Thermoresponsive nanogels based on hyperbranched polyglycerol for dermal protein delivery

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ABSTRACT SUMMARY
A thermoresponsive nanogel made of poly(N-isopropylacrylamide) and hyperbranched polyglycerol was made to encapsulate proteins, protect them, and trigger release in dermal applications. Efficient encapsulation in the nanogels and subsequent release of the proteins without loss of protein activity was achieved.

INTRODUCTION
Therapeutically relevant proteins such as antibodies, growth factors or enzymes play an increasing role in the treatment of malignant and autoimmune diseases. However, they often suffer from insufficient stability and poor bioavailability. A suitable method to increase protein stability might be the non-covalent encapsulation into polyglycerol-based nanogels. For drug release, external stimuli such as pH or a thermal trigger can be used to initiate a controlled and targeted release.¹² By crosslinking poly(N-isopropylacrylamide) (pNIPAm), a thermosensitive polymer, with hyperbranched polyglycerol (hPG), thermoresponsive nanogels (NG) were developed.³

The model proteins bovine serum albumin (BSA), L-asparaginase, and lysozyme were incorporated and release, protein stability and dermal drug delivery were investigated.

EXPERIMENTAL METHODS
pNIPAm-hPG NG were synthesized following the procedure described by Calderón et al. by precipitation polymerization.³ After purification of the NG the proteins were encapsulated by diffusion.

The particle sizes and the dispersity of the NG were measured before and after encapsulation at 25 °C using a Nano-ZS 90 Malvern. The thermoresponsive properties were determined with a temperature ramp from 25 – 40 °C (1 °C/min).

For protein release characterization, 50 µl of the samples were analyzed with HP-SEC using a TSKgel® G4000 PWXL column. Isocratic elution with 20 mM Na₂HPO₄, 150 mM NaCl, pH 7.4, 0.003 mM NaN₃ was performed with a flow-rate of 0.4 ml/min. The protein concentration was determined using UV (280 nm) and fluorescence (ex. 295 nm, em. 348 nm) detection.

The enzymatic activity of the L-asparaginase was determined according to the protocol published by [1], according to the Sigma-Aldrich protocol “Enzymatic Activity of Lysozyme” (EC 3.2.1.17).

Skin penetration experiments were performed using pig skin mounted onto static-type Franz cells (diameter 15 mm, volume 12 ml, PermeGear Inc., Bethlehem; PA, USA) and according to validated test procedures (Schäfer-Korting et al., 2008). For data analysis, cross-sections (5 µm) were subjected to normal and fluorescence light microscopy (magnification 200x, excitation (RhB ITC) 560 nm, BZ-8000, Keyence, Neu-Isenburg). For semi-quantitative protein amount determination, arbitrary pixel brightness units (ABU) were evaluated using image analysis software BZ Analyser (Keyence, Neu-Isenburg, Germany) within the SC, viable epidermis and dermis. To simulate barrier-disrupted skin tape-stripping was performed by 30 times tape stripping (10 cm length; tesapack® 4120 PVC, Tesa SE, Hamburg) of the skin surface.

RESULTS AND DISCUSSION
The particle size of the NG increase from 100 nm to 207 nm after the protein encapsulation. Examining the thermoresponsive properties, the temperature at which the NG are expected to shrink due to a conformational change of the thermosensitive cross-linker, DLS revealed a sharp decrease in particle size to 170 ± 3 nm (PDI 0.1) between 34–35 °C (Figure 1).

Figure 1. hPG-NG size change upon heating from 25-42 °C (n=3).

Preliminary release experiments showed successful drug release up to 100%. As intended, the NG stayed stable at temperatures below the trigger point of 34 °C. At a higher temperature around 37 °C, the NG immediately released about 85% of the protein.

The lysozyme showed a reduced activity after the release at 37 °C (54.2± 3.0%) compared to the native lysozyme (91.2±1.0%). As additional FT-IR analysis revealed no changes in secondary structure indicating sustained bioactivity, the protein decreased activity most probably is due to problems with the activity assay as repeatedly turbidity interfering with the hPG occurred. Thus, a colorimetric assay will be established to verify bioactivity sustainment.

To test the drug delivery efficiency, the penetration of the proteins encapsulated in the NG into pig skin was investigated. Figure 2 displays that in tape-stripped skin the protein penetration into the epidermis at 32 °C was twice as much as for the control. In further experiments, a temperature gradient during incubation ranging from 32 °C up to 37 °C will be used to meet the temperature trigger and to elucidate the role of temperature-triggered drug release.

Figure 2. Effect of NG on skin penetration of BSA-RhB ITC (n=3) at 32 °C. PEE = penetration enhancing effect

CONCLUSION
Our data show that the thermosensitive hPG-NG are suitable and promising carrier systems for labile drugs such as biomacromolecules. Despite harsh chemical conditions efficient encapsulation in the NG and subsequent release of the proteins without loss of protein activity was achieved. In terms of skin penetration, first experiments showed promising results for the thermosensitive NG.

REFERENCES

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