Investigation of dye labelled dPG-Nanogels as pH sensors in hair follicles

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Summary

Dermal and transdermal drug delivery is an important field aiming at transporting drugs through the skin for targeted treatment of skin diseases and other conditions with reduced side effects. Smaller nanoparticles may diffuse through the skin via an intercellular pathway whereas bigger nanoparticles can diffuse through the hair follicles (HF) into deeper skin areas[1]. However, it is not known how the pH in the HF changes with depth in comparison to the skin surface. The accurate determination of the pH values can be used to develop smart nanotransporters which possibly makes use of this environment to have triggered release of drugs. In the present study, we developed a new pH responsive nanogel (NG) to study the pH in the HF using an *ex vivo* porcine ear model. The devised nanogels were synthesized through an inverse nanoprecipitation method under mild and surfactant free conditions via a thiol-michael reaction[2-5]. The study results showed that the pH of the hair follicle increased from 6.5 at the surface of the skin to 7.4 in deeper areas of the HF (penetration depth of 531 µm).

To study the pH change inside the hair follicle, pH sensitive nanogels were devised. The results of the different chemical and biological tests are shown in the following:

Synthesis of dye labelled Nanogels

The dPG nanogels were synthesized via a thio-michael nanoprecipitation process. The pseudo click reaction occurs under mild conditions and did not need any catalysts or surfactants. dPG-amine was conjugated with two different dyes (Cy3 and pH Cy5.25) due to an NHS ester coupling. The dye labelled dPG-amines were thiolated *in situ* and coupled with an dPG-acrylate. The nanogel preparation was conducted in a precipitation polymerization in a non-solvent.

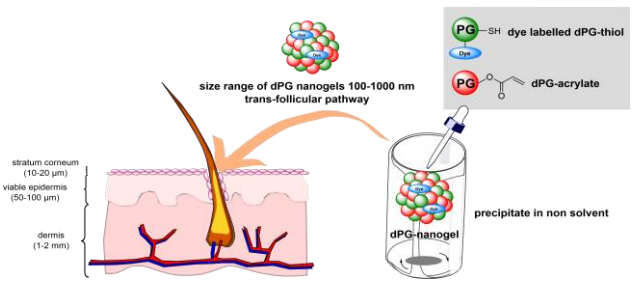

Figure 1: Synthesis of dye labelled nanogels and application on the skin.

Physical characterization of the NGs

After purification the nanogels were duly characterized using a combination of techniques (DLS, NTA and AFM). The Elmanns test was used to observe and track the pseudo click reaction.[1]H-NMR showed full conversion of the acrylate groups which were involved in the thio-michael reaction. With higher concentration of the macromonomers different sizes of NGs were obtained. As dye labelled NGs were not measurable with the DLS due to experimental limitations (overlap in the absorption region of the laser and the coupled dyes) their size was measured by NTA and AFM. The sizes of labeled nanogels corresponded to the size of the nonlabeled NGs synthesized in the same condition. The dye labelled NGs were also analyzed with UV measurement. Different buffers were used to reveal the decrease of the absorbance intensity of the pH dependent Cy 5.25 dye with the increase of the pH (Fig 2).

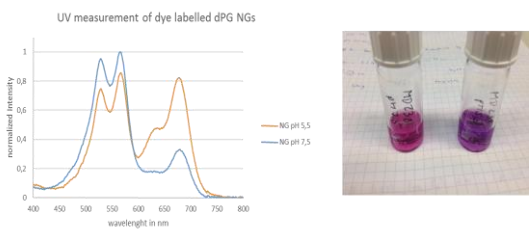

Figure 2: pH dependent behavior of the dye conjugated dPG-NGs.

Construction of calibration curve

Preceding determination of the pH values in the HF a calibration curve was constructed. During the process, the nanogels maintained in varying buffer solutions were applied on freshly obtained porcine ear skin tape strips (n=2) and the solution was allowed to dry in the dark at room temperature. Then microscopic images of the dyes were obtained using a Confocal Laser Scanning Microscope (CLSM) (Fig 3A) and the emission intensity from both dyes was quantified using ImageJ software: only the black and white picture of the two different channels (channel1: Cy3 dye, channel2: pH Cy5.25 dye) were analyzed to achieve the value of the integrated density of the mean gray values. Finally using the ratios of intensities of the dyes at different pH values the calibration curve was calculated (Fig 3B).

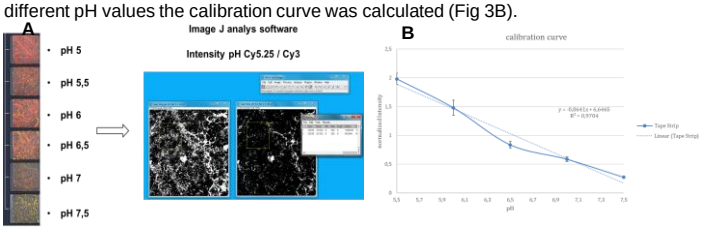

Figure 3: Flowchart to evaluate the data for the calibration curve **(A)** and the Calibration curve for nanogels in different PBS buffers **(B)**.

Determination of HF pH values

To determine the pH value in the HF, the nanogels were applied on porcine ear skins (n=3) and biopsies were obtained. Then histological sections (n=10) containing hair follicles were cut out at different depths using a microtome (a total of 70 hair follicles at various depths were analyzed) and the hair follicles (Fig 4A) were observed under a CLSM. Then the emission intensity of both dyes at selected depths were calculated as described above to obtained the intensity ratios and the corresponding pH values (Fig 4B).

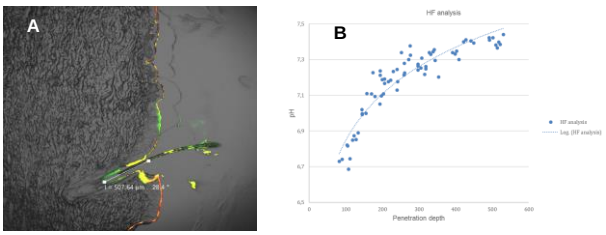

Figure 4: Confocal picture of the pig ear skin section with applied NGs **(A)** and the graph of the pH change during the follicular penetration route **(B)**.

Conclusions

We have developed a new approach to synthesize dPG-nanogels via a thiol-michael nanoprecipitation method which works under mild conditions and does not need any catalyst or surfactant. This enabled the development of dye labelled dPG-nanogels that were used as pH senor to probe the pH in an *ex vivo* porcine ear model. The investigation showed that the NGs penetrated in the HF up to 530 µm. With the help of software analysis of CLSM images an accurate determination of the pH inside the HF was achieved. A pH gradient in the hair follicle from 6.5 on the skin surface to 7.44 in deeper areas of the HF (penetration depth of 531 µm) was obtained.

References

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