International Journal of Pharmaceutics 532 (2017) 466–477

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Research paper

Synthesis and preparation of biodegradable hybrid dextran hydrogel incorporated with biodegradable curcumin nanomicelles for full thickness wound healing

Mona Alibolandi[a,*], Marzieh Mohammadi[b], Seyed Mohammad Taghdisi[c], Khalil Abnous[a], Mohammad Ramezani[a,b,*]

[a] Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Trgeted Drug Delivery Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Keywords:
Dextran
Hydrogel
Curcumin
Wound healing
Micelle
Hybrid hydrogel

ABSTRACT

There is a clinical need for a novel, more efficient therapy for full thickness wound healing. In the current study, curcumin encapsulated PEG-PLA [poly(lactide)-block-poly(ethylene glycol)] nanomicelles were incorporated into dextran hydrogel for a full thickness dermal wound healing application. To assess the application of the hydrogel as a therapeutic wound dressing, its morphology, swelling pattern, kinetics of degradation, and capacity to control curcumin release were evaluated.

It was found that the prepared hybrid hydrogel had acceptable biocompatibility, incorporation capacity of curcumin nanomicelles, and mechanical properties.

An *in vitro* release experiment also demonstrated the sustained release of curcumin from dextran hydrogel, which was first controlled by the diffusion of curcumin from hydrogel and continued through hydrogel matrix erosion at the terminal phase.

An *in vivo* wound healing experiment was carried out using dressing hydrogels on full thickness wounds in BALB/c mice.

An histological study demonstrated that the application of curcumin nanomicelles incorporated hydrogel could significantly augment the re-epithelialization of epidermis and collagen deposition in the wound area.

Expression of CD31 and vimentin in wound tissue was investigated using immunohistochemistry tests on the eighth day post wounding. The results obtained demonstrated that curcumin nanomicelles incorporated hydrogel could significantly accelerate angiogenesis, fibroblast accumulation, and the process of wound healing.

Together, the data indicate that the prepared hybrid curcumin PEG–PLA nanomicelles incorporated dextran hydrogel is a promising candidate for full thickness wound treatment that increases re-epithelialization, collagen deposition, angiogenesis, and tissue granulation.

1. Introduction

Wound healing is a complex biological process composed of interrelated and overlapping phases: inflammation, migration, proliferation, and maturation (Izumi et al., 2015). In severe wounds, such as large skin injuries, second or third degree burns, or diabetes foot ulcers, wound healing is a slow process and rarely leads to the complete restoration of tissue function (Ribeiro et al., 2013; El-Refaie et al., 2015). In some cases, such as resistant infections, insufficient blood flow, or local edema, the wound healing process has failed to occur in a timely manner (Chereddy et al., 2013; De Cicco et al., 2014). It is estimated that chronic wounds affect approximately 6.5 million people in the united states alone (Jung et al., 2016).

Wound dressings help maintain the moisture of the wound bed, which has an important effect on the healing process (Gong et al., 2013a). Although has been demonstrated that gauze and cotton wool are still the most widely used wound dressings, the use of novel dressings, such as foams, alginates, superabsorbents, etc., has received a geat deal of interest (Zhang et al., 2015).

Hydrogels are three-dimensional polymeric networks that can swell in aqueous environment/biological fluids by absorbing the wound exudates while preventing wound dehydration. Such materials are non-

* Corresponding authors at: Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
E-mail addresses: alibolandim@mums.ac.ir, mona.alibolandi@gmail.com (M. Alibolandi), ramezanim@mums.ac.ir (M. Ramezani).

http://dx.doi.org/10.1016/j.ijpharm.2017.09.042
Received 18 May 2017; Received in revised form 10 September 2017; Accepted 16 September 2017
Available online 18 September 2017
0378-5173/

adherent and protect the damaged tissue from bacterial infection, which might lead to significant pain reduction (Ribeiro et al., 2013; Zhang et al., 2015). Recently, dextran hydrogels have attracted considerable interest in wound healing applications due to their biocompatibility and soft and pliable properties (Sun et al., 2011). Dextran is a natural, hydrophilic polymer that has been used as a plasma extender for many years (Alibolandi et al., 2017). Dextran hydrogel scaffolds have been used to enhance angiogenic properties (Sun et al., 2011) or as a platform to incorporate drug/growth factor loaded particles in skin regeneration (Ribeiro et al., 2013).

Previously it was demonstrated that the dextran hydrogel enhances neutrophil infiltration, neutrophils provide hydrogel digestion, and this leads to vascular cell infiltration. Thus, in contrast to the clinically implemented scaffold, dextran hydrogels facilitate the migration of endothelial cells to the wound area and increase neovascularization during treatment (Sun et al., 2011).

There are many studies investigating different wound dressings containing various antibiotics, growth factors, and anti-inflammatory agents (Ribeiro et al., 2013; Gong et al., 2013a; Zilberman et al., 2015; Namazi et al., 2016; Xie et al., 2015; Kant et al., 2014). It has been reported that topically administered curcumin, a natural polyphenol and the active component of *Curcuma longa*, is a promising wound healing agent (El-Refaie et al., 2015) with anti-inflammatory, antimicrobial, anti-oxidant, and antineoplastic effects. Nevertheless, its therapeutic efficacy is hindered by curcumin's poor aqueous solubility, low oral bioavailability, and rapid metabolism (Gong et al., 2013a). One approach to improve the delivery efficiency of curcumin through the skin layers is to encapsulate it inside nanoparticles. The small size and high surface-to-volume ratio of the nanoparticles are ideal specifications for topical drug delivery.

To this goal, El-Refaie et al. produced hyaluronic acid-based gel-core hyalusomes loaded with curcumin. They demonstrated that these nanogels enhanced the skin deposition of curcumin five folds more than the conventional gel made of Tween 80 and Lipoid s100 (Krausz et al., 2015a). Krausz and coworkers developed a silane-hydrogel nanoparticle as a curcumin delivery vehicle and evaluated its antibacterial effect. They reported that curcumin nanoparticles inhibited the growth of methicillin-resistant *Staphylococcus aureus (MRSA)* and *Pseudomonas aeruginosa in vitro*. The *in vivo* results showed that it inhibited MRSA growth *in vivo* in a murine mouse model leading to an improved wound healing process (Krausz et al., 2015b).

One widely-used approach to enhance the water solubility of curcumin is to incorporate it into polymeric micelles. Polymeric micelles are nanoscale assemblies of polymeric chains with an amphiphilic nature that are arranged in spherical forms in aqueous solutions and produce core–shell structures. Hydrophobic drugs are encapsulated in the core, while hydrophilic segments of the polymer provide the stability of the micelle in aqueous solutions. Nanomicelles enhance the cellular uptake of hydrophobic agents and improve the *in vivo* circulation time (Gong et al., 2013b).

In the current study, we fabricated a novel wound dressing made of dextran hydrogels and incorporated curcumin-loaded nanomicelles made of polylactic acid–polyethylene glycol (PLA–PEG) into the hydrogel as a drug delivery vehicle. Thereafter, we used this platform as a wound dressing *in vivo* in mice bearing skin injuries.

2. Materials and methods

2.1. General

Dextran (M_W = 50000 Da) was purchased from Pharmacosmos (Holbaek, Denmark). Adipic acid dihydrazide (AAD), sodium periodate, polyethylene glycol (M_W 5000 Da), DL-lactide, and stannous octanoate (Tin[II] ethyl hexanoate) were obtained from Sigma-Aldrich (Germany). All other chemical reagents and analytical and HPLC (high performance liquid chromatography) grade solvents were purchased from Merck (Germany) and were implemented as received.

2.2. Preparation of PEG–PLA nanomicelles of curcumin

2.2.1. Synthesis and characterization of PEG–PLA

The PEG–PLA copolymer was synthesized under microwave irradiation as previously reported (Alibolandi et al., 2015a). Briefly, to synthesize the PEG5000–PLA5000 diblock copolymer, polyethylene glycol (2.5 g) was introduced into a two-neck round-bottom flask equipped with a condenser and was irradiated in a Milestone Microsynth microwave (Italy) for 10 min at 1000 W and 120 °C to prepare anhydrous viscous PEG. In the next stage, 7.5 g DL-lactide and 10 μL Sn (Oct) were added to the flask and the mixture was further irradiated at 1000 W and 130 °C and stirred (30 rpm) for 25 min.

The synthesized polymer was purified as follows: copolymer was dissolved in 20 mL chloroform and was precipitated via the addition of 200 mL cold diethyl ether. The aforementioned purification method was repeated three times. The purified copolymer was freeze dried for 48 h, and kept at −20 °C until use.

The synthesized PEG–PLA copolymer was characterized using ^{1}H-nuclear magnetic resonance (^{1}H NMR). In this regard, the ^{1}H NMR spectra of the diblock copolymer was recorded at room temperature using a Bruker AC 80 nuclear magnetic resonance spectrometer (Germany) in chloroform.

The ^{1}HNMR spectrum of the synthesized copolymer was used to calculate M_n of copolymers using the integration ratio of proton resonances of CH in PLA block at 5.4 ppm and CH_2 in the ethylene glycol at 3.8 ppm according to the method developed by Jeong and colleagues (Jeong et al., 1999).

The molecular weights and polydispersity of the PEG–PLA copolymer were estimated using the Agilent gel permeation chromatography (GPC) add-on system and refractive index signal detector recording at 212 nm coupled to the PL gel columns and operated at a temperature of 25 °C. The molecular weights were calibrated with polystyrene standards. Tetrahydrofuran was used as eluent (flow rate: 1 mL/min), and the sample injection volume was 10 μL.

2.2.2. Curcumin encapsulation in PEG–PLA nanomicelles

In order to improve the water solubility of curcumin, a nano-precipitation method was implemented to prepare the nanomicelle of curcumin using mPEG–PLA as the carrier (Gou et al., 2011).

PEG–PLA copolymer (20 mg) and curcumin (6 mg) were dissolved in 2 mL of acetone. The solution was added drop-wise through a syringe into 25 mL of distilled water under certain mixing rates and was continuously stirred magnetically at room temperature for the evaporation of the organic solvent. After removing the acetone using rotary vacuum evaporation, the resulting yellowish aqueous solution was filtered through a 0.45 mm filter membrane to remove the unloaded curcumin (CUR). Curcumin-loaded micelles were obtained by the self-association of amphiphilic PEG–PLA copolymers. The resulting suspensions were freeze-dried to obtain the final dried form of the formulation.

Two parameters–the drug loading ratio and the encapsulation efficiency of the micelles–were used for the determination of the drug loading and encapsulation efficiency of curcumin in the micelles and were calculated as follows:

$$\text{DL } (\%) = \frac{\text{Total amount of CUR} - \text{Free CUR inpercipitant}}{\text{Total amount of CUR}} \times 100 \quad (1)$$

$$\text{DL } (\%) = \frac{\text{Total amount of CUR} - \text{Free CUR inprecipitatnt}}{\text{Mass of Final formulation}} \times 100 \quad (2)$$

2.2.3. Characterization and physicochemical properties of the nanoparticles

The size measurement of prepared micelles was performed using dynamic light scattering (DLS). Briefly, 50 μL of the prepared micelles (10 mg/mL) was suspended in 950 μL of deionized water and then