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Functionalization of gauzes with liposomes entrapping an anti-inflammatory drug: A strategy to improve wound healing

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ABSTRACT

From ancientness, suitable materials have been developed to cover the wounds in order to prevent infections and promote proper wound healing. In this study, the successful development of functionalized nonwoven gauzes with liposomes entrapping anti-inflammatory piroxicam is reported. Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) that can suppress a persistent inflammatory response, leading to improved wound healing. The results demonstrated that the highest NSAID concentration released is achieved when gauzes were previously cationized with poly(diallyldimethylammonium chloride) (PDDA) and high concentration of phospholipid (≈3000 μM) and multilamellar liposomes (MLVs) were used. MLVs were also the best vehicle considering their biocompatibility with skin human fibroblasts, where no toxicity was observed for neither of the tested conditions.

The developed functionalized gauzes can be, therefore, a good strategy to treat chronic wounds.

1. Introduction

Wound healing is a complex process that includes inflammation, which is basically a defensive phenomenon yet often leading to serious pathological conditions if unbalanced. In fact, the inflammatory response is needed for the recruitment and activation of dermal cells to the wound site, which leads to the secretion of extracellular matrix, cell proliferation, angiogenesis, granulation tissue formation and eventual reepithelization of the wound [1]. Additionally, it has been well documented that an inflammatory response is needed to prevent infection, which if not eradicated impairs the healing process [1–3]. On the other hand, the state of chronic inflammation, that characterize chronic wounds, creates a proteolytic environment that is mediated by infiltration of inflammatory cells at the wound site, as well as prolonged overexpression of pro-inflammatory cytokines and chemokines that inhibit the normal progression of wound healing [2]. Therefore, non-steroidal anti-inflammatory drugs (NSAIDs), which are the principal pharmacological treatment for inflammatory diseases, could suppress the imbalances of chronic inflammation, leading to wound healing and restoration of the skin barrier function. This feature is very important, once that skin serves as a protective barrier against the environment and loss of cutaneous integrity, in the absence of adequate repair, can lead to major disability or even death [4].

Topical administration of NSAIDs can control severe side effects, as observed in gastrointestinal tract and kidney [5,6]. Besides this advantage over conventional oral and intravenous dosage forms, topical drug delivery offers many other benefits, such as first pass metabolism avoidance, pain minimization and patient compliance [7]. Consequently, there is a growing interest in the optimization of NSAID-carrier formulations to improve cutaneous delivery of these drugs. An example is liposomal formulations that have gained a considerable interest over the last decades. Additionally, advanced medical textiles is an area in expansion in the field of wound healing. Textile materials favour wound occlusion, exit of bacteria, exudates transport and drug dispensation with much reduced distress to the patient [8–10]. In addition, prolonged contact time of a drug with a bodily tissue, through the use of gauzes, for example, can significantly improve the clinical performance of many drugs. These improvements range from better treatment of local pathologies to improved drug bioavailability and controlled release, to enhanced patient compliance [11]. Thus, textiles' functionalization with drugs that counteract the progression of chronic wound inflammation is a promising approach to develop new textile-based wound dressings.

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In this context, the aim of this study was to entrap a NSAID, specifically piroxicam, into liposomes and then proceed to the attachment of those delivery systems to the nonwoven gauzes. Two types of liposomes were used, namely multilamellar liposomes (MLVs) and large unilamellar liposomes (LUVs), to investigate the best liposomal vehicle for the NSAID under study. MLVs present great variations in the vesicle size, size distribution and lamellarity, while LUVs are formed by a single bilayer of lipids, with well defined size [12,13]. Besides the liposomes, the influence of EPC concentration (≈1500 and 3000 μM) in liposomes properties and NSAID release it was also investigated. The production was followed by liposomes characterization which included the determination of size, polydispersity index (PDI), zeta-potential, morphology, drug entrapment efficiency and cytotoxicity. Liposomes were then attached to nonwoven gauzes to produce a functionalized biomaterial, with great potential for biomedical application. It was also explored if activation of the surface gauzes with a cationic product could lead to an enhancement of liposome attachment onto nonwoven gauzes. Finally, besides to evaluate the presence of liposomes in the gauzes surface, it was proceeded to the quantification of piroxicam released.

2. Experimental

2.1. Materials and equipment

The chemical reagents were purchased from Sigma–Aldrich and all were used as supplied. The nonwoven gauzes (composed by 67% of viscose and 33% of polyester, 3,1915 ± 0,0077 g/m^2) were commercial available in a local pharmacy. The BJ5ta cell line (telomerase-immortalized human normal skin fibroblasts) was purchased from the European Collection of Cell Cultures (ECACC) and cultured according to American type culture collection (ATCC) recommendations.

The absorbance measurements were recorded at 25.0 ± 0.1 °C with a Heλios γ ThermoSpectronic spectrophotometer (Unicam). A Spectraflash spectrophotometer (illuminant D_{65} at 600 nm for Comassie Brilliant Blue G250 and 540 nm for Reactive Red 66), from Datacolor International, was used to measure the *K*/*S* values.

The Malvern zetasizer NS (Malvern Instruments) equipment was used to determine the size distribution and the zeta-potential of the particles, using photon correlation spectroscopy (PCS) and electrophoretic laser doppler anemometry, respectively, at 25.0 ± 0.1 °C.

The shape and morphology of liposomes, as well as their attachment into gauzes were observed using a scanning electronic microscope model NOVA Nano SEM 200 FEI.

The ultrasound equipment was composed of a probe type (20 kHz Sonics and Materials Vibracell CV 33) fitted with a 3 mm diameter titanium micro-tip. Power delivery was controlled as percentage amplitude (40%) and 19 mm was the depth, measured from the base of the vessel, used. The reaction vessel was an open glass cell (diameter 19 mm and height 75 mm), containing 16 mL of sample solution. The sonochemical reactor temperature was controlled via a thermo-stated water bath with a freezer exchanger placed within a thermo jacket cell.

The laboratory scale machine used to dye was an AHIBA Spectradye, from Datacolor International (Loorenstrasse 9, CH-8305 Dietlikon, Switzerland), with infrared temperature control.

2.2. Liposomes preparation and drug incorporation

Liposomes were prepared by the thin film hydration method [14]. A known amount of EPC was dissolved in chloroform and was mixed with a chloroform solution of piroxicam to obtain liposomes entrapping this drug. The organic solvent was then evaporated in a rotary evaporator and the lipid film formed was left at least 3 h to remove residual traces of the chloroform. The resulting dried lipid film was dispersed by addition of phosphate buffered saline solution (PBS; 0.01 M, pH 7.4) and the resultant mixture was then vortexes above the phase transition temperature (room temperature) to yield MLVs. The sonication of MLVs suspension was carried out with a total treatment of 21 min monitored in 3 min increments, at 25 ± 1 °C. A pulsed duty cycle of 8 s on, 2 s off was used for all the experiments with indicated power delivery of 40%. After LUVs preparation, the suspension was submitted to a centrifugation (2500g, 15 min) to remove larger lipid aggregates and titanium particles released from the sonicator probe [15].

Liposomes were separated from non-entrapped drug by size exclusion chromatography using a Sephadex G-25 M column containing 0.15% kathan Gc as preservative (GE-Healthcare) and equilibrated with PBS buffer before use.

2.3. Determination of size, PDI and zeta-potential

Size distribution and zeta-potential values of EPC liposomes, with and without incorporated drug, were determined at pH 7.4 (PBS), at 25.0 ± 0.1 °C. Lipid concentration was kept constant at approximately 500 μM.

2.4. Quantification of NSAID entrapment efficiency in liposomes

The entrapment efficiency of piroxicam into vesicular systems was evaluated by UV spectrophotometry, at 353 nm. Entrapment efficiency refers to the ratio of quantity entrapped/adsorbed drug in relation to the total (theoretical) amount of drug used for liposomes production. Therefore, the entrapment efficiency of piroxicam was determined by:

$$\text{Entrapment efficiency } (\%) = \frac{[\text{Piroxicam}]_i - [\text{Piroxicam}]_f}{[\text{Piroxixam}]_f} \times 100 \quad (1)$$

where $[\text{Piroxicam}]_i$ and $[\text{Piroxicam}]_f$ is the initial and final NSAID concentration, respectively, in supernatant after separation of the vesicular systems.

Measurements were recorded in triplicate and the results were expressed as mean value ± standard deviation (SD).

2.5. Determination of liposomes morphology

The characterization of the overall structure and shape of liposomes was performed using STEM. For this analysis, the liposomes suspension was dropped in Copper grids with carbon film 400 meshes, 3 mm diameter.

2.6. Chemical activation of gauzes

The chemical activation of nonwoven gauzes comprised the cationization, as well as the determination of cationization efficiency on the gauzes surface.

The gauzes were cationized using the exhaust method already described [16]. The cationization was performed in sealed, stainless steel pots of 120 cm^3 in capacity using a laboratory scale dyeing machine, previously referred in Section 2.1. The treatment was carried out using a material:liquor ratio of 1:20, 10% (of weight of the fabric) of poly(diallyldimethylammonium chloride) (PDDA) and 5 g L^{-1} of sodium hydroxide, over 60 min at 50 °C. Afterwards, the gauzes were collected and washed thoroughly with deionised water.