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Development and *in vitro* evaluation of lipid nanoparticle-based dressings for topical treatment of chronic wounds

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ABSTRACT

This research addresses the development and *in vitro* evaluation of lipid nanoparticle (NP)-based dressings to optimize the delivery of human recombinant epidermal growth factor (rhEGF) for the topical treatment of chronic wounds. The systems investigated were rhEGF-loaded solid lipid nanoparticles (rhEGF-SLN) and rhEGF-loaded nanostructured lipid carriers (rhEGF-NLC) formulated in wound dressings comprising either semi-solid hydrogels or fibrin-based solid scaffolds. Following detailed characterisation of the NP, *in vitro* diffusion cell experiments (coupled with dermatopharmacokinetic measurements), together with confocal microscopic imaging, conducted on both intact skin samples, and those from which the barrier (the *stratum corneum*) had been removed, revealed that (a) the particles remained essentially superficially located for at least up to 48 h post-application, (b) rhEGF released on the surface of intact skin was unable to penetrate to the deeper, viable layers, and (c) sustained release of growth factor from the NP "drug reservoirs" into barrier-compromised skin was observed. There were no significant differences between the *in vitro* performance of rhEGF-SLN and rhEGF-NLC, irrespective of the formulation employed. It is concluded that, because of their potentially longer-term stability, the fibrin-based scaffolds may be the most suitable approach to formulate rhEGF-loaded lipid nanoparticles.

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1. Introduction

The skin is an attractive route for the administration of drugs intended for both local and systemic effects (Campbell et al., 2012). In particular, the topical route for local treatment lowers the risk of systemic side effects, because the *stratum corneum* (SC), the most superficial skin layer, provides a significant barrier to drug penetration (Curdy et al., 2004). Given that nanoparticles (NP) larger than 10 nm are unable to penetrate either intact or partially impaired skin to any great extent (Campbell et al., 2012; Prow et al., 2011), novel drug delivery systems for local effect based on this technology have been proposed as topical reservoirs from which the sustained release of an active compound may be achieved over a prolonged period of time. These characteristics support a strategy, therefore, of using biodegradable, drug-loaded nanoparticles for the topical treatment of skin disease-associated lesions and chronic wounds.

The administration of growth factors, such as epidermal growth factor (EGF), to accelerate wound healing has been extensively described (Choi et al., 2008; Chu et al., 2010; Gainza et al., 2013; Hardwicke et al., 2008; Hori et al., 2007; Johnson and Wang, 2013), however, topical delivery of EGF is severely limited by its high molecular weight, hydrophilicity and, above all, its short half life at the wound site (Al Haushey et al., 2010; Choi et al., 2012; Ulubayram et al., 2001). To address these shortcomings, the nanoencapsulation of growth factors, like EGF, may enhance their stability at the wound and may allow their controlled release, thereby optimising efficacy. In this regard, the *in vivo* performance of topically applied recombinant human EGF (rhEGF)-loaded solid

Abbreviations: NP, nanoparticle; rhEGF-SLN, rhEGF-loaded solid lipid nanoparticles; rhEGF-NLC, rhEGF-loaded nanostructured lipid carriers; SC, *stratum corneum*; EGF, epidermal growth factor; EE, encapsulation efficiency; F, occlusion factor; PDI, polydispersity indices; LSCM, laser scanning confocal microscopy; NR, nile red; 16-NBD, 16-NBD palmitic acid.

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lipid nanoparticles (rhEGF-SLN) and nanostructured lipid carriers (rhEGF-NLC) in a superficially wounded animal model has been reported (Gainza et al., 2014).

The topical administration of a NP-based delivery system may be facilitated by their incorporation into either semi-solid hydrogels or solid scaffolds. Bioadhesive hydrogels (*e.g.* Noveon® AA-1 polycarbophil, a high molecular weight polymer of acrylic acid chemically cross-linked with divinyl glycol) are widely used as wound dressings and their prolonged residence time in the skin offers the opportunity for sustained drug release (Ceschel et al., 2001; Padamwar et al., 2011). Semisolid hydrogels can be prepared using amphiphilic surfactants, such as Pluronic F-127 (Poloxamer 407), the reversible thermo-gelling behaviour of which creates an extremely versatile material for drug delivery (Antunes et al., 2011; El-Kamel, 2002; Kant et al., 2014). Solid scaffolds, such as fibrin-based biomaterials with slow degradation kinetics, have also been frequently used as immune-compatible polymeric dressings from which drug delivery can be controlled (Briganti et al., 2010; Moura et al., 2014). A further advantage of this approach is that fibrin is an important haemostatic mediator acting as a matrix for tissue repair, providing support for new capillaries, and generating an array of cell signalling compounds and growth factors following an injury (Brown and Barker, 2014).

The present work aimed to further advance the development of local therapies with rhEGF. For this, the previously developed rhEGF-SLN and rhEGF-NLC (Gainza et al., 2014) were embedded in three different vehicles Noveon® AA-1 hydrogels, Pluronic F-127 hydrogels and fibrin-based solid scaffolds proposed as potential wound dressings. The performance of these integrated wound dressing–delivery systems was characterized and compared to that of the corresponding nanoparticles suspension. This allowed investigating whether incorporation of the nanoparticles into semi-solid hydrogels and fibrin scaffolds modified the rate and extent of rhEGF release as well as the nanoparticle disposition through intact and partially damaged skin. Finally, we aimed to establish whether their hypothetical role of these systems as drug reservoirs for topical therapies could be demonstrated.

2. Materials and methods

2.1. Chemicals

Precirol® ATO 5 was from Gattefossé (Nanterre, France); Noveon® AA-1 Polycarbophil, USP, was purchased from Lubrizol (Barcelona, Spain); Pluronic F127, fibrinogen from bovine plasma, and thrombin, also from bovine plasma, were acquired from Sigma–Aldrich, Chemie GmbH (Steinhelm, Germany); Nile Red (analytical grade) was obtained from Sigma–Aldrich (St. Louis, MO, USA), 16-NBD palmitic acid from Avanti Polar Lipids, Inc. (Alabaster, AL, USA), and rhEGF was supplied by the Center for Genetic Engineering and Biotechnology, Cuba.

2.2. Skin

Dorsal, full-thickness porcine skin was obtained post-sacrifice from locally sourced female pigs. The skin was cleaned under cold running water and the subcutaneous fat was removed with a scalpel. The remaining tissue was then dermatomed to a thickness of ~750 μm and stored frozen at −20 °C for up to at most one month before use.

2.3. Lipid nanoparticle (NP) preparation

rhEGF-SLN and rhEGF-NLC were prepared as previously described (Gainza et al., 2014). Briefly, rhEGF-SLN were obtained by emulsifying 1% w/v Tween® 80 in Milli-Q water with an organic phase comprising 0.1% (w/v) rhEGF and 5% (w/v) Precirol® ATO 5 in dichloromethane using a 30 s period of sonication at 50 W (Branson® 250 Sonifier, CT, USA). The resulting emulsion was then vigorously stirred for 2 h to evaporate the organic solvent. Subsequently, the rhEGF-SLN were collected by centrifugation/filtration at 2500 rpm for 10 min using a filter with a 100 kDa pore size (Amicon® Ultra, Millipore, Spain), and washed three times with Milli-Q water. Finally, particles were freeze-dried using trehalose (15% w/w of the lipid weight) as a cryoprotectant.

rhEGF-NLC were prepared at 40 °C by adding an aqueous solution of 0.67% w/v poloxamer and 1.33% w/v Polysorbate 80 to a lipidic blend of melted Precirol® ATO 5 (200 mg) and Miglyol® 182 (20 mg). Subsequently, 100 μl of rhEGF in Milli-Q water (20 mg/ml) were added to the aqueous/lipidic mixture, which was then emulsified with sonication for 15 s at 50 W. The resulting emulsion was stored for 12 h at 4 °C to allow lipid re-crystallisation and NLC formation. Finally, particles were collected, washed and freeze-dried as previously described.

In the experiments examining the disposition of the nanoparticles on the skin, the lipid phase of the formulations was labelled with two fluorophores: Nile Red (0.5% w/w of the lipid weight) and 16-NBD-palmitic acid (1% w/w of the lipid weight).

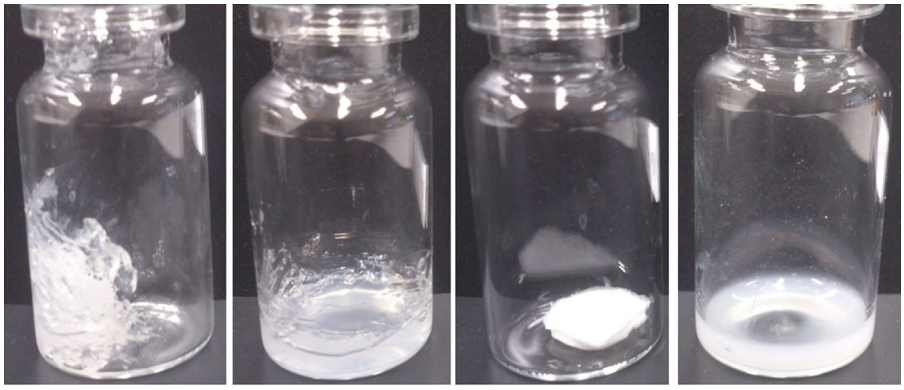

Fig. 1. rhEGF-SLN and rhEGF-NLC integrated wound dressing—delivery systems: Noveon® AA-1 hydrogel, Pluronic F-127 hydrogel, fibrin-based scaffold, and reconstituted NP suspension.

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