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Polymeric micellar nanocarriers of benzoyl peroxide as potential follicular targeting approach for acne treatment

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ABSTRACT

The aim of this work was to optimize polymeric nano-sized micellar carriers of the anti-acne compound benzoyl peroxide (BPO) and to examine the ability of these carriers to deposit into hair follicles with the objective of improving skin delivery of BPO. BPO loaded polymeric micelles composed of Pluronic® F127 were prepared by the thin film hydration method and characterized in terms of size, loading capacity, morphology and physical stability. The optimized micelle formulation was then selected for skin delivery studies. The penetration of BPO loaded micellar carriers into skin and skin appendages across full thickness porcine skin was examined *in vitro*. Confocal microscopy images confirmed the penetration of Nile Red into hair follicles, which was loaded into micellar carriers as a model fluorescent compound. The relative safety of the polymeric micelles was evaluated with the MTT viability test using mouse embryonic fibroblasts. The results indicated that nano-sized polymeric micelles of BPO composed of Pluronic® F127 offer a potential approach to enhance skin delivery of BPO and that targeting of micelles into hair follicles may be an effective and safe acne treatment.

1. Introduction

Acne vulgaris (acne) is a common and chronic inflammatory skin disorder occurring within *pilosebaceous* units in the skin, including hair follicles and *sebaceous* glands [1]. The primary causal factors during the development of acne include hyperactivity of the *sebaceous* gland, follicular epidermal *hyperproliferation* and inflammation of pilosebaceous units caused by pathogens, such as *Propionibacterium acnes* [2,3].

Topical therapy is considered the first option in the treatment of mild and moderate acne due to the drawbacks of systemic medication delivery, such as adverse effects of drugs. Benzoyl peroxide (BPO) is one of the active ingredients in medication that has been prescribed for the topical treatment of mild and moderate acne since the 1960s [3]. Its main mechanism of action is related to antimicrobial activity against *Propionibacterium acnes* in sebaceous follicles. However, BPO has many side effects, such as skin dryness, itching, burning, erythema, scaling, and contact allergy [4]. BPO also causes mild to moderate skin irritation depending on the amount applied onto the skin surface and the type of medication formulation. Due to the aforementioned side effects, patient compliance to therapy may be low [3,4]. In addition, BPO is a chemically unstable compound and has poor aqueous solubility [5]. The hydrophobic character of drugs such as BPO limits their incorporation into an acceptable formulation and reduces efficient drug delivery into the *stratum corneum*. This effect could be due to a decrease in the thermodynamic activity of the formulation because of the excipients used for solubilisation [6]. Regarding the poor physicochemical features of BPO and the potential side effects observed with the use of conventional topical dosage forms, the development of novel BPO carriers may enhance therapeutic efficiency and improve patient compliance. Microsponge delivery systems of BPO [7,8], niosomal gel formulations [9,10] and solid lipid nanoparticles [11] have been reported in the literature. However, the targeting efficiency of BPO loaded nano-sized carriers has not yet been explored.

Nano-sized polymeric carriers have been investigated as alternative vehicles for targeted cutaneous drug delivery due to the improved stability of chemically unstable active ingredients, the limitation of side effects, and the sustained drug release pattern over prolonged periods of time [12–14]. Although the skin delivery of nano-sized particulate carriers has been debated in the literature [15–19], hair follicles are regarded as an efficient storage and potential penetration pathway for nanocarriers [17,20,21]. Thus, follicular drug delivery targeting of nanocarriers is considered to be an efficient strategy, particularly for improving the topical deliv-

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ery of drugs used in the treatment of dermatological diseases, such as acne, allopecia, psoriasis, vitiligo, folliculitis, and skin tumours [18,21–23].

Polymeric micelles are nano-sized (10–100 nm) carriers consisting of self-assembling amphiphilic polymers, including hydrophobic sections in the core of micelles surrounded by hydrophilic sections at the critical micelle concentration in aqueous solution [24]. The major advantages of micellar carriers are the improved aqueous solubility of hydrophobic and lipophilic drugs, the prevention of chemical degradation of drugs due to environmental factors (oxidation, *etc.*), and the facilitation of drug targeting to a preferred site. There has been a considerable amount of research studies performed to address the delivery of drugs loaded into nano-sized micellar carriers *via* parenteral [25,26], oral [27], ocular [28,29], pulmonary [30], and nasal [31,32] routes. In recent years, researchers have also focused on the targeted cutaneous delivery of active compounds *via* polymeric micellar carriers as an efficient strategy for the treatment of skin diseases, such as *psoriasis*, fungal infections, and acne [33–37]. The deposition of micellar carriers composed of PEG-dihexPLA (methoxy poly(ethylene glycol)-dihexyl substituted polylactide) in skin has been demonstrated, and the follicular pathway has been described as the main transport route for micellar carriers [33–35,37]. However, the skin delivery and follicular targeting of micelles based on Pluronic® F127, a polymer that has been approved by the FDA (Food and Drug Administration), has not yet been investigated.

The current study was designed to develop micellar carriers of BPO with Pluronic® F127 by improving the aqueous solubility of BPO by encapsulating it into the core of the micelles and to explore the potential skin transport of the optimized micelle formulation of BPO targeted to hair follicles. The characterisation (particle size, size distribution, encapsulation efficiency, and morphology) of optimized polymeric micelles was evaluated, and then, *in vitro* penetration and deposition of the polymeric micelles into full thickness porcine skin were quantified. Confocal laser microscopy analysis was performed using optimized micelles loaded with Nile Red to demonstrate the deposition of micellar carriers in the skin and skin *appendages*. The cytotoxicity of polymeric micelles was also assessed and compared to the cytotoxicity of sodium dodecyl sulphate (SDS), which is a severe irritant of skin.

2. Materials and methods

2.1. Materials

Benzoyl peroxide (BPO) was a gift from Embil Pharmaceutical Company (Turkey). Nile Red and bovine serum albumin (BSA) were purchased from Sigma Aldrich (St. Louis, USA). Pluronic® F127 was kindly provided by BASF (Ludwigshafen, Germany). The phosphate buffer solution (PBS, Ca-Mg free, pH 7.2) and cell culture media used in the *in vitro* cytotoxicity studies, sterile plastic materials and sodium dodecyl sulphate were obtained from Wisent Bioproducts (Quebec, Canada), Greiner (Frickenhausen, Germany) and Merck (Billerica, MA, USA), respectively. Dichloromethane, acetone, chloroform, tetrahydrofuran, methanol and acetonitrile were purchased from Sigma Aldrich (St. Louis, USA). The mouse embryonic fibroblast cells (BALB/3T3, clone A31) were supplied by American Type Culture Collection (ATCC® CCL-163™, USA). All other chemicals were of analytical grade.

2.2. Preparation of polymeric micelles

BPO-loaded micelle formulations were prepared according to the thin film hydration method [31]. Briefly, 50 mg of Pluronic® F127 and BPO (0.75–2.50 mg) were dissolved in 2 mL of a series of organic solvents (dichloromethane, acetone, chloroform, tetrahydrofuran or acetonitrile) and mixed for 30 min. A thin film layer was then obtained in a round-bottom flask after acetonitrile was evaporated in a rotary evaporator at 40 °C and 100 mbar. The film was kept in a vacuum desiccator overnight to remove the residual solvent. The film layer then was hydrated with 5 mL of ultrapure water and the formulation was filtered through a regenerated cellulose filter membrane (Millex® Millipore, 0.45 μm, Darmstadt, Germany) to remove the free drug. The formulation of Nile Red loaded (1.25 $\mu g\,mL^{-1}$) micelles for confocal microscopy studies was also performed using acetonitrile as the organic solvent according to the procedure explained above.

2.3. Characterization of the micellar carriers

2.3.1. Particle size and polydispersity index (PDI)

The hydrodynamic diameters of the particle and polydispersity index (PDI) of micelles were measured using dynamic light scattering by the NanoZS ZetaSizer (Malvern Instruments, Malvern, UK). The size and PDI values of polymeric micelles prepared using different solvents were measured immediately after the preparation procedure and at the end of 48 h. Three measurements were performed on each sample at 25.0 ± 0.1 °C.

2.3.2. Zeta potentials

The zeta potentials of BPO loaded Pluronic® F127 micelles were determined at 25 ± 0.1 °C by a Zetasizer NanoZS (Malvern Instruments, Malvern, UK) using the electrophoretic light scattering method. The parameters for the zeta potential measurements were as follows: refractive index, 1.330; viscosity, 0.887 cP; and dielectric constant of water, 79; f(ka), 1.50 (*Smoluchowski value*). The voltage and time parameters were automatically set. Each sample was measured in triplicate.

2.3.3. Drug encapsulation efficiency

BPO (0.5 mL) loaded polymeric micelles were diluted in methanol to dissociate the polymeric micelles, and the solution was stored in 10 mL vials. The samples were filtered through membrane filters (0.45 μm, PTFE Millex LCR, Merck Millipore, Darmstadt, Germany). The amount of BPO in the samples was then quantified by HPLC using the method described in the *2.5 Analytical method* sections. The experimental study was performed at least three times. The encapsulation efficiency (%) of the micelles was calculated according to the following equation:

$$\text{Encapsulation Efficiency}\,(\%) = \frac{\text{The amount of BPO} - \text{loaded into polymeric micelles}\,(\text{mg})}{\text{The amount of BPO used for formulation}\,(\text{mg})} \times 100$$

2.3.4. Morphology of polymeric micelles

Topographical image analyses of the BPO loaded polymeric micelles were performed by Atomic Force Microscopy (AFM) (SPM-9600, Shimadzu, Kyoto, Japan) using the dynamic mode and phase imaging at the resonance frequency of 320 kHz. Micelle formulations were diluted at a ratio of 1:10 in ultrapure water before analysis. The mica surface was washed with 20 μL of ultrapure water three times, and 20 μL of magnesium chloride solution (10 mM) was applied to increase adhesion on the mica surface. Twenty microliters of a diluted formulation were deposited on the mica surface, which was dried at room temperature for 5 min before image acquisition.