Materials Science and Engineering C 72 (2017) 160–167

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Topical hydrogel matrix loaded with Simvastatin microparticles for enhanced wound healing activity

S Yasasvini [a], RS Anusa [a], BN VedhaHari [a], PC Prabhu [b], D RamyaDevi [a,*]

[a] School of Chemical and Biotechnology, SASTRA University, Thanjavur 613401, Tamil Nadu, India
[b] Central Animal Facility, SASTRA University, Thanjavur 613401, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 8 November 2016
Accepted 10 November 2016
Available online 12 November 2016

Keywords:
Chitosan
Hydrogel
Microparticles
Poly vinyl alcohol
Simvastatin
Wound healing

ABSTRACT

A prolonged release drug delivery system was developed by loading Simvastatin-chitosan microparticles into poly vinyl alcohol (PVA) hydrogels for enhanced wound healing efficiency. The microparticles prepared by ionic gelation method with varying composition of chitosan and surfactants (Tween 80/Pluronic F-127) were optimized for entrapment efficiency, morphology and drug-polymer interactions. Microparticles prepared with 0.3% between 80 and 0.5:5 chitosan: drug ratio showed maximum entrapment efficiency of 82% with spherical morphology and mild interaction between drug and chitosan. 5% PVA solutions loaded with pure drug and drug loaded microparticles at three different doses (2.5 mg, 5 mg and 10 mg equivalent of drug) were chemically cross linked using gluteraldehyde and HCl. The formulated hydrogels were optimized for swelling, *in vitro* release behavior and *in vivo* wound healing effect. Hydrogels containing 2.5 mg equivalent dose of Simvastatin microparticles exhibited maximum cumulative percentage drug release of 92% ($n = 3$) at the end of 7 days. The *in vitro* drug release data was supported by the higher swelling index of the low dose hydrogels. The *in vivo* wound healing study was performed using Wistar rats ($n = 30$, 5 groups with 6 animals in each group) for the formulated hydrogels (at 3 doses) and compared with the untreated animals and the positive control group treated with conventional topical Simvastatin ointment (1%). The wound healing effect was comparable to the *in vitro* results, wherein the animals treated with low dose hydrogels (replaced every 7 days) exhibited considerable reduction in the wound area compared to medium and high dose hydrogels. Statistically significant difference ($P < 0.05$) was observed in the wound area of the animals treated with low dose hydrogels compared to 1% ointment and untreated animals, as estimated by two-way ANOVA. The histopathology images of the different groups of animals also displayed the comparative changes in the wound healing process. Hence, the incorporation of Simvastatin-chitosan microparticles in PVA hydrogels has demonstrated significant wound healing efficiency at optimum dose.

1. Introduction

Wound repair is a complex process in which the skin (or other organ) regenerates itself after an injury. There are four main stages of wound healing process namely hemostasis, inflammatory response, proliferative stage and the remodeling of tissues [1]. Wound healing therapy could be initiated based on the type of wounds such as incision, excision, burns, punctures, blunt wound and abrasion [2]. Several drugs and herbal medicines have been reported for the effective wound healing process through different mechanisms [3].

Simvastatin is a lipid lowering agent that exhibits its mechanism of action as a competitive inhibitor of HMG-CoA reductase that catalyses the conversion of HMG-CoA to mevalonate. It has been reported to improve vascular endothelial growth factor (VEGF) production thus stimulating angiogenesis, reduce oxidative stress, improve micro vascular function and improve endothelial function, thereby improving wound healing efficiency [4]. It also exhibits anti-inflammatory activity in both acute and chronic wounds by up regulating the pro-inflammatory cytokines such as IL-1,-6 and 8 and TNFα. It also inhibits the migration of trans-endothelial cells of leukocytes, which happens by reducing the expression of adhesion molecules (ICAM-1), thus resulting in reduced inflammation [5].

Hydrogels are three-dimensional cross-linked polymeric network and highly biocompatible or biodegradable, which are used for effective controlled drug delivery and wound healing applications. Hydrogels support the wound healing process by providing sufficient mechanical properties, preventing the fluid loss from wound and also maintaining optimum moisture/dryness in the area [6]. Owing to the moisture provided to the wound site by hydrogel dressings, the granulation, epidermis repair phases get easier to progress and the risk of wound infection gets eventually reduced. They also act as flexible dressings without changing the shape on absorbing wound fluids and furthermore, they do not stick to the wound site [7]. Certain demerits of hydrogels in

* Corresponding author.
E-mail address: ramya@scbt.sastra.edu (D. RamyaDevi).

http://dx.doi.org/10.1016/j.msec.2016.11.038
0928-4931/

wound dressing include biodegradation product induced non-specific reactions, adherence of some polymer to wound and the cost of the product. Hydrogels offer higher flexibility in drug delivery application owing to their remarkable features like varying degree of swelling based on pH, temperature and other stimuli. Poly vinyl alcohol (PVA) hydrogel has gained more attention of the researches in past decades, as a promising biomaterial suitable for application in many fields like drug delivery, tissue engineering, vascular cell culturing, orthopedic implants, *etc.* due to its excellent biocompatibility and mechanical properties [8–10]. Chitosan containing hydrogels have been reported as a prominent polymer for prolonged release of drug and wound healing process [11]. Chitosan itself increases the collagen synthesis by accelerating the infiltration of poly morpho nuclear (PMN) cells at the early wound healing phase, thereby enhancing the epithelialization [12]. It takes charge of accelerated and well organized deposition of collagen over dermis layer at the wound site.

Polymeric microparticles have been well-explored as promising and novel tool for controlled and sustained delivery of enormous drugs [13,14]. They have also been cited as unique carrier systems and adapted for targeted delivery to specific sites [15]. Microparticles of anti-tumor drugs namely, doxorubicin and 5-fluoro uracil were reported for therapeutic targeting to the organs. Also certain microparticles were adopted as markers for diagnosis, analysis and detection [16]. Intracellular gene delivery, ribozyme delivery, drug delivery into cell organelles like mitochondria and vaccine adjuvant therapy were also well accomplished using polymeric microparticles [17]. Chitosan microparticles are explored with several applications in the areas of novel and controlled drug delivery, wound dressings, biomedical tools, food and cosmetics [18,19]. The active pharmaceutical ingredients have been loaded into the chitosan polymer microparticles through various methods like ionotropic gelation, spray drying, emulsion phase separation, simple and complex coacervation process, electro spinning, *etc.* [20,21]. The drug loaded chitosan microparticles offer high flexibility in drug loading and optimization, good physical and chemical stability and controlled release of drugs.

The chronic treatment for wound healing generally requires repeated administration of drugs at regular time intervals for longer duration. The extended release therapy for long period could reduce the dosing frequency and ensure the presence of drug exposure at the site of action (wound area) continuously [22,23]. Based on this background, the present work aims to combine the effect of microparticles and hydrogel for dual controlled delivery system of Simvastatin for topical wound healing application. The rationale for this work is based on the significance of the polymers, PVA and chitosan, used for hydrogels and microparticles, respectively, as they are proved to support the wound healing process in addition to the active drug Simvastatin at lower doses. The incorporation of drug-polymer microparticles in the hydrogel could provide prolonged release with minimum amount of drug loaded in the formulation, reduced dosing frequency and maintenance of required therapeutic concentration in plasma, thereby improving the efficacy of Simvastatin in wound healing therapy. Simvastatin loaded microparticles are developed first followed by the incorporation of the particles in hydrogels and optimization of their characteristics for *in vivo* wound healing study.

2. Materials and methods

2.1. Materials

Simvastatin (Batch No.: SIM/030313A.R.NO.13/FP/SIM/0303, Date: Feb 2013, Manufacturer: Swapnrop Drugs & Pharmaceuticals, Aurangabad, India) was obtained as gift sample from Glukem Pharma, Hyderabad, India. Chitosan was purchased from Sigma Aldrich, Mumbai, India. Poly vinyl alcohol (PVA) was obtained from SD Fine Chem. Pvt. Ltd. Mumbai, India. All other chemicals and reagents used in the study were of analytical grade.

2.2. Preparation of Simvastatin-chitosan microparticles

Simvastatin loaded chitosan microparticles were prepared by modified ionic gelation method. Sodium tri poly phosphate (STTP) was dissolved in distilled water containing the required quantity of surfactant to obtain the aqueous phase. Chitosan dissolved in 1% w/v acetic acid and Simvastatin dissolved in methanol were mixed and then sonicated to obtain the homogenous acidic non-aqueous phase. The aqueous STTP-surfactant mixture was added drop wise to the chitosan-drug mixture under constant stirring for 3 h using magnetic stirrer (Khera instruments Pvt. Ltd., India) at room temperature. The organic solvent evaporated completely and the uniform sized micro particles were obtained through the ionic gelation process [24]. The obtained suspension was centrifuged to separate the microparticles as pellet. The collected microparticles were washed with distilled water to remove the unentrapped drug, unreacted chitosan and surfactant and finally freeze dried.

In this process, the addition of surfactant (Tween 80 or Pluronic F-127) favors the formation of stable, spherical and uniform sized particles, due to their optimum hydrophilic-lipophilic balance and surface interaction with the drug and chitosan. The addition of surfactants Tween 80 could enhance effective drug delivery [25] and Pluronic F-127 could support the wound healing activity [26]. Chitosan was selected as the polymer for controlled drug release owing to its wide biomedical applications [27]. The microparticles were formulated and optimized with respect to varying surfactant concentration (0.1, 0.3, 0.5, and 0.7% of Tween 80 or Pluronic F127) and chitosan: drug ratio (0.5:5, 1:5, and 2:5).

2.3. Preparation of Pure Simvastatin loaded hydrogels

Poly vinyl alcohol (PVA) was chosen for the preparation of Simvastatin loaded hydrogels by chemical cross linking method since it is highly biocompatible [28] and also has potential biomedical applications along with chitosan [29]. PVA hydrogels were prepared with three different concentrations, precisely 5, 7 and 9% w/v. The polymer PVA was completely dissolved in distilled water by warming in hot water bath at 50 °C for approximately 45 min, followed by bath sonication to remove the air bubbles. Pure Simvastatin was added to the PVA solution and dispersed uniformly by stirring manually with glass rod. The mixture was poured into cylindrical molds to obtain the dose of 5 mg drug per hydrogel patch. Gluteraldehyde (2%) was used as the cross linking agent along with concentrated hydrochloric acid (2%) as catalyst for the chemical cross linking mechanism. The cross linked PVA hydrogels loaded with Pure Simvastatin were washed with distilled water to remove the traces of gluteraldehyde/HCl, air dried over-night at room temperature and then stored in the desiccator for swelling studies and further characterization.

2.4. Preparation of Simvastatin microparticles loaded hydrogels

The microparticles loaded hydrogels were prepared by chemical cross linking method, as mentioned in the previous section. Based on the *in vitro* release profile of plain drug loaded hydrogels, 5% PVA was identified to be optimized. Simvastatin-chitosan microparticles (SMT-5) prepared with 0.3% of Tween 80 and chitosan: drug ratio of 0.5:5 was selected for incorporation into the hydrogels due to the high entrapment of drug. To provide an initial release of drug from the hydrogels, specific quantity of pure drug was also added in the PVA solution before cross linking. Henceforth, the selected drug loaded microparticles and pure drug (at equal proportions) equivalent to total dose of 2.5 mg, 5 mg and 10 mg per hydrogel patch were loaded in the 5% PVA hydrogels. Incorporation of pure drug along with the microparticles into the hydrogel could provide the burst release followed by sustained release of the drug from dual controlled release system for prolonged period of time.