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Active wound dressings based on bacterial nanocellulose as drug delivery system for octenidine

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ABSTRACT

Although bacterial nanocellulose (BNC) may serve as an ideal wound dressing, it exhibits no antibacterial properties by itself. Therefore, in the present study BNC was functionalized with the antiseptic drug octenidine. Drug loading and release, mechanical characteristics, biocompatibility, and antimicrobial efficacy were investigated. Octenidine release was based on diffusion and swelling according to the Ritger–Peppas equation and characterized by a time dependent biphasic release profile, with a rapid release in the first 8 h, followed by a slower release rate up to 96 h. The comparison between lab-scale and up-scale BNC identified thickness, water content, and the surface area to volume ratio as parameters which have an impact on the control of the release characteristics. Compression and tensile strength remained unchanged upon incorporation of octenidine in BNC. In biological assays, drug-loaded BNC demonstrated high biocompatibility in human keratinocytes and antimicrobial activity against *Staphylococcus aureus*. In a long-term storage test, the octenidine loaded in BNC was found to be stable, releasable, and biologically active over a period of 6 months without changes. In conclusion, octenidine loaded BNC presents a ready-to-use wound dressing for the treatment of infected wounds that can be stored over 6 months without losing its antibacterial activity.

1. Introduction

The increasing resistance of microorganisms against antibiotics in the treatment of acute and chronic colonized wounds has led to the renaissance of antiseptic drugs for local application in the prophylaxis and the treatment of wound infections.

Octenidine dihydrochloride (octenidine) was introduced for skin, mucous membrane, and wound antisepsis more than two decades ago (for review, see Hübner et al., 2010). Due to its cationic charge, it exhibits strong interactions with negatively charged components of microbial cell walls and membranes, leading to an inhibition of vital cell functions and a broad antimicrobial activity against Gram-positive and Gram-negative germs, plaque-forming bacteria, and fungi (Harke, 1989; Sedlock and Bailey, 1985; Slee and O'Connor, 1983). Furthermore, octenidine is effective against Methicillin-resistant *Staphylococcus aureus* (MRSA) (Al-Doori et al., 2007) and exhibits a moderate virucidal effectivity against enveloped viruses (Hübner et al., 2010). Its activity was not found to be compromised by interfering substances like albumin or mucin (Pitten et al., 2003).

A vast amount of knowledge on efficacy, tolerance, and safety has been collected from preclinical cell culture and animal studies as well as clinical trials (Hübner et al., 2010; Koburger et al., 2010; Vanscheidt et al., 2012). The following properties support the rationale for the selection of octenidine in this study: octenidine was found to be superior to polyhexanide (polyhexamethylene biguanide, PHMB), the agent of first choice for chronically poorly healing wounds and burns in terms of its therapeutic spectrum (Kramer et al., 2006). Consequently, it is nowadays an established

Abbreviations: BNC, Bacterial nanocellulose; MRSA, Methicillin-resistant Staphylococcus aureus; PHMB, Polyhexamethylene biguanide (polyhexanide); LS, Lab-scale; US, Up-scale; HoLiR, Horizontal lift reactor; SEM, Scanning electron microscopy; MLN, Microplate laser nephelometry; IC_{50}, Half maximal inhibitory concentration; AUC, Area under the curve; LC_{50}, Half maximal lethal concentration; SAV, Surface area to volume.

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broad spectrum antiseptic in a wide range of applications and represents a valuable alternative to other antiseptics especially for sensitive patient groups due to its (i) excellent skin compatibility and (ii) 24 h long skin remanent effect as well as (iii) the absence of resistance induction or (iv) local resorption with subsequent systemic side effects (Harke, 1989; Hübner et al., 2010). Due to its superior safety and biocompatibility in comparison to older antiseptics it is hence thought that it will gradually replace conventional antiseptics like triclosan, PVP-iodine or chlorhexidine in the near future (Hübner et al., 2010). Octenidine is usually applied as gel or solution by wiping, spraying, pouring or compresses soaked with commercially available preparations immediately prior to application.

Modern wound dressings are designed to fulfill many different requirements such as to promote a rapid and painless wound healing, maintain a moist environment, optimum pH and temperature, form an effective bacterial barrier, and provide protection from potentially irritating wound exudates (Wiegand and Hipler, 2010). Within the last years, a broad variety of modern wound dressings in different forms such as gels, foams or thin films and based on materials such as collagen, alginates, polyurethane, silicone or polyacrylates successfully entered the market. Against this, traditional wound dressings such as cotton wool, natural or synthetic bandages, lint or gauzes, designed to solely keep the wound dry, allow the evaporation of wound exudates and to prevent the entry of harmful bacteria into the wound significantly lost importance (Boateng et al., 2008). However, the search for the "ideal" wound dressing material is still ongoing, since most of the modern wound dressings also possess some drawbacks. Depending on the material used and its form, important criteria such as high moisture vapor transmission and fluid affinity, well balanced liquid uptake and retention without drying out the wound, mechanical stability (e.g., tensile strength) in combination with high softness following the limp contours, availability in different shapes as well as non-allergenic and sterile composition cannot be provided by all in one type of those dressings.

Bacterial nanocellulose (BNC) fleeces produced by Gram-negative, aerobic strains of *Komagataeibacter xylinus* have been proven to be a perfectly suitable biomaterial that fulfills all performance requirements of ideal modern wound dressings (Bielecki et al., 2013). With fibers, 100-times thinner than for conventional cellulose dressings, BNC presents a fully biocompatible and mechanically stable hydropolymer that acts as a barrier to microbial contamination and traumas, but also provides gaseous exchange and maintains a moist wound environment while simultaneously absorbing exudates (Bielecki et al., 2013). While BNC itself has no antimicrobial activity, several approaches using silver (Berndt et al., 2013), silver sulfadiazine (Luan et al., 2012), benzalkonium chloride (Wei et al., 2011), and montmorillonite (Ul-Islam et al., 2013) were described to equip this biopolymer with antimicrobial properties. Up to now, only one BNC product containing polyhexanide (Suprasorb® X + PHMB) has made it to the market as antiseptic wound dressing for critically colonized or infected, superficial or deep wounds with low to moderate exudation (Dissemond et al., 2010), but more products containing active drugs will surely follow in the future.

In the present study, an active wound dressing based on bacterial nanocellulose loaded with the antiseptic octenidine was developed as drug delivery system for the treatment of acute and chronically infected wounds. The octenidine containing BNC fleeces were produced in 24-well plates under laboratory conditions [lab-scale (LS) BNC] and investigated regarding drug loading performance, controllable drug release, mechanical characteristics, biocompatibility, and antimicrobial efficacy. Additionally, the preservation of the drug release characteristics and the antimicrobial activity were determined in a six month accelerated stability study. Furthermore, loading and release were compared to that of octenidine loaded BNC samples produced in a large scale process [up-scale (US) BNC] and cut into dimensions (100 mm × 100 mm) typically used in clinical applications.

2. Materials and methods

2.1. Preparation and characterization of BNC fleeces

For the biosynthesis of BNC, *K. xylinus* DSM 14666 (culture collection of the Friedrich-Schiller-University of Jena, deposited at the DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) was cultivated at 28 °C in Hestrin–Schramm medium (Kralisch et al., 2009). Under lab-scale static conditions, 24-well plates, 12-well plates (compression studies) (both from Sarstedt, Nuembrecht, Germany) or dumbbell-shaped culture containers (dimensions according to EN ISO 527-1, test specimen type 1A) consisting of polytetrafluoroethylene (RS Components, Moerfelden-Walldorf, Germany) (tensile tests) were used for cultivation of the bacteria as described before (Kralisch et al., 2009; Müller et al., 2013). In an up-scale process BNCs of two different thicknesses (4 mm: US4, 7 mm: US7) were produced as endless fleece under semi-continuous steady state conditions with continuous harvesting using the Horizontal Lift Reactor (HoLiR) process (Kralisch et al., 2009). After biosynthesis, the endless fleece was cut into pieces (100 mm × 100 mm). All types of BNC were purified as described previously (Kralisch et al., 2009), sterilized by autoclaving (121 °C, 20 min, 2 bar), and stored at 4 °C until use. Compressed US BNC (USpress) was obtained by compression of the US7 fleeces between the plates of a hydraulic press (LaboPress P200S, Vogt Maschinenbau, Berlin, Germany) at 3 bar and 20 °C for 3 min to form fleeces with a final thickness of about 3 mm. All BNC fleeces were characterized regarding their dimensions (edge length or diameter, height, and weight) as described before (Müller et al., 2013). For calculation of surface area and volume, the geometrical formulas of a circular cylinder or a cuboid were used (Müller et al., 2013).

2.2. Quantification of octenidine

Octenidine was purchased from Schuelke & Mayr (Norderstedt, Germany) as a stock solution containing 0.5% octenidine. For quantification of the octenidine concentrations ultraviolet and visible (UV/vis) spectra of octenidine between 200–800 nm and calibration curves (3.4–11.1 μg/mL) were recorded in 0.1 M phosphate buffered solution (PBS) pH 7.4 (Carl Roth, Karlsruhe, Germany) using the Beckman DU 640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). The time and storage temperature dependent stability of octenidine solutions was investigated by UV/vis measurements after storage at −20 °C, 4 °C, 20 °C, and 32 °C over 1, 2, 3, 4, 7, 9, 11, and 16 days and after autoclaving (121 °C, 2 bar, 15 min). All experiments were run in triplicates and repeated once.

2.3. Loading and release experiments

BNC fleeces produced in 24-well plates (LS samples) were incubated under submersed conditions in 10.0 mL 0.5% octenidine solution at 20 °C on a temperature-controlled orbital shaker (KS 4000 ic control, IKA®-Werke, Staufen, Germany) at 70 rpm for 48 h. Fleeces loaded only with PBS were used as control. The drug loading is equal to the difference of the octenidine concentration of the loading solution before and after the loading process. To determine the octenidine release from BNC fleeces at 32 °C, loaded BNC was removed from the loading solution and transferred into 20.0 mL PBS pH 7.4 as release medium. Samples were incubated