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Research paper

Impact of structural differences in hyperbranched polyglycerol–polyethylene glycol nanoparticles on dermal drug delivery and biocompatibility

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ABSTRACT

Polyglycerol scaffolds and nanoparticles emerged as prominent material for various biomedical applications including topical drug delivery. The impact of slight structural modifications on the nanoparticles' properties, drug delivery potential, and biocompatibility, however, is still not fully understood.

Hence, we explored the influence of structural modifications of five structurally related polyglycerol-based nanoparticles (PG–PEG, SK1–SK5) on dermal drug delivery efficiency and biocompatibility. The PG–PEG particles were synthesized via randomly and controlled alkylated chemo-enzymatic approaches resulting in significantly varying particle sizes and interactions with guest molecules. Furthermore, we observed considerably improved dermal drug delivery with the smallest particles SK4 and SK5 (11 nm and 14 nm) which also correlated with well-defined surface properties achieved by the controlled alkylated synthesis approach. The consistently good biocompatibility for all PG–PEG particles was mainly attributed to the neutral surface charge. No irritation potential, major cytotoxicity or genotoxicity was observed. Nevertheless, slightly better biocompatibility was again seen for the particles characterized by alkyl chain substitution in the core and not on the particle surface.

Despite the high structural similarity of the PG–PEG particles, the synthesis and the functionalization significantly influenced particle properties, biocompatibility, and most significantly the drug delivery efficiency.

1. Introduction

Topical drug delivery is highly interesting for local and systemic therapies. Due to the unique composition and properties of the human skin, however, the ability of substances to penetrate into or through the skin is limited and strongly depends on the physicochemical properties of the respective substance. Sufficient skin absorption is solely achieved by applying moderately lipophilic drugs (log*P* 1–3) with a molecular weight ⩽500 g/mol. The total cutoff for dermal absorption is 800 g/mol. Large and hydrophilic drugs including proteins and peptides are therefore nowadays excluded from topical applications. To overcome these obstacles various nanoparticulate drug delivery systems have been developed in order to improve the drug delivery into or through the skin [1–3]. Particularly hyperbranched polymers and dendrimers represent a promising opportunity [4–6] due to tailorable particle size and shape, monodispersity, and the possibility for surface modifications [7].

Despite the huge variety of dendrimers, the majority of studies have investigated two types of dendrimers for topical drug delivery: poly(amido amine) (PAMAM) and polyglycerol [7]. Aside from the above-mentioned advantages, PAMAM exhibits strong cytotoxic effects which limits its applicability [8]. A recent study described that PAMAM G2.5 shell tecto-dendrimers are tolerated well by spontaneously transformed keratinocytes (HaCaT cells) and a human colonic adenocarcinoma cell line but is still toxic for a melanoma cell line [9]. In general, the biocompatibility of

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nanoparticulate carrier systems strongly depends on the surface charge of the particles. For example, cationic dendrimers are highly cytotoxic and hemolytic, whereas anionic [10] and PEGylated [11] particles appear to be better tolerated. Hence, dendritic polyglycerol (PG) shows high biocompatibility and is an excellent candidate for the formation of drug delivery systems due to its easy accessibility and possible variations in the degree of branching and molecular weight [12,13].

Good biocompatibility is particularly important for topical application onto diseased or barrier deficient skin. Here, the carrier system can easily come into contact with cells of the viable epidermis which particularly requires low toxicity [14]. It is still highly debated if nanoparticles are able to overcome the outermost layer of intact human skin, the stratum corneum (SC), and penetrate into deeper dermal layers. Various groups have investigated this question but obtained highly controversial results. However, evidence has emerged that nanoparticle penetration into viable layers of intact human skin is very limited [15,16]. Nevertheless, hyperbranched dendritic core–multishell (CMS) nanotransporters which are composed of a dendritic PG core surrounded by an internal C18 alkyl shell and an outermost methoxy polyethylene glycol (mPEG) shell [17] overcame the SC after a prolonged contact time of 24 h [18]. Biocompatibility studies showed no toxic effects as well as no local irritation following the topical application of CMS nanotransporters [19]. Moreover, CMS nanotransporters efficiently transport lipophilic and hydrophilic agents into the skin. For example, loading of the lipophilic model drug Nile red resulted in a 13-fold enhanced penetration into the viable epidermis [20].

Based on these promising results, we evaluated in the present study the drug delivery efficiency of five different PG–PEG nanoparticles which were composed of a dendritic PG core that was functionalized with linear PEG blocks and varying alkyl branches. We aimed to unravel the impact of the structural organization of the alkyl and PEG chains on drug loading, delivery, and biocompatibility. Therefore, we employed PG–PEG nanoparticles for skin penetration studies using the lipophilic model dye Nile red (log*P* 3.8, molecular weight: 318 g/mol). Additionally, we performed a comprehensive toxicity screening to assess cytotoxicity (MTT and neutral red uptake test), local irritation potential (red blood cell test, HET-CAM test), and genotoxicity (Comet assay) of the PG–PEG particles.

2. Materials and methods

2.1. Materials

PG (Mn ≅ 5.000 g/mol, Mw/Mn = 1.9) was prepared as previously described, using 1,1,1-tris(hydroxyl methyl)propane (TMP) as initiator [21]. Novozyme-435 was purchased from Codexis (Redwood City, CA, USA). Lewatit K1131 acidic ionic exchange resin was received from Bayer AG (Berlin, Germany). The solvent tetrahydrofuran, pyridine, methanol, and chloroform were purchased from Acros (Geel, Belgium). Dialysis was performed using Spectra/Pro membrane or benzoylated cellulose tubing (molecular weight cut-off 2000 Da), Sigma–Aldrich (Taufkirchen, Germany) changing the solvent three times over a period of 24 h. Texapon ASV 50 (INCI: sodium laureth sulfate, sodium laureth 8-sulfate, magnesium laureth sulfate, magnesium laureth 8-sulfate, sodium oleth sulfate, and magnesium oleth sulfate) was purchased from Cognis (Düsseldorf, Germany). Nile red was obtained from ABCR (Karlsruhe, Germany). Sodium dodecyl sulfate, acetone dimethyl acetal and 4-toluenesulfonic acid (PTSA), sodium hydroxide, neutral red and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and all chemicals and solvents were obtained from Sigma–Aldrich (Taufkirchen, Germany). Water of Millipore quality was used in all experiments and for the preparation of all samples. Buffers of 0.01 and 0.10 M phosphate were prepared by weight from $Na_2HPO_4{\cdot}7H_2O$ and $NaH_2PO_4{\cdot}H_2O$.

2.2. Nanoparticle synthesis, dye loading and particle characterization

Five different amphiphilic PG–PEG nanoparticles (SK1–SK5) synthesized via two different chemo-enzymatic approaches were investigated. The synthesis, characterization, solubilization, and release profile of PG–PEG nanoparticles SK1 and SK2 using Nile red as a hydrophobic drug model have been reported earlier (Fig. 1) [22]. Here, the alkyl chains were at random positions, i.e., by randomly substituting alkyl groups on terminal and hydroxyl groups of PG. A more controlled chemo-enzymatic approach was followed for the synthesis of SK3–SK5 as described in Fig. 2. Encapsulation and release of Nile red for SK5 have been studied in detail by UV–VIS, fluorescence, atomic force microscopy, and dynamic light scattering [23] (Fig. 2). For non-covalently loading, a film method was applied. Nile red was dissolved in dry tetrahydrofuran and the organic solvent was evaporated generating a thin film of Nile red. Subsequently the aqueous polymer solutions were added. Afterward, the aqueous solution was stirred for at least 18 h at room temperature. PG–PEG particles SK3 and SK4 have been reported for first time in this article. The synthesis of SK3–SK5 is a more controlled procedure, the terminal hydroxyl groups were protected first, and alkyl chains were introduced in the core. Subsequently, removal of the protection resulted in an alkyl substitution only in the core and not of the terminal functional groups. For control of the synthesized polymers, ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken at 25 °C using an ECX 400 spectrometer (Joel USA, MA, USA). The synthesis of the CMS nanotransporters and non-covalently Nile red loading (0.004%) of all nanoparticles were performed according to previously published procedures [1,17]. For a detailed description see the supplementary data.

For particle characterization, dynamic light scattering (DLS) measurements were taken using a Zetasizer Nano instrument (Malvern Instrument, United Kingdom).

2.3. Biological material

For skin penetration studies, pig skin of the axillary region from mature donor animals (breed: "Deutsche Landrasse") was provided by the Department of Comparative Medicine and Facilities of Experimental Animal Sciences, Charité (Berlin, Germany). Following the removal of subcutaneous fat, the skin was stored at −20 °C until usage.

Normal human keratinocytes (NHK) isolated from juvenile foreskin were expanded in keratinocyte growth medium (KGM BulletKit, Lonza, Cologne, Germany). Normal human dermal fibroblasts (NHDF from foreskin) and murine Balb/c 3T3 fibroblasts (Sigma–Aldrich, Taufkirchen, Germany) were cultivated in Dulbecco's Modified Eagle Medium (DMEM, Sigma–Aldrich) supplemented with 7.5% fetal calf serum, L-glutamine (5 mM), and 100 I.U./ml penicillin/100 μg/ml streptomycin (Biochrom, Berlin, Germany). Human umbilical vein endothelial cells (HUVEC) were cultivated in endothelial growth medium (EGM-2 BulletKit) purchased from Lonza.

Human blood was purchased from the German Red Cross (Berlin) and fertilized chicken eggs for the HET-CAM test were purchased from Lohmann livestock breeding (Cuxhafen, Germany).

2.4. Skin penetration studies

The efficiency of Nile red loaded PG–PEG particles for dermal drug delivery was evaluated according to validated test procedures using the Franz cell setup and full-thickness pig skin [24]. On the