use as a matrix for controlled release or local delivery of large compounds. The interactions between NFC and specific therapeutic compounds are possible and should be investigated further.

1. Introduction

The use of hydrogels as nanostructured scaffolds and particles in tissue engineering and delivery of therapeutic agents is an emerging field in biomedicine (Geckil et al., 2010; Lu et al., 2013), as many hydrogels have innate structural similarities with physiological matrices (Slaughter et al., 2009). However, there is an ongoing research to improve the properties and quality of these applications, such as structural integrity, biocompatibility, and biodegradability. Recently, cellulose and cellulose-based materials have gained an increasing interest in modern medicine, mostly due to their versatility and inherent properties (Charreau et al., 2013).

Cellulose is the most abundant naturally occurring biopolymer on earth. The discovered structural features and properties have enabled the creation of novel cellulose-based materials and applications, particularly the emerging investigation of nanoscale celluloses (Charreau et al., 2013). The cellulose nanomaterials, mostly films and hydrogels, have already shown importance in industrial, pharmaceutical, and biomedical research (Klemm et al., 2011). In the biomedical field, injectable hydrogels have shown some potential (Jain et al., 2013); especially considering the challenges of non-invasive delivery of peptide and protein therapeutics, such as monoclonal antibodies and recombinant human proteins (Jain et al., 2013; Kumar et al., 2006; Muller and Keck, 2004).

Modern medicine involving drug delivery and therapy with implants and hydrogels, the applications must be non-toxic and biocompatible, while still providing the desired properties and functions for successful treatment. Currently, the modern medicine related research on nanostructural cellulose hydrogels has mostly focused on the use of bacterial celluloses (Innala et al., 2013; Muller et al., 2013; Pretzel et al., 2013). However, plant-derived nanofibrillar cellulose (NFC) prepared from wood pulp is also one of the emerging nanomaterials with properties for potential biomedical applications (Bhattacharya et al., 2012). High water content of NFC, like with many hydrogels, provides soft tissue like mechanical behavior and similarity, which inherently improves biocompatibility (Jain et al., 2013). Furthermore the viscoelastic properties of NFC resemble the physiological properties of extracellular matrices (Bhattacharya et al., 2012; Miron-Mendoza et al., 2010). The NFC aqueous suspensions behave as 1-compartmental hydrogels with pseudoplastic and thixotropic properties (Pääkkö et al., 2007). Pseudoplasticity induces a shear thinning

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effect which reduces viscosity with increased shear stress. Shear thinning therefore enables NFC hydrogels to be easily injected (Bhattacharya et al., 2012) as the extruding force of the syringe is enough to change NFC flow properties to lower the viscosity. While in static conditions, NFC retains higher viscosity due to the rearrangement of the fibers, which reverts the shear thinning effect. As an injectable hydrogel, NFC is able to deliver cells or therapeutic agents (e.g. proteins or peptides) into easily accessible target sites, such as under the skin. Additionally NFC hydrogels are biocompatible, non-toxic, and structurally durable (Märtson et al., 1999; Vartiainen et al., 2011). As a plant derived material, the NFC hydrogels are obtained from a non-animal and non-human source, being thus xeno-free. Additionally, cellulose based materials offer a broad modification capacity (Klemm et al., 2011), which is advantageous when designing new biomaterials.

Currently, in biomedical and -pharmaceutical research, the hydrogels under investigation for the potential use of controlled release matrices can prove to be problematic in terms of gel activation properties (Hennink and van Nostrum, 2002), especially with injectable hydrogels. The need for an external source of activation presents additional complications and toxicity as crosslinking agents often used are potentially toxic compounds (Van Tomme et al., 2008), that need to be extracted from the gels before usage. This could prove to be difficult in the case of parenteral delivery, such as subcutaneous injections. Furthermore, the crosslinkers may react with the imbedded drug compounds within the hydrogel, which may result to unwanted consequences or ineffective treatment. NFC overcomes this obstacle, as there is no need for activation methods such as the use of UV irradiation or chemical crosslinking due to the pseudoplasticity of the material. After administration (e.g. subcutaneous injection), NFC "gels" spontaneously, as the fibers rearrange to form a viscous gel; therefore avoiding all the complications with removing the crosslinking agents, potential toxicity or interactions between the crosslinking agents and the drug compounds in use.

The aim of this study was to investigate the properties of plant-derived NFC hydrogel as an injectable platform or "implant" for drug release, in addition to examine the utility of SPECT/CT imaging to illustrate the behavior of hydrogels *in vivo*. We investigated the use of NFC hydrogel as a potential device for local delivery and/or sustained drug release in an *in vivo* study with the use of BALB/c inbred mice. Furthermore the use of radiolabeled wood pulp NFC hydrogel as a potential biomedical device amongst other biomedical applications has not been demonstrated before. However, the biocompatibility and toxicity of bacterial and plant-derived cellulose materials have been documented both in vitro and *in vivo* use with of small animals (Märtson et al., 1999; Vartiainen et al., 2011; Alexandrescu et al., 2013; Roman et al., 2010; Kovacs et al., 2010; Pértile et al., 2011; Helenius et al., 2006; Moreira et al., 2009). In addition, we demonstrate a reliable and efficient method for NFC radiolabeling for the purpose of molecular imaging with a small animal SPECT/CT.

2. Materials and methods

2.1. ^{99m}Tc-NFC labeling

To image NFC in animals by SPECT/CT, NFC was labeled with ^{99m}Tc-NFC according to a previously described procedure for ^{99m}Tc-labeled carboxymethyl-cellulose (Schade et al., 1991) with slight modifications. 1.6% NFC stock hydrogel (GrowDex®, UPM-Kymmene Corporation, Finland) was used to prepare 1% NFC hydrogel with added stannous chloride stock (17.5 μg/ml in saline solution) and ^{99m}Tc-pertechnetate ($^{99m}TcO_4^-$) stock (~80 MBq/ml in saline solution) to a final volume of 1 ml. Briefly, 590 μl of the stock NFC was added to 285 μl of stannous chloride dehydrate solution (Angiocis®, IBA Molecular, Belgium) followed with 10 min incubation and mixing. Subsequently, 125 μl of $^{99m}TcO_4^-$ was added to the reaction mixture to reach the NFC concentration of 1% and incubated while mixing for 30 min.

To optimize the method for ^{99m}Tc-NFC labeling, various conditions were tested during the labeling procedure, such as buffer pH ranging from 4.74 to 8.05, different incubation times for $^{99m}TcO_4^-$/NFC reaction mixture (5, 10, 15, 20, 25 and 30 min) and stannous chloride concentrations ranging from 50 to 0.05 μg/ml.

The stability of the radiolabel was investigated in neutral isotonic pH by incubating the 1% ^{99m}Tc-NFC samples for 24 h. Samples were prepared in stock solutions as described above in saline or in fetal bovine serum (FBS) (Sigma–Aldrich, Finland). Radiochemical purity and efficiency was tested at every time point (0, 15, 60, 120, 240 min and 24 h).

TLC determined labeling efficiency and radiochemical purity of ^{99m}Tc-NFC with ITLC-SG chromatography plates (Agilent Technologies, Santa Clara, CA, USA) in methylethylketone (MEK) solvent system. Plates were cut in smaller equally sized pieces and placed in standard RIA tubes for radioactive measurement with a gamma counter (RiaCalc. WIZ, Wallac 1480 WIZARD® 3″, Finland).

2.2. In vivo imaging, drug release and kinetics

Animal studies were approved by the Finnish National Animal Experiment Board and performed in accordance with the Animal Welfare Act (247/1996) and Good Laboratory Practices for Animal Research. The release properties of plant-derived NFC implants were investigated with the use of radiolabeled small compounds. The use of ^{99m}Tc-NFC allows localization of the NFC in animals. Hydrogel implants were injected subcutaneously in the pelvic region, and the mice were observed non-invasively over a 24 h period with the use of a SPECT/CT system. After the 24 h period, the mice were sacrificed by cervical dislocation.

A total of 20 female BALB/c inbred mice were obtained from a professional stockbreeder (Harlan Laboratories, Netherlands) and quarantined for two weeks prior to the start of the experiment. The mice were divided into 7 groups, A, B, C, D, E, F ($n = 3$) and G ($n = 2$). The mice in groups A and C were injected with a mixture of saline solution and Iodine-123-Sodium Iodine (^{123}I-NaI) or with a cocaine analogue Iodine-123-(2-beta-carbomethoxy-3-beta-(4-iodophenyl)-tropane) (^{123}I-β-CIT) (MAP Medical Technologies Oy, Finland), respectively. The mice in groups B and D were injected with a 5:1 mixture of 1% NFC and ^{123}I-NaI or ^{123}I-β-CIT, respectively (final mixture of 0.83% NFC hydrogel with added study compound). Group E was injected with a mixture of ^{123}I-NaI and ^{99m}Tc-NFC for dual-radionuclide SPECT/CT. Groups F and G were injected similarly with 5:1 mixture of 1% NFC and ^{99m}Tc-labeled human serum albumin (HSA) (Sigma–Aldrich, Finland) or ^{99m}Tc-labeled HSA in a saline solution, respectively (final mixture of 0.83% NFC hydrogel with added study compound). All mice received 50–60 MBq/200 μl injections.

^{99m}Tc-HSA was prepared, and radiochemical purity was tested according to the manufacturer's instructions (Vasculocis®, CIS bio international, France). Radiochemical impurities were found below the allowed 5% of the total activity.

SPECT/CT imaging was performed with a four-headed small animal scanner (NanoSPECT/CT®, Bioscan, USA), outfitted with 1.0 mm multipinhole apertures. All mice were sedated with isoflurane, and SPECT images were acquired 0 h (with 5 or 6 acquisitions at 15 min intervals), 5 h and 24 h post-injection in 16 projections using time per projection of 45, 90 and 180 s, respectively. CT imaging was accomplished with 45 kVp tube voltage in 180 projections. For 3D co-registration and analysis, the SPECT images were reconstructed with HiSPECT NG software (Scivis GmbH, Germany) and fused with CT datasets by using the molecular imaging suite