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Short communication

Pharmaceutical use of nanocellulose produced by enzymes

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Introduction

Cellulose is an indispensable gelling agent in pharmaceutical hydrogels (HGs). There are two main strategies to gain cellulose: the top-down process from plants and the eco-friendly bottom-up synthesis out of single sugar molecules. By these methods celluloses of different degree of polymerization (DP) can be obtained. The production and use of short-chained cellulose (DP<50), called nanocellulose (NC), is a promising, rather new development. NC shows a high biocompatibility, liquid absorption capacity, porosity and mechanical strength. The latest development to achieve more defined NC products and controlled yield is an enzymatic bottom-up in-vitro production. Highlight of this technique is the possibility to produce soluble (DP ≤ 6) as well as insoluble (DP > 6) NC, the uniformity of DP, short production periods and defined modifications of the cellulose (Serizawa et al., 2017; Zhong et al., 2019).

HG prepared of insoluble, enzymatically synthesized NC, showing a self-assembled nanoribbon structure, bear new potentials for drug delivery. This work focused on the preparation and characterization of HG from enzymatically synthesized, insoluble NC with a DP of 7–10 and their use as drug delivery systems. To ensure purity and inertness of the NC, a process to remove possible production residues was established in a first step. After purification and freeze-drying of the NC, HGs were produced by addition of water. These HGs were intensively characterized by rheological measurements. Afterwards different active pharmaceutical ingredients (APIs) were successfully loaded into the HGs and their release was studied in permeation and diffusion tests.

Materials and methods

The studied NC was enzymatically synthesized under conditions described by Zhong et al. (2019) using cellobiose as primer. Unless stated, all materials used in this work were purchased from Merck KGaA (Darmstadt, Germany) or Carl Roth GmbH + Co. KG (Karlsruhe, Germany) in highest purity. All tests were at least duplicates.

Purification and freeze drying

For purification of the NC, four washing steps with acetic buffer pH 4.5 were performed at a mass ratio NC:buffer=1:1. After addition of buffer and short vortexing at 3000 rpm, the samples were centrifuged at room temperature (RT) and 14000 rpm. The purified NC pellet was frozen in liquid nitrogen and freeze-dried in a Lyovac GT2 (SRK Systemtechnik GmbH, Riedstadt, Germany).

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Determination of removed phosphate and enzyme

The supernatants of the purification were subjected to a quantitative, colorimetric phosphate assay (Zhong et al., 2019). Quantification of the enzyme was done by Roti®-Quant. All photometric measurements were performed by a Nanophotometer Implen P-Class (Implen GmbH, München, Germany).

Hydrogel preparation and drug loading

The freeze-dried NC was reconstituted with MilliQ®-water to its original weight. For drug loading, 80% of the total water amount was added to the NC at first. The samples were vortexed and heated up to 95 °C for 5 minutes. The missing 20% of water, including gentamicin-sulfate or diclofenac-sodium purchased from G.L. Pharma GmbH (Lannach, Austria), were added and vortexed again. HGs containing 3 mg/mL gentamicin (GM) and 1% diclofenac-sodium, respectively, were achieved.

Rheological characterization

Rheological measurements were performed by a Physika MCR 301 (Anton Paar GmbH, Graz, Austria) using a cone-plate device (CP-25). Flow curves with increasing shear rates (0.01 – 2000 s^{-1}) were set up. Additionally, rotation and oscillation recovery tests after high shear load (2000 s^{-1}) were conducted.

Drug release

According the gentamicin gel, a diffusion test on Müller-Hinton agar plates was carried out using *Staphylococcus epidermidis* as test germ. For this test 3.33 mg gel (10 µg GM) and 10 mg gel (30 µg GM) were used. The drug permeation of the diclofenac gel was tested in Franz cells (PermeGear, Pennsylvania, USA) in combination with the hydrophilic-lipophilic multilayer membrane PermeaPad® (innoME GmbH, Espelkamp, Germany).

Results and discussion

The enzymatically synthesized NC was purified by several washing steps with acetic buffer to remove the by-product phosphate and the enzymes. While phosphate could be eliminated rather completely, only 50% of enzymes were washed off. After lyophilization of the insoluble NC, HGs were produced by addition of MilliQ®-water. Rheological measurements of these HGs showed a dynamic viscosity of about 600 Pa*s at 0.01 s^{-1} and RT and revealed a pseudoplastic-thixotropic flow-behavior. As G', representing the elastic portion of the gel, was higher than G'', representing the viscous portion, before and directly after high shear load, the HG seemed to reconstruct quickly.

For inclusion of an API, the lyophilized NC was reconstituted with aqueous API-solutions including diclofenac-sodium and gentamicin-sulfate, respectively. During the drug loading heat of 95 °C was applied to inactivate the remaining 50% of enzymes. The API solution was taken up completely by the NC under swelling. The drug release of the gentamicin gel was successfully proven by diffusion tests. *Staphylococcus epidermidis* was not growing within an inhibition zone of at least 14 mm, proving a qualitative drug release and the activity of the antibiotic API. Referring to the diclofenac gel, the API-permeation tested in Franz cells reached 4% after 6 h. Additionally, a 1% diclofenac solution was tested. Within 6 h approximately 26% of the API permeated from the diclofenac solution through the membrane. This is a 6.5 times higher permeation rate than for the NC gel, indicating a retardation effect of the gel.

Conclusion

To the best of our knowledge, this is the first time an enzymatically synthesized NC was used for the preparation of drug-loaded hydrogels. The results shown are a promising starting point for further investigations of these NC hydrogels as drug delivery systems as they are capable of taking up APIs in therapeutic concentrations, show a pseudoplastic-thixotropic flow-behavior and a prolonged drug release after topical application.

References

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