Iontophoretic Drug Delivery for the Treatment of Scars

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ABSTRACT: Topical treatment of hypertrophic scars is challenging because of poor penetrability of drugs into the scar tissue. The objective of the study was to investigate the effectiveness of iontophoresis to deliver medicaments across the scar epidermis. Initially, biophysical studies were performed to investigate the differences between scar and normal skin epidermis obtained from cadaver. In case of scar skin epidermis, the transepidermal water loss was not significantly different from the normal skin epidermis, whereas the electrical resistivity was significantly higher. The passive permeation flux of sodium fluorescein was approximately one-third of that across the normal skin epidermis. Scanning electron microscopy studies revealed that the two membranes were alike except that the scar skin epidermis lacked follicles. Cathodal iontophoresis enhanced the delivery of sodium fluorescein across the scar skin epidermis by approximately 46 folds [51.90 ± 8.82 ng/(cm^2 h)]. However, the transport of sodium fluorescein across the scar skin epidermis was about an order of magnitude less than the normal skin epidermis. Overall, the studies suggest that iontophoresis could be utilized to overcome the barrier resistance of scar skin epidermis and treat the scar regionally. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1638–1642, 2014

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INTRODUCTION

Scar formation is the terminal point of the wound healing process where collagen deposition, reepithelialization and restoration of anatomical and structural integrity of the skin occur.[1] Keloid, contracture, acne, and hypertrophic scars are different types of scars and these may be formed because of trauma, severe acne, burn injury, lacerations, abrasions, surgery, and vaccinations or may be because of the combined effects of skin tension, biochemical, endocrinologic, and genetic factors.[2,3] Currently, multiple invasive and noninvasive therapies exist for the treatment of scars. Invasive techniques like laser therapy, radiotherapy, cryotherapy, pressure therapy, and intralesional injections are not only expensive but also cause discomfort to the patient.[2,4] The success rate with topical application of flavonoids, imiquimod, and other drugs is not encouraging owing to the poor penetrability of drugs into the highly resistive barrier of scar skin.[4] Scar skin epidermis possess significantly higher degree of collagen orientation index compared with that of the normal skin epidermis. Collagen orientation in normal skin appears less parallel in deep dermis compared with superficial dermis.[5] The morphological differences of scar skin also are likely to be responsible for low drug penetration into scar.

Iontophoresis is a promising method for enhancing drug delivery across the skin and other biological membranes.[6–8] Generally, iontophoresis is known to enhance the drug delivery across skin via preexisting follicular pathways and other appendageal pathways.[9–11] Recently, iontophoresis was found to enhance drug permeation across the nail fold skin that lacks any appendageal pathways similar to scar skin, indicating that iontophoresis can enhance the drug delivery even across the bulk of the epidermis as well.[12] Therefore, in the present study the feasibility of drug delivery by iontophoresis across the scar skin epidermis was investigated as a potential method for treating scars. The biophysical difference between scar and normal skin epidermis was investigated as well.

MATERIALS

Scar and normal skin epidermis excised from the thigh region of human cadaver (Female, 41 years life time) supplied by Science Care (Phoenix, Arizona) was stored at −20°C and used within a week. Sodium fluorescein (molecular weight 376.27) was obtained from Sigma–Aldrich Inc. (St. Louis, Missouri). Phosphate-buffered saline (PBS) with phosphate buffer concentration of 0.01 M, sodium chloride concentration of 0.154 M, and pH 7.4 were purchased from Sigma–Aldrich Inc. All other chemicals and reagents used were of analytical grade and were procured from Sigma–Aldrich Inc.

METHODS

Biophysical Studies

In Vitro Transepidermal Water Loss Measurements

Scar and normal skin epidermis was mounted between the donor and receiver compartments of the Franz diffusion cell. The receiver compartment was filled with 5 mL of PBS and allowed to equilibrate for 2 h at 37 ± 1°C. Transepidermal water loss (TEWL) was measured using a Vapometer® (Delfin

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technologies INC., Stamford, Connecticut). The Franz cell adapter of the Vapometer® was placed on the donor compartment and the respective TEWL measurements were recorded at regular time intervals.[13]

Electrical Resistance

Scar or normal skin epidermis was sandwiched between the donor and receiver compartments of the Franz diffusion cell. Donor and receiver compartments were filled with 400 μL and 5 mL of PBS, respectively. The electrical resistance across the epidermis was carried out at 37 ± 1°C and estimated by placing a load resistor R_L (100 kΩ) in series with epidermis. The voltage drop across the whole circuit (V_O) and epidermis (V_{ep}) was approximated with the aid of a waveform generator and a digital multimeter. A voltage of 100 mV was applied at 10 Hz and the skin resistance in kΩ was approximated. After the inherent electrical resistivity of the epidermis was recorded, the resistance drop across the epidermis during iontophoresis was investigated by application of iontophoresis at 0.5 mA/cm^2.

The epidermal resistance was approximated using the formula:

$$R_{ep} = \left(V^*_{ep} R_L\right) / \left(V_0 - V_{ep}\right)$$

where R_{ep} is epidermal resistance and R_L is the load resistance in kΩ.

Scanning Electron Microscopy

Normal and scar skin specimen, collected from the same anatomical location, was fixed in 5% (v/v) glutaraldehyde solution overnight. After fixing, skin specimen was dehydrated in a series of ethanol (200° proof)/water mixture (10%, 30%, 50%, 70%, 90%, 100%, v/v) and then dried using critical point drying apparatus. The samples were then mounted on aluminum stubs and fixed using glued carbon tapes. Further, it was coated with gold using Hummer 6.2 sputter coater (Anatech USA, Union City, California). The sputter coater chamber was supplied with argon gas throughout the coating process. Photomicrographs of the microsphere specimens were prepared using a model JSM-5600 scanning electron microscopy (JEOL Ltd., Tokyo, Japan).[14]

In Vitro Transport Studies

Passive Permeation Studies

Scar and normal skin epidermis isolated from the human cadaver (heat stripping method) were mounted on a Franz diffusion cell (Logan Instruments Ltd., New Jersey) with an active diffusion area of 0.64 cm^2 to carryout *in vitro* permeation studies. The temperature of the chamber was regulated at 37 ± 1°C by water circulation. Ag/AgCl wire electrodes (Alfa Aesar, Wardhill, Massachusetts) with a diameter of 0.5 mm were fixed at a distance of 2 mm from the epidermis in donor and receiver compartment. Sodium fluorescein (500 μL, 2 mg/mL) in PBS was placed in the donor compartment and the receiver compartment was filled with PBS. The receiver compartment buffer was sampled at different time points and measured by fluorescence spectroscopy. The amount of sodium fluorescein retained in the epidermis was estimated using the extraction and analytical method as mentioned below. Passive permeation experiments were run in parallel with iontophoresis that served as control.

Iontophoretic Transport Studies

The iontophoretic study setup was similar to that of the passive permeation studies mentioned above. In addition, a constant DC with a current density of 0.5 mA/cm^2 was applied by connecting cathode and anode of an Iomed Phoresor® II (Iomed Inc., Salt Lake City, Utah) dose controller to donor and receiver chamber respectively.

Extraction of Sodium Fluorescein

After the *in vitro* transport studies, the epidermis was washed five times with alcohol and water to get rid of the drug adhering on the surface. The washing protocol was standardized to wash only the adhering drug on the surface of the epidermis. Followed by washing, the epidermal active diffusion area was punched using a metric punch and weighed. The tissue samples were cut into small pieces, immersed in PBS, and incubated for 24 h at 37°C. After 24 h, the homogenate was collected and quantified for sodium fluorescein. The same procedure was repeated using untreated scar and normal epidermis to serve as control.

Analytical Method

Sodium fluorescein in the receiver compartment was measured using a PerkinElmer (Waltham, MA) fluorescence spectrophotometer. The fluorescence emission intensity was measured at 520 nm with an excitation at 494 nm. Standard calibration of sodium fluorescein in PBS as solvent was plotted and the linearity was ($R^2 > 0.99$) over the concentration range of 1–100 ng/mL. The intensity of the receiver samples were compared with that of a standard calibration curve plotted with known concentrations of sodium fluorescein.

Data Analysis

Enhancement of drug delivery was calculated as ratio of the iontophoresis value to its corresponding control. Statistical analysis was carried out by Mann–Whitney *U*-test (Statistical software). p Value less than 0.05 was considered statistically significant. The data points provided in the graphs are an average of three trials. The error bars represent the SD.

RESULTS AND DISCUSSION

Scars are normal and inevitable outcome of mammalian tissue repair and arise after almost every dermal injury. Excessive deposition of collagen occurs in the hypertrophic scar, which is known to be due to the imbalance between biosynthesis and degradation of collagen. Scarring is considered to be a major medical as well as a cosmetic issue. The surgical procedures of scar removal are intricate by the composite geometry and stress states in different parts of the body. Apart from invasive treatments, topical therapies, in particular, would be most preferred because of their ease of use, noninvasiveness, and relatively lesser cost. However, clinical success with the existing products has been poor. Silicone and nonsilicone patches are available for use on the wound to suppress the formation of a scar. However, these patches are not effective in the treatment of completely formed hypertrophic scars.[15,16] Some medicated formulations such as imiquimod and steroids are generally suggested for treating scars.[3,4,15,16] The poor absorption of therapeutic agents into the scar as compared with the normal skin is one of the major factors responsible for poor success rate of topical therapy of scars. Therefore, in this study, the ability of