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Research paper

Wound dressings based on silver sulfadiazine solid lipid nanoparticles for tissue repairing

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ABSTRACT

The management of difficult to heal wounds can considerably reduce the time required for tissue repairing and promote the healing process, minimizing the risk of infection. Silver compounds, especially silver sulfadiazine (AgSD), are often used to prevent or to treat wound colonization, also in presence of antibiotic-resistant bacteria. However, AgSD has been shown to be cytotoxic *in vitro* toward fibroblasts and keratinocytes and consequently to retard wound healing *in vivo*. Recently, platelet lysate (PL) has been proposed in clinical practice for the healing of persistent lesions. The aim of the present work was the development of wound dressings based on AgSD loaded in solid lipid nanoparticles (SLNs), to be used in association with PL for the treatment for skin lesions. SLN were based on chondroitin sulfate and sodium hyaluronate, bioactive polymers characterized by well-known tissue repairing properties. The encapsulation of AgSD in SLN aimed at preventing the cytotoxic effect of the drug on normal human dermal fibroblasts (NHDFs) and at enabling the association of the drug with PL. SLN were loaded in wound dressings based on hydroxypropylmethyl cellulose (HPMC) or chitosan glutamate (CS glu). These polymers were chosen to obtain a sponge matrix with suitable elasticity and softness and, moreover, with good bioadhesive behavior on skin lesions. Dressings based on chitosan glutamate showed antimicrobial activity with and without PL. Even though further *in vivo* evaluation could be envisaged, chitosan based dressings demonstrated to be a suitable prototype for the treatment for skin lesions.

1. Introduction

Foot ulceration affects 15–20% of diabetic patients and precedes up to 85% of amputations in these patients, reducing the quality of life [1]. The costs for medical care due to the associated complications, including peripheral neuropathy, are very high. Timely resolution of diabetic foot ulceration avoids infection and lower limb amputation. Current guidelines recommend the use of pressure relieving devices and wound dressings to promote healing and prevent infection, and, when appropriate, debridement, drainage, and revascularization [2]. Commonly, a delay in wound healing can occur: this could depend on low patient compliance to treatment regimens, scarcely controlled glycemic levels and poor tissue oxygenation [2]. The impaired immune response to injury, which is common in diabetic patients, frequently affects formation of granulation tissue that is a prerequisite for epithelialization or complete skin healing [1,2].

The healing process can also be delayed by infections of the lesions due to both aerobic and anaerobic bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* and *Enterococcus* species, *Pseudomonas aeruginosa*, *Peptostreptococcus* and coliforms [1,2]. On the contrary, the restoration of bacterial burden to an acceptable level promotes healing.

Burn injury can occur in all age individuals, can have many causes and different severity. The most severe lesions require the highest levels of intensive care and surgery [3]. The severity of the burns depends on the depth and on the size of the body surface area involved. The management of burns can have a considerable influence on the time taken for the wound to heal. This is a critical point, since the healing enhancement minimizes the risk of burn wound infection.

Healing is a dynamic process composed of a sequence of events that result in the restoration of skin compartments (i.e., dermis and epidermis), anatomical continuity, and skin functions. In response to a skin injury, neighboring cells initiate a cascade of biological events (inflammatory processes, formation of new tissue, remodeling) to restore global skin morphology and function. At the dermal level, the wound repair process is characterized by the significant activation of quiescent fibroblasts and their differentiation into

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myofibroblasts, which then leads to wound contraction and extracellular matrix (ECM) remodeling [4]. (Myo)fibroblasts are responsible for the production of ECM components, including collagen and elastin, which give the ECM strength. They also synthesize highly hydrated molecules, such as proteoglycans and glycosaminoglycan (GAG). The ECM can directly bind to and release several growth factors, which may serve to link and protect growth factors from degradation. [5].

Growth factors (GFs) influence many of the processes common to both tissue repair and disease, including angiogenesis, chemotaxis, and cell proliferation, while they also control the synthesis and degradation of extracellular matrix proteins [6].

Platelets constitute a source of multiple GF and proteins involved in tissue regeneration. Platelet lysate (PL) is a solution of bioactive molecules obtained after platelet destruction by freeze-thawing, usually starting from a platelet-rich plasma (PRP) in presence of an anticoagulant agent [7–9]. PL can be prepared from the patient (autologous) or from donors (allogenic). Recently, platelet lysate (PL) has been proposed in clinical practice for the healing of oral mucositis [10]. Moreover, it has been suggested for the treatment for occipital decubitus ulcers in a male child born with antenatal congenital left diaphragmatic hernia [11]. Several attempts have been done to develop effective formulations intended for corneal [12,13], buccal [14], and skin applications [15].

The use of topical anti-infective chemotherapy is fundamental in the management of severe lesions to prevent infections [1]. Silver may be used to prevent or to treat wound colonization that could impair healing, also in presence of antibiotic-resistant bacteria [1–3,16]. In fact, silver is an effective antimicrobial due to its ability to strongly interact with thiolic groups present in respiratory enzymes of the bacterial cell. Additionally, silver has been shown to interact with structural proteins and preferentially bind DNA bases in order to inhibit replication [1]. For these reasons, it is reported to be effective against almost all known bacteria including fungi and some viruses [1]. Silver compounds such as silver nitrate and silver sulfadiazine are commonly used for chronic lesions and burns [1].

However, silver sulfadiazine (AgSD) has been shown to be cytotoxic *in vitro* toward fibroblasts and keratinocytes and consequently to retard wound healing *in vivo* [16].

The aim of the present work was to develop wound dressings based on silver sulfadiazine (AgSD) loaded in solid lipid nanoparticles (SLNs), to be associated with PL for the treatment for skin lesions. SLN were prepared using chondroitin sulfate and sodium hyaluronate, as bioactive polymers characterized by well-known tissue repairing properties [17,18]. The encapsulation of AgSD in nanosystems should prevent the cytotoxic effect of the drug, and moreover, it should enable the association of the drug with PL. SLN were loaded in wound dressings based on hydroxypropylmethyl cellulose (HPMC) or chitosan glutamate (CS glu). These polymers were chosen to obtain a sponge matrix with suitable elasticity and softness. AgSD SLN were characterized for biocompatibility on normal human dermal fibroblasts (NHDFs) and for antimicrobial activity with and without PL. Wound dressings were moreover characterized for elasticity (tensile measurements) and resistance to penetration, as well as for hydration and bioadhesion properties.

2. Experimental part

2.1. Materials

Silver sulfadiazine (Sigma Aldrich, I) was used as drug.

SLN: Glyceryl Behenate, Compritol® 888 ATO, a kind gift from Gattefossè (I), was chosen as lipidic phase. Poloxamer 188 (F68) (Pluronic® F 68, BASF, I) was used as surfactant. Chondroitin-6-Sulfate Sodium (CSS) (Bovine 100 EP, Bioiberica, ES, kindly gifted from Prochifar s.r.l., I) and hyaluronic acid sodium salt (HA) (low molecular weight: intrinsic viscosity 4.0 dl/g; purity >94%; Bioiberica, ES, kindly gifted from Prochifar s.r.l., I) were used in aqueous phase.

Matrix forming polymers: Chitosan (CS) (MW 251 kDa measured by viscosimetry; deacetylation degree: 98%; Giusto Faravelli, I) salified with a stoichometric amount of glutamic acid); Hydroxypropylmethyl cellulose (HPMC) (Metolose 60 SH-4000 Shin-Etsu, JP).

Platelet lysate (PL): obtained from the Apheresis Service of Immunohaematology and Transfusion Service Center for transplant immunology, by employing a sterile connection technique. Aliquots of hyperconcentrated platelets (high platelet concentration in small plasma volume and minimal leukocyte contamination) were obtained from apheresis, carried out on regular blood donors. The platelet pool was frozen at −80 °C for 5 h and subsequently defrozen in a sterile water bath at 37 °C. An automated platelet count and tests for aerobial, anaerobial, and fungi contamination were performed after saline dilution.

2.2. Methods

2.2.1. SLN preparation

SLN were prepared by means of hot homogenization and ultrasound technique [19]. 2.5 g of C888 (10% w/w) was the lipidic phase. As aqueous phase 12.5 ml of a solution containing HA and CSS (both at 0.75% w/w concentration) and different amounts of F68, ranging from 0.625 g (2.5% w/w) to 1.25 g (5% w/w), were used. After melting C888 at 85 °C, the lipidic phase was homogenized with the aqueous phase by means of an Ultraturrax equipment (T25, IKA Labortechnik, DE) at 24000 rpm for 5 min. The emulsion was cooled by diluting the system with distilled water (12.5 ml) at 2–8 °C. The preparation was maintained at 2–8 °C for 5 min and sonicated for 10 min in order to stabilize the particles and to eliminate aggregation, respectively.

AgSD was loaded by adding 16 mg of the drug solubilized in 200 μl NH_4OH 30% (Sigma Aldrich, I) into the lipidic phase before melting. The excess of drug not encapsulated was eliminated by centrifugation at 3000 rpm for 10 min (ALC 4218, Jouan, I).

2.2.2. Wound dressing preparation

HPMC and CS glu were hydrated in distilled water at 1% w/w concentration. SLN were mixed with each polymeric solution or with distilled water in a 1:3 weight ratio. The mixtures were poured in Petri dishes to have 0.4 g/cm^2 and frozen at −40 °C for 24 h. The systems were then lyophilized (Heto 15, Analitica De Mori, I) for 24 h.

2.2.3. SLN characterization

2.2.3.1. Physical–chemical properties. Particle size (PS) and polydispersity index (PI) were determined at 25 °C by Photon Correlation Spectroscopy (Beckman Coulter N5, Instrumentation Laboratory, I) at an angle of 90°, after dilution of formulations with bidistilled and filtered (0.22 μm) water.

The zeta potential of SLN dispersions was measured by PALS technology using a Brookhaven ZetaPALS equipment (Brookhaven Instrument Co., Holtsville, NY, USA).

The morphological examination of the SLN was performed using a transmission electron microscope TEM (Jeol JEM-1200 EX II, JP) equipped with TEM CCD camera Mega View III (Jeol, JP) [19]. The samples were placed on copper grids for TEM analysis.

2.2.3.2. AgSD assay. AgSD was assayed by an HPLC method (USP 35) using a Series 200 apparatus (PerkinElmer, I) equipped with a UV detector set at 254 nm and a C18 column (B10C18*30QS, Microbondapak, Interchrom, Interchim, Stepbio, I). The mobile phase,