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Effects of thermoresponsivity and softness on skin penetration and cellular uptake of polyglycerol-based nanogels

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ABSTRACT

Nanogels are water soluble cross-linked polymer networks with nanometer size dimensions that can be designed to incorporate different types of compounds and are promising carrier systems for drugs and biological molecules. In this study, the interactions of thermoresponsive nanogels (tNGs) with the human skin barrier and underlying epidermis cells were investigated with the aim of using such macromolecules to improve dermal and transdermal drug delivery. The investigated tNGs were made of acrylated dendritic polyglycerol, as water soluble cross-linker, and of oligo ethylene glycol methacrylate (OEGMA) as subunit conferring thermoresponsive properties. tNGs with different polymer transition temperatures were tagged with Rhodamine B (RhdB) and analyzed for their physicochemical properties. We found that tNGs with cloud point temperatures (Tcps) of 38 °C (tNG–RhdB-T38) lost softness (measured by PeakForce quantitative nanomechanics, QNM) and aggregated to bigger sized particles (measured as increase of particle average size by dynamic light scattering, DLS) when temperature changed from 15 to 37 °C. On the contrary, at the same conditions, tNGs with higher Tcps (tNG–RhdB-T55) did not show any significant changes of these characteristics. Applied on excised human skin, both tNGs penetrated deep in the stratum corneum (SC). Small amounts of tNGs were detected also in cells of the viable epidermis. Interestingly, whereas tNG softness correlated with higher penetration in SC, a better cellular uptake was observed for the thermoresponsive tNG–RhdB-T38. We conclude that soft nanocarriers possess a high SC penetration ability and that thermoresponsive nanogels are attractive carrier systems for the targeting of drugs to epidermis cells.

1. Introduction

Nanocarriers are considered to be key tools in several medical and pharmaceutical research fields, allowing new treatment strategies and improving the administration of therapeutic, imaging, and theranostic agents [1]. Some of the most appreciated features of nanocarriers are the possibilities to target drugs to specific body compartments or cell types as well as to deliver defined amounts of drugs in a sustained manner [2]. For good outcome, nanocarriers might therefore be used to deliver a drug persistently into skin. Several treatments of frequent skin conditions such as acne, psoriasis, and atopic dermatitis would benefit from new drug delivery strategies. Improved topical drug delivery, aiming at a more selective and controlled drug delivery by use of nanocarriers, would help to maximize the drug concentration at the target site and reduce systemic side effects [3].

In addition, nanocarriers might also be used for transdermal drug delivery. Especially for biopharmaceuticals which can be degraded in the gastrointestinal tract or by the hepatic first-pass metabolism, alternative routes, *e.g.* the transcutaneous one, might be preferred to the oral route. In the case of Rivastigmine, where small daily amounts of drug are needed, transdermal delivery by means of patches has been shown to promote prolonged and constant blood levels, resulting in improved therapeutic effects [4]. Nevertheless, in order to fully exploit the efficiency of nanocarriers for transdermal drug delivery, safe and non-invasive methods enhancing skin penetration are required.

The stratum corneum (SC), the outermost skin layer made of several dozen corneocytes layers, interconnected by corneodesmosomes and a highly structured lipid extracellular matrix, provides a very efficient barrier function [5,6]. Most of the investigated nanoparticles were found to penetrate in the SC and to accumulate in furrows and hair follicle (HF) canals [7], which are considered to be reservoir for topically applied carrier systems [8]. Nanoparticle physicochemical properties (size, surface charge, polymer hydrophobicity, and stability) were

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identified as crucial parameters for the control of particle penetration depth in the HF canal and the targeting of drugs to different HF areas and cell populations [3,9,10]. It is generally believed that nanoparticles cannot cross the intact skin barrier and that they rather gather in between the corneocyte layers or disassemble before their components penetrate the skin [11,12]. Nevertheless, the search for non-invasive improved transdermal drug delivery has shown that nanocarrier coatings (*e.g.* penetrating peptides), incubation conditions (*e.g.*, occlusion) or application methods (*e.g.*, microneedles) may promote nanocarrier penetration and thus increase drug delivery [13–15].

In this study, polyglycerol-based thermoresponsive nanogels (tNGs) were investigated. Nanogels can be described as polymeric particles which consist of entangled or chemically cross-linked polymer networks [16]. They are promising carrier systems because of their high loading capacity, biocompatibility, and physical stability in biological environment. Depending on the constituting units, nanogels with responsiveness to stimuli such as ionic strength, pH or temperature can be prepared. They can incorporate high volumes of water and may act as moisturizing agents enhancing SC hydration. In addition, they can be loaded with water-soluble drugs as well as biomacromolecules such as nucleotides and proteins [17,18]. Drug loading can be achieved by covalent conjugation, incorporation or by adsorption [16]. Properties such as size, degree of branching, viscosity and swelling behavior can be controlled by varying subunit chemical composition and feeding ratio [19]. By combining thermoresponsive polymers with dendritic polyglycerol (dPG), our group has developed a series of dendritic tNGs that are suitable for biomedical applications [20–22]. The presence of dPG as a macro-crosslinker provides high biocompatibility, hydrophilicity and chemical functionality for further chemical modification. In this study, acrylated dendritic polyglycerol (dPG-Ac), a water-soluble linker, oligo ethylene glycol methacrylate (OEGMA), and diethylen glycol methacrylate (DEGMA), a the thermoresponsive units, were polymerized in aqueous media by using the redox system of ammonium persulfate (APS) and N,N,N′,N′-tetramethylethylenediamine (TMEDA) as the radical initiator system, as well as sodium dodecyl sulfate (SDS) as colloidal stabilizer [19,23]. By modifying the DEGMA monomer mol fraction as previously described [23], thermoresponsive systems were synthesized with different cloud point temperatures (Tcp = 38 and 55 °C). At these temperatures, tNGs undergo a phase transition which results in release of eventually loaded molecules and a decrease of colloidal stability in water with consequent aggregation [23]. A Tcp of 38 °C was chosen because it is close to that of body temperature during inflammatory processes (>37.8 °C), whereas tNG–RhdB-T55 with Tcp above the *in vivo* temperature served as a non-responsive control.

A special characteristic of these nanogels is that they consist of one single entity. In contrast, most organic nanocarriers, *e.g.* liposomes, polymer- and lipid-based particles, are supramolecular structures consisting of several physically interacting components (polymer chains, ceramides, phospholipids) that are not covalently bound to each other but kept together by physical interactions, *e.g.* hydrogen bonds, ionic interactions, and Van der Waals forces. It is known that liposomes and solid lipid nanoparticles change their physical aggregation form once they get in contact with the SC [24,25]. A similar behavior was found for polylactic acid (PLA) particles [26]. We expected that tNGs might interact with skin barrier in a different way than liposomes and PLA particles. Hence, the main objective of this study was to investigate the parameters influencing the penetration of tNGs in the SC, their eventual uptake by epidermal cells, and to verify whether thermoresponsivity may influence these interactions.

2. Material and methods

The synthesis and chemical structure characterization of the nanogels are reported in Supplementary Materials.

2.1. Dynamic light scattering (DLS)

Size, and size distribution, measurements of tNGs were performed on a Malvern Nano-ZS 90 equipped with a He–Ne laser (λ = 633 nm) under scattering angle of 173° at 25,36 and 60 °C. All the samples were maintained for stabilization at the designated temperature for 5 min before testing. The samples were prepared dissolving 1 mg of dry nanogel in 1 mL of 10 mM phosphate buffer pH = 7.4 one day prior to the experiments. Particle sizes and size distribution are given as the average of 3 measurements (each measurement containing 11 runs with a duration of 10 s per run) of the intensity distribution curves. The intensity distribution curves of particle sizes represent the first order result from DLS experiments.

2.2. Transmission electron microscopy (TEM)

Transmission electron microscopy samples were prepared on carbon-coated copper grids (300 mesh, Quantifoil) by blotting samples in 1% aqueous uranyl acetate and visualized using the TEM mode of the Hitachi Scanning Electron Microscope (SU8030) (20 kV). 1 mg of each nanogel was dissolved in 1 mL of deionized water one day before the TEM experiment. The sample was dried in an oven at 30 °C for 2 min.

2.3. Atomic force microscopy (AFM)

The AFM tapping mode images were recorded in the air under ambient conditions, with a MultiMode 8 AFM equipped with a Nanoscope V controller from Veeco Instruments, Santa Barbara, California. The results were analyzed by means of Nano-Scope Analysis 1.3 software. The nanogels were spread on pieces of silicon wafers (1 cm × 1 cm) *via* spin-coating from aqueous solution (ambient conditions, room temperature). The wafers (Si-100, p (boron), 30 Ω cm), with artificially oxidized surfaces (wet oxidation) with an oxide layer thickness of about 300 nm were obtained from Silchem, Freiberg, Germany. Before the spin coating procedure, the wafers were treated with a piranha solution (3:1 mixture of sulphuric acid and 30% hydrogen peroxide) in order to eliminate any organic impurities from the surface. For size distribution analysis 293 particles of tNG–RhdB-T55 and 2027 particles of tNG–RhdB-T38 have been analyzed using the ImageJ software 1.48v.

2.4. PeakForce quantitative nanomechanics (QNM)

The material properties of the investigated nanogels were obtained with an AFM Nanoscope MultiMode 8 from Bruker operated in PeakForce mode. All measurements were performed in liquid using a fluid cell at controlled temperature. The thermal application controller (Bruker) was used to maintain a constant temperature through all experiments. Experiments with the tNG were performed at 37 °C and 15 °C. For the preparation of the samples HOP-Graphite (spi Supplies West Chester, PA, USA) glued to metal disks was used as substrate, since tNGs remained firmly attached to its surface without any further chemical modification. Regular adhesive tape was first used to remove the most external layers of the graphite surface until a flat surface was obtained. Then 10 μL of the tNG solution (1 mg mL^{-1}) were deposited and incubated for at least 20 min. After incubation, the surface was repeatedly rinsed with ultrapure water (Milli-Q) and finally incubated with 30 μL of ultrapure water (Milli-Q). Therefore samples were at any moment allowed to dry. Afterwards, the samples were mounted onto the AFM head and a fluid cell was assembled. The temperature controller was set to a desired temperature and the system was allowed to reach equilibrium for at least 10 min. Prior to any measurements, calibration of the cantilever spring constant was performed at the corresponding temperature and using the already well established thermal noise method [27]. AFM tips SNL-10 (Bruker) with a tip radius of 2–12 nm were used. Operation with PeakForce QNM was performed using the ScanAsyst auto control with a PeakForce set point value of