Colloids and Surfaces B: Biointerfaces 172 (2018) 323–329

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Hyaluronic acid-containing ethosomes as a potential carrier for transdermal drug delivery

Jiesi Xie[a,b], Yujie Ji[a,b], Wei Xue[a,b], Dong Ma[a,b,*], Yunfeng Hu[c,*]

[a] Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Department of Biomedical Engineering, Jinan University, Guangzhou 510632, China
[b] Guangdong Provincial Engineering and Technological Research Center for Drug Carrier Development, Jinan University, Guangzhou 510632, China
[c] Department of Dermatology, The First Affiliated Hospital of Jinan University, Jinan University, Guangzhou 510630, Guangdong, China

ARTICLE INFO

Keywords:
Transdermal drug delivery
Hyaluronic acid
Ethosomes

ABSTRACT

A hyaluronic acid-containing ethosomes (HA-ES) as the transdermal drug delivery system was prepared in this work, and rhodamine B (RB) was used as a model drug to be encapsulated. The obtained HA-ES-RB was then characterized by the surface morphology, entrapment efficiency, drug loading and the stability. Results showed that the prepared HA-ES-RB was spherical and showed good dispersion as well as the stability, with a particle size of below 100 nm. The skin permeation experiments were carried out *in vitro* with the Franz diffusion cells and the rat dorsal skins were used. It was found that the penetration effect of HA-ES-RB was much better than that of ES-RB. The fluorescence microscopy image showed that HA-ES-RB penetrated into the deepest dermis. The excellent transdermic drug delivery effect of HA-ES-RB maybe attributed from its smaller size, hydration of hyaluronic acid as well as greater potential targeting to skin and skin appendages of liposomal carriers. Moreover, the HA-ES delivery system showed non-cytotoxicity to normal cells, indicating a good biocompatibility. This work provded a hyaluronic acid-containing ethosomes which can offer a quick, high efficient, safe and self-administered transdermal drug delivery system.

1. Introduction

Transdermal drug delivery system (TDDS) is a route of administration that drugs are applied on the skin surface and continuously delivered into the body through the skin, followed realizing the topical superficial or systemic therapy effects. Compared with the traditional injection and oral administration methods, TDD showed some advantages as follows: reducing the pain caused by injection, releasing the medication at a constant rate, avoiding the liver first-pass effect and preventing drug degradation in the gastrointestinal route et al. [1,2]. Stratum corneum is the outermost layer of the epidermis, which is directly contact with the external environment and is the most important protective structure of the skin. Stratum corneum cells have a property similar to the semipermeable membrane, which resulted into that the hydrophilic macromolecules are hard to be delivered across the skin and the transdermal rate cannot meet the treatment requirements. Therefore, some methods are attempted to increase the efficiency of transdermal administration: change the structure of the stratum corneum, deliver the medication by external force or modifies/wraps the medication. In the past several decades, various technologies have been utilized to improve transdermal or topical drug delivery, including the chemical enhancers, iontophoresis, electroporation, sonophoretic and magnetophoresis [3–5].

Ethosomes (ES) have been reported as the carriers for TDDS to increase the amount of drug permeation through skin [6,7]. ES showed the higher capacity of liquidity and deformability as well as the high encapsulation efficiency. In addition, ES can significantly promote the drug to penetrate the skin, increase the drug accumulation in the skin [8]. The ethanol contained in ES can change the tight arrangement of stratum corneum lipid molecules and enhance the lipid fluidity. Moreover, the high concentration of ethanol also enhances the flexibility and fluidity of the ES membrane [9]. However, its poor stability and low entrapment efficiency limit its application in TDD.

Recently, it has been reported that hyaluronic acid (HA) was used as an effective transdermal delivery carrier [10,11]. HA is a natural polysaccharide that has been widely used in biomedicine with excellent biocompatibility, biodegradability and hydrophilicity [12,13]. It is a significant component of extracellular matrix and is present in the skin at high concentrations. As the TDDS, HA has the high adhesion to some biological macromolecular drugs which could not only delay the drug release but also improve the transdermal absorption ability and targeting [14]. HA can form the hydration film on the surface of the skin to

* Corresponding authors.
E-mail addresses: tmadong@jnu.edu.cn (D. Ma), 103257712@qq.com (Y. Hu).

https://doi.org/10.1016/j.colsurfb.2018.08.061
Received 26 May 2018; Received in revised form 23 August 2018; Accepted 27 August 2018
Available online 29 August 2018

increase the moisture content of stratum corneum, thereby changing the permeability of the skin stratum corneum. In addition, HA showed the amphiphilic property due to it contained the hydrophobic patch domain of CH group. Some reports have shown that the structural hydrophobic patch domain of HA could make a complex with phospholipids [15]. Therefore, HA can complex with ES to increase the ability of drugs to penetrate the skin.

In this study, we prepared a HA-ES by using HA to make a complex with ES to improve the transdermal delivery effect of the drugs. To enhance the transdermal efficiency of HA-ES, three different mass ratios of HA to ES were designed. At the same time, rhodamine B (RB) was used as the model drug for the carrier to facilitate the observation of the carrier in various representations. Transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) were used to characterize the structure and morphology of HA-ES and to determine whether the drug was encapsulated. The encapsulation efficiency and drug loading rate were determined by UV–vis spectrophotometer. *In vitro* transdermal delivery assay was used to evaluate the permeation of HA-ES. Finally, the biocompatibility and cytotoxicity of ES were determined by CCK-8 assays.

2. Materials and methods

2.1. Materials

Lecithin (from soybean, > 98%), cholesterol (AR, > 95%) and Rhodamine B (RB) were purchased from Aladdin (Shanghai, China). Doxorubicin hydrochloride was obtained from Guangzhou Wandong Biological Technology Co., Ltd (Guangdong, China). Sodium forms of HA (Mw = 150 kDa) was bought from Macklin Biochemical Technology Co., Ltd (Shanghai, China). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and Trypsine-EDTA (0.25%) were purchased from Gibco (BRL, MD, USA). Cell counting kit-8 (CCK-8) was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Ethanol and isopropanol were of analytical reagent grade.

2.2. Preparation of ES-RB, HA-ES-RB and FITC-HA

ES were prepared by the ethanol injection method. Lecithin (2%, w/v), cholesterol (0.5%, w/v) and RB were mixed into 3 mL of ethanol solvent and then heated by water bath to make it completely dissolved. After that, the mixture was added dropwise into 7 mL of phosphate buffer solution (PBS), and the mixture was stirred for 30 min at 50 °C and 300 rmp. Finally, the suspension was homogenized and downsized by ultrasonic dispersion, and then ES-RB was obtained.

HA-ES-RB was prepared by mixing HA and ES with different mass ratio (ES:HA = 10:1, 5:1 and 2.5:1) in PBS.

FITC-HA was obtained according to the reported method [16]. Subsequently, FITC-HA-ES and FITC-HA-ES-RB were also prepared according to the above method.

2.3. Characterization of ES-RB and HA-ES-RB

The particle sizes and zeta potentials of the ES-RB, HA-ES-RB (ES:HA = 10:1, 5:1 and 2.5:1) and free RB in alcohol were determined by dynamic laser scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK), the detection temperature was 25 ℃ and each sample test was repeated for three times. The physical stability of ES-RB and HA-ES-RB (ES:HA = 5:1) were investigated by monitoring the particle sizes during the storage at various time points (1, 2, 3, 4, 5, 6 and 7 days) after the preparation. The ES-RB and HA-ES-RB (ES:HA = 5:1) from three batches were stored at 4 °C for 7 days. The surface morphology and particle distributions of the samples were analyzed by the transmission electron microscopy (TEM). A drop of samples dispersion was applied to a carbon film-covered copper grid, and the samples were negatively stained with one drop of 3% aqueous solution of sodium phosphotungstate before the observation.

2.4. Confocal laser scanning microscopy and fluorescence microscope observation

FITC-HA-ES and FITC-HA-ES-RB were prepared with FITC-labeled HA. The freshly prepared samples were added to a laser confocal culture dish and imaged with the FV10i Fluoview confocal microscopy. In the confocal images, FITC-labeled HA displayed green fluorescence with excitation/emission wavelengths at 539/570–620 nm. Those stained with RB presented red fluorescence with excitation/emission wavelengths at 470/490–540 nm.

2.5. Drug loading

The drug loading amount (DL) and entrapment efficiency (EE) for HA-ES-RB were studied. The DL refers to the unit weight or unit volume of the drug loaded by the microspheres [17]. The EE was the percentage fraction of the total amount of RB found in the studied formulations at the end of the preparation procedure. To assess the DL and EE, fresh samples were centrifuged at 8000 rpm for 30 min at 20 °C and then the supernatant was collected. The supernatant solution was appropriately diluted and detected the absorbance by UV–vis spectrophotometer at 375 nm. The concentration of the unloaded RB was then calculated from the standard curve (y = 0.0069 x + 0.0029, R^2 = 0.999) constructed with freshly prepared RB solution. The DL and EE were calculated by the following formula:

$$\text{EE}\,(\%) = \frac{\text{Intial amount of drug} - \text{drug in the supernatant}}{\text{Initial amount of drug}}$$

$$\text{DL}\,(\%) = \frac{\text{Initial amount of drug} - \text{drug in the supernatant}}{\text{Weight of the liposomes}}$$

2.6. In vitro skin permeation assay

2.6.1. Skin preparation

The hairs of dead rat dorsal surface were removed by a razor and the full thickness of skin was surgically removed. The dermis side was wiped with isopropanol to remove the residual adhering subcutaneous fat. Subsequently, the obtained skin was repeatedly washed with PBS (pH = 7.4) and wrapped in aluminum foil and stored at - 20 ℃ until further use.

2.6.2. Cumulative drug delivery

In vitro permeation experiments of HA—ES—RB and ES—RB were carried out through the rat dorsal skin using the Franz diffusion cells with a permeation area of 1.767 cm^2 (Fig. 1). The receptor compartments were filled with PBS (pH = 7.4), which were stirred continuously at 200 rpm and maintained at the temperature of 37 ℃ by a circulating water bath. The appropriate size of the skin specimen was sandwiched between the donor and receptor of the Franz diffusion cell system, with the stratum corneum facing the formulations [18]. HA—ES—RB with different ES/HA ratio (10:1, 5:1 and 2.5:1) were carried out. ES—RB, aqueous free RB and free RB in 30% alcohol were used as control groups. For all formulations, 2 mL of those was added in the donor compartment above the skin, which was then covered with plastic wrap to prevent the evaporation. At appropriate interval, 1 mL of the receptor fluid was withdrawn *via* the sampling port at and then replaced by equal volume of fresh buffer. The receptor solutions were analyzed for drug content by a UV–vis spectrophotometer.

The cumulative drug permeation (Qt) through the skin was calculated as follows:

$$\text{Qt} = V_r C_t + \sum_{i=0}^{t-1} V_s C_i$$