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Electrospun poly-L-lactide scaffold for the controlled and targeted delivery of a synthetically obtained Diclofenac prodrug to treat actinic keratosis ☆

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ABSTRACT

Actinic Keratosis' (AKs) are small skin lesions that are related to a prolonged sun-damage, which can develop into invasive squamous cell carcinoma (SCC) when left untreated. Effective, specific and well tolerable therapies to cure AKs are still of great interest. Diclofenac (DCF) is the current gold standard for the local treatment of AKs in terms of costs, effectiveness, side effects and tolerability. In this work, an electrospun polylactic acid (PLA) scaffold loaded with a synthetic DCF prodrug was developed and characterized. Specifically, the prodrug was successfully synthetized by binding DCF to a glycine residue via solid phase peptide synthesis (SPPS) and then incorporated in an electrospun PLA scaffold. The drug encapsulation was verified using multiphoton microscopy (MPM) and its scaffold release was spectrophotometrically monitored and confirmed with MPM. The scaffold was further characterized with scanning electron microscopy (SEM), tensile testing and contact angle measurements. Its biocompatibility was verified by performing a cell proliferation assay and compared to PLA scaffolds containing the same amount of DCF sodium salt (DCFONa). Finally, the effect of the electrospun scaffolds on human dermal fibroblasts (HDFs) morphology and metabolism was investigated by combining MPM with fluorescence lifetime imaging microscopy (FLIM). The obtained results suggest that the obtained scaffold could be suitable for the controlled and targeted delivery of the synthesized prodrug for the treatment of AKs.

Statement of Significance

Electrospun scaffolds are of growing interest as materials for a controlled drug delivery. In this work, an electrospun polylactic acid scaffold containing a synthetically obtained Diclofenac prodrug is proposed as a novel substrate for the topical treatment of actinic keratosis. A controlled drug delivery targeted to the area of interest could enhance the efficacy of the therapy and favor the healing process. The prodrug was synthesized via solid phase, employing a clean and versatile approach to obtain Diclofenac derivatives. Here, we used multiphoton microscopy to image drug encapsulation within the fibrous scaffold and fluorescence lifetime imaging microscopy to investigate Diclofenac effects and potential mechanisms of action.

1. Introduction

The medical term "actinic keratosis (AK)" identifies small skin lesions, which appear as round, rough spots between 5 mm and 1 cm in diameter. AKs are characterized as pre-cancerous or as early-stage tumors [1,2]. This pathology is also known as "solar keratosis" or "senile keratosis" because it is more common to peo-

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ple over 50 with fair skin and is related to a prolonged sun-damage [1]. This process, also known as photoaging, leads to an accumulation of oncogenic changes [2]. Changes related to photodamage are most evident in the extracellular matrix (ECM) [3]. The accumulation of these changes leads to a pathological ECM mainly due to the degradation and fragmentation of its components such as elastic fibers [4]. As a result, the normal repair and regenerative capacity of the ECM is inhibited [4,5]. Skin ageing processes also have a significant impact on cellular mechanisms such as DNA repair, gene expression and immune response modulation [5]. AK is considered a key event in the progression from photoaged skin to the invasive squamous cell carcinoma (SCC) [2,6]. SCC affects the keratinocytes of the epidermis layer and is the second most diffused skin cancer after the basal cell carcinoma (bcc) [1,7]. However, there are many more AKs than SCCs and it is difficult to predict exactly which lesions will progress to invasive cancer [1,2]. About 15% of the men and 6% of the women in Europe are affected by AKs. The percentages rise respectively to 34% and 18% in the European population over 70 years of age [7]. Since the average life expectancy is increasing, it is predicted that even more people will be affected by AKs in the next years [1,2,7]. Although a number of treatments are already available [8–10], the development of effective, more targeted and well-tolerable therapies for the treatment of AKs is still of great interest. The first therapy approved by the Food and Drug Administration (FDA) for the topical treatment of AKs was 5-Fluorouracil in 1962 [8] followed by Imiquimod in 1997 [8], Diclofenac (DCF) in 2002 [9,10] and Ingenol Mebutate in 2012 [9]. Among them, DCF is currently the therapy of choice in terms of costs, effectiveness, side effects and tolerability [10]. DCF belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) and is one of the most commonly used in the world [11]. It is still not clear how DCF affects AKs, but its activity seems to be related to anti-inflammatory and anti-angiogenic effects [12]. Induced apoptosis has been also proposed as a possible DCF mechanism of action in the treatment of AKs [13]. The absorption of a drug through the outermost layer of the skin, the stratum corneum (SC), is limited [14]. Thus a specific formulation of DCF commercially known as Solaraze® is needed for the treatment of AKs. This formulation is supplied with hyaluronic acid that enables DCF accumulation in the epidermis [15]. Considering these aspects, a chemical modification of DCF that enhances its permeation through the SC is of interest since it could improve its efficacy against AKs.

Over the last two decades, electrospinning has gained growing interest as a potential polymer processing technique for applications in tissue engineering [16,17] and drug delivery [18]. Electrospinning is a process that uses a high voltage source to apply a charge of a certain polarity into a polymer solution or melt, which is then accelerated toward a collector of opposite polarity. Electrospinning is an easy way to fabricate fiber-containing scaffolds with a fiber diameter in the nano- to micrometer size scale that mimic the structure and morphology of the ECM components in the skin [16,17,19]. Biodegradable and natural materials can be electrospun, and a wide range of molecules like drugs and proteins can be incorporated in the scaffolds [18]. Polylactic acid (PLA) is a biodegradable, biocompatible polymer with beneficial mechanical properties. Moreover, it is stable over a long time and its degradation proceeds through the hydrolysis of the ester linkage in the polymer's backbone resulting in a non-toxic degradation product called lactic acid [20].

In this study, we aimed to encapsulate a chemical modified and synthetically produced DCF prodrug in an electrospun PLA scaffold in order to obtain a suitable drug delivery system to locally treat AKs. A controlled and targeted drug delivery to the region of interest could reduce the undesired side-effects and enhance the efficacy of the therapy.

2. Materials and methods

2.1. Synthesis of DCF-Glycine (DCF-Gly) prodrug

All coupling reagents were purchased from Novabiochem (EMD Millipore by Merck KGaA, Darmstadt, Germany). 2-Chlorotrityl chloride resin preloaded with glycine (H-Gly-2ClTrt Resin), the solvents and DCF sodium salt (DCFONa) were purchased by Sigma Aldrich (Steinheim, Germany). DCF free acid was obtained by the dissolution of the sodium salt in water followed by acidification and extraction [21]. For the synthesis, H-Gly-2ClTrt resin (227 mg, 0.250 mmol, substitution 1.1 mmol/g, mesh 75–150) was suspended in a solvent mixture (Dimethylformamide(DMF): Dichloromethane (DCM) = 1:1, Vf = 10 mL) using a glass frittered disk sealed in glass column equipped with a faucet. The suspension was stirred (250 rpm) for 2 h using an orbital shaker. The solvent was then removed by filtration and 8 mL of a DCM:DMF = 1:1 solution containing 148 mg (0.5 mmol) of DCF, 520 mg (1 mmol) of Ben zotriazol-1-yloxy-tripyrrolidino- phosphoniumhexafluoropho sphate (PyBOP) and 150 mg (1 mmol) of 1-Hydroxybenzotriazole hydrate (HOBT) were added to the resin. After shaking the mixture manually for a few minutes, 175 µL (1 mmol) of N,N-Diisopropylethylamine (DIPEA) was added and the mixture was shaken (280 rpm) using an orbital shaker for 20 h. Thereafter, the solvent was removed by filtration, the resin was washed (2x10 mL DMF, 3x10 mL Methanol (MeOH), 2x10 mL DMF, 3x10 mL DCM), and the functionalized amino acid was cleaved by adding 10 mL of a mixture of acetic acid (AcOH):2,2,2-Trifluoroethanol (TFE):DCM = 1:1:8. The solution was shaken (250 rpm) employing an orbital shaker for 30 min. The cleavage mixture was then filtered, and the resin was washed with DCM (3x5 mL). The filtrates were combined and evaporated under reduced pressure to 5% of the initial volume, and 10 mL of double distilled water was then added to precipitate the desired product. The final product was lyophilized twice to remove any solvent residual, and recovered as a white fluffy powder in a final yield of 98% (86.5 mg, 0.245 mmol).

2.2. Nuclear magnetic resonance (NMR) analysis

The crude DCF-Gly was characterized by ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) analysis. The ^{1}H- and ^{13}C- NMR spectra were acquired at room temperature on an Inova 500 NMR spectrometer (Varian, Agilent technologies, Inc., Palo Alto, CA, USA), equipped with a 5 mm triple resonance probe and z-axial gradients operating at 500 MHz for ^{1}H nuclei and 125 MHz for ^{13}C nuclei. Reference peaks for ^{1}H and ^{13}C spectra were respectively set to δ 2.49 and δ 39.5 for DMSO-d_6. Two-dimensional double quantum filtered spectroscopy (DQF-COSY) spectra were collected in the phase-sensitive mode using the States method [22]. Typical data were 2048 complex data points, 32 transients and 256 increments. Shifted sine bell squared weighting and zero filling to 2 k × 2 k were applied before Fourier transform. The 2D ^{1}H-^{13}C gradient heteronuclear single quantum correlation (adiabatic version) (gHSQCAD) experiment was carried out using the pulse sequences from the Varian user library [23]. The acquired spectra were processed with ACD®/NMR Processor Academic Edition (Advanced Chemistry Development, Inc., Toronto, Canada) and are reported in the supplementary materials (Suppl. Fig. 1A–D).

2.3. Electrospun scaffolds production

All reagents and solvents were purchased from Sigma Aldrich. The electrospun scaffolds were obtained from a 15% w/v PLA (Poly(L-lactide), #93578, Mn 59.000, Sigma Aldrich) solution