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ORIGINAL ARTICLE

Electroporation of polymeric nanoparticles: an alternative technique for transdermal delivery of insulin

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Abstract

Purpose: The aim was to study transdermal electroporation of insulin-loaded nanocarriers as a methodology for delivering macromolecules. *Methods*: The efficacy of electroporation of insulin as solution and nanoparticles was compared in vitro and in vivo. Histology and confocal laser scanning microscopy were used to assess the effects of electroporation on skin structure, whereas the latter also demonstrated the depth of permeation of the nanoparticles. In vivo studies were performed on streptozotocin-diabetic male Wistar rats and compared with subcutaneous administration. *Results*: A linear increase in insulin flux was noted on increasing the applied voltage (R^2 = 0.9514), the number of pulses (R^2 = 0.8515), and the pulse length (R^2 = 0.9937). Electroporation of nanoparticles resulted in fourfold enhancement in insulin deposition in rat skin in contrast to solution. In vivo studies showed maximum reduction of 77 ± 5% (87.2 ± 6.4 mIU/mL, t = 2 hours) and 85 ± 8% (37.8 ± 10.2 mIU/mL, t = 4 hours) in blood glucose levels for solution and nanoparticles, respectively, with therapeutic levels maintained for 24 and 36 hours. *Conclusion*: Overall, electroporation of polymeric nanosystems proved to be an ideal alternative to injectable administration.

Key words: *Electroporation; in vitro and in vivo evaluation; insulin; nanoparticles; transdermal*

Introduction

Twenty-first century has seen a boom in the research on transdermal delivery of peptides and proteins. Though transdermal delivery has its inherent advantages of controlled rate of drug input, ease of application with no associated fear of needles, and reduced degree of side effects, the formidable barrier of stratum corneum (SC) poses a challenge[1]. One of the major targets has been the delivery of insulin, a 6 kDa protein, for the management of diabetes mellitus. Virtually all developed technologies have been tested for delivering therapeutic concentrations of this macromolecule. Sizeable numbers of approaches, physical (iontophoresis, electroporation, radiofrequency, ultrasound, and microneedles), chemical (iodine, trypsin, chemical enhancers), and their combinations, have been tried[2–13]. Because the results obtained vary from technique to technique, the target to deliver insulin transdermally is still unsolved and has adequate scope for research.

Various nanosized vesicles have proven credible for transdermal applications: to name a few, flexible liposomes, transferosomes, and ethosomes[14]. Amphiphilic block copolymer vesicles are polymeric counterparts of liposomes and have been successfully used to deliver hydrophilic and hydrophobic drugs by parenteral route[15]. Because of their tunable block chemistries, they can be designed with varying features such as prolonged circulation time, biorecognition, and stimuli sensitivity[16]. Their applications for alternative routes of delivery have not been studied in detail. Recently, we have shown that polymer vesicles can be flexible in nature and can permeate across epidermal layers[17]. However, their movement was found to be slow with accumulation in the lower layers of epidermis. It is therefore expected that application of physical forces such as current or high voltage might fasten their movement as well as deliver them systemically. Use of combination techniques has been reported in literature. In a latest report by Chen et al.[18], noninvasive delivery of

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positively charged insulin-containing liposomes by a combination of iontophoresis and microneedles has been investigated. In this study, we will be investigating the use of electroporation for driving polymer vesicles across the skin barrier.

To achieve the purpose, vesicles of block copolymers of poly(ε-caprolactone) (PCL) and polyethylene glycol (PEG), loaded with insulin were prepared. In vitro experiments on full-thickness rat abdominal skin model were carried out to optimize the electroporation parameters for insulin solution and subsequently compared with electroporative permeation of nanoparticles. Detailed histological studies were performed to assess the alterations in skin structure upon electroporation. Confocal microscopy on cadaver epidermis was performed to visualize the movement of polymerosomes. Reduction in blood glucose and changes in serum insulin were noted to evaluate the efficiency of the techniques in streptozotocin (STZ)-diabetic adult Wistar rats.

Materials and methods

Materials

PEG (M_n 2000), ε-caprolactone, human recombinant insulin (activity 28.6 IU/mg, expressed in *Escherichia coli*), STZ, and nile red (NR) were purchased from Sigma Chemicals (St. Louis, MO, USA). PEG and ε-caprolactone were purified by azeotropic distillation in toluene and calcium hydride under reduced pressure, respectively. All other chemicals of analytical grade were locally procured and used as received.

Synthesis and characterization of polymer vesicles

Triblock copolymers PCL-PEG-PCL of varying molar ratio of CL:EG and uniform PEG content were synthesized by a metal catalyst free method[19]. The synthesis and characterization of the polymers and the preparation of polymerosomes have been described in earlier reports[20]. Briefly, 1 mg of insulin suspended in 0.1 M HCl was emulsified with Ultraturrax (IKA Corporation, Staufen, Germany) in 50 mg of the copolymer in dichloro methane (DCM) using 2% (v/v) of Span 80 as the emulsifier at 14,000 rpm for 1 minute. The primary emulsion was further emulsified in 20 mL of 1% aqueous solution of Tween 80 at 6000 rpm for 2 minutes. Thereafter, the formulation was allowed to stir at room temperature for 4 hours for formation of nanoparticles. To aid evaporation of DCM from the system, excess of deionized water (30 mL) was added. The nanoparticles were isolated by high-speed centrifugation (3K30, Sigma, USA) at 60, 360 $\times$ *g* for 15 minutes and washed to remove the adsorbed surfactant. The process was repeated twice followed by freeze-drying (Freezone, Labconco Corporation, Kansas City, MO, USA) for 10 hours at –80°C. The samples were suspended in deionized water (0.1 mg/mL) and sonicated for 20 minutes to ensure deaggregation for size and charge measurements. The hydrodynamic diameter and ζ-potential of the nanoparticles were determined by differential light scattering (DLS, Zetasizer Nano ZS, Malvern, Worcestershire, UK) at an angle of 173° at 37 ± 0.5°C (physiological temperature).

NR-loaded vesicles were prepared for confocal laser scanning microscopy (CLSM) studies. Briefly, 8 mL of acetone containing the polymer (0.5%, w/v) and NR was added to 20 mL of 2% aqueous solution of Tween 80 and allowed to stir at room temperature for 6 hours to ensure complete evaporation of acetone with formation of polymer vesicles. The final concentration of the dye was 20 ng/10 mg of polymer.

Skin preparation

All protocols followed in the study were approved by Institutional Animal Ethics Committee, All India Institute of Medical Sciences, New Delhi, India. In vitro percutaneous absorption studies were carried out on full-thickness rat skin (adult Wistar male rats weighing 200–250 g). The rats were killed by excessive ether anesthesia and their hair trimmed. Full-thickness abdominal skin was excised. Subcutaneous fat was carefully removed with scissors. The skin samples were used freshly and not stored for more than a week at –20°C.

For CLSM studies, cadaver skin was obtained from the mortuary after permission from the Institutional Ethical Committee, All India Institute of Medical Sciences, New Delhi, India. Epidermis was removed by heat separation method by placing full-thickness skin in water at 60°C for 1 minute; the epidermis teased off carefully; it was washed and stored at –20°C until use[21].

Electroporation protocol

Electroporation of insulin and insulin-loaded nanoparticles was carried out by a stainless steel pin electrode array (4 $\times$ 2; Figure 1) clamped to an ECM 830 (BTX Harvard Apparatus, Genetronics, San Diego, CA, USA) square wave pulse generator. The effect of voltage, pulse length, and number of pulses was determined by insulin flux, and the depth of penetration of nanoparticles and cell separation was noted by fluorescence microscopy.

In vitro skin permeation and deposition

Permeation studies were carried out using unjacketed Franz diffusion cells (diffusion area 1.54 cm^2) placed on a magnetic stirrer (Remi 2ML, Remi, Mumbai, India) at

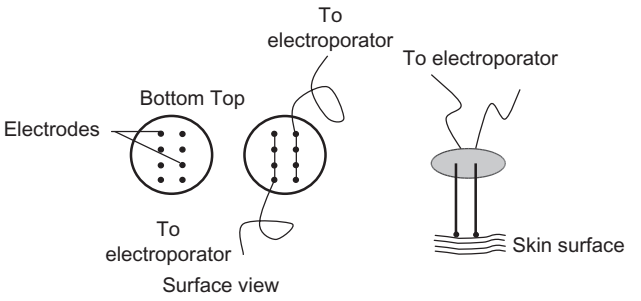

Figure 1. Diagrammatic representation of the electrodes used in the study and their setup. Blunt end stainless steel needles (4 × 2) of length 2.5 cm and diameter 1 mm were applied onto the skin surface for in vitro and in vivo studies.

37 ± 0.5°C as shown in Figure 1. Phosphate-buffered saline (10 mL) pH 7.4 containing sodium azide (0.0025%; bactericidal) was used as the receptor medium for the studies. Rehydrated rat skin was equilibrated for 1 hour with constant stirring. Free insulin or insulin-loaded polymerosomes (equivalent to 1 mg free insulin) were suspended in 2% carboxymethyl cellulose (CMC) gel and placed in the donor chamber for permeation studies. No shift in pH was noted in the donor and receptor media because of pulsing. Samples from the receptor solution (0.3 mL) were taken at regular intervals up to 24 hours after the pulses and were replaced with equal volume of fresh buffer.

To assess the extent of deposition, the area of skin exposed to pulsing was removed after the experiment, homogenized in ice bath, and treated with 0.1 M HCl to extract the insulin present.

The insulin concentration was determined by a solid-phase two-site immunoassay (Human Insulin Kit, Mercodia, Uppsala, Sweden). The test is based on direct sandwich technique consisting of two monoclonal antibodies directed against separate antigenic determinants on insulin. It is followed by the reaction of the substrate 3,3′,5,5′-tetramethyl benzidine with bound horse-radish peroxidase conjugated enzyme. The insulin concentration is determined from absorbance at 450 nm (Microplate reader, ECIL, Hyderabad, India) corresponding to a colorimetric end point obtained by stopping the above reaction.

Assessment of permeation by CLSM

The electroporation of nanovesicles was studied by CLSM and the depth of penetration was determined. Cadaver epidermis was fitted onto the Franz diffusion cells similar to permeation studies. NR-loaded polymerosomes (1%) suspended in deionized water were applied on the SC surface followed by electroporation electrodes. The microscope LSM 510 (Carl Zeiss, Jena, Germany) equipped with a HeNe laser (477 nm) was used to excite the fluorophore. The as-such treated samples after surface washing were mounted inverted onto the platform with the SC side facing the objective lens system (Plan Neofluar 20×/0.5) and 50–90% laser intensity. NR was detected by a low-pass filter of wavelength 505 nm. Optical sectioning was done parallel to the *xy* plane of the skin surface. The point $z = 0$ was fixed for all the samples to assess the depth of penetration of the fluorescent probe into the epidermis. All the images were obtained at the same pinhole aperture, filter, lens, and scan speed with an average of 8 scans per image.

Histology studies

Freshly removed full-thickness rat abdominal skin was used for light microscopic investigations to study the effects of the electroporation protocols. The skin was sandwiched between the donor and receiver compartments of Franz diffusion cells with the SC side facing the donor compartment. Phosphate-buffered saline (pH 7.4) was filled in both the donor and receiver compartments. The donor and receiver compartments contained 0.5 and 5 mL of phosphate-buffered saline, respectively. Electroporation pulses were applied as described earlier. The area of the skin exposed to the pulses was 0.6 cm^2. Each specimen was fixed in a 10% buffered formaldehyde solution at pH 7.4 for at least 48 hours. Each section was dehydrated using ethanol, embedded in paraffin wax, and stained with hematoxylin and eosin. Three different sites for each sample were examined (Nikon TE-2000E, Nikon Instruments Inc., Melville, NY, USA) and photographed by a digital camera (Olympus, Center Valley, PA, USA).

In vivo delivery of insulin

Male adult Wistar rats with body weight ranging from 200 to 250 g were selected for the study. The animals were fasted for 16 hours with free access to water prior to the experiment. Diabetes mellitus was induced by an intraperitoneal (i.p.) injection of 50 mg/kg of STZ in citrate buffer pH 4.5 in fasted animals under light ether anesthesia[22]. The buffer was mixed to STZ just prior to the injection. The body weight and blood glucose levels (BGLs) of the animals were monitored regularly for the complete duration. Animals with BGLs above 200 mg/dL were included in the study.

Rats were administered with ketamine (80 mg/kg) 15 minutes prior to the start of the experiments. Subcutaneous (s.c.) insulin (5–7 IU) was injected in control animals for comparison. Gel containing insulin/insulin-loaded nanoparticles was applied followed by pulsing with pin electrodes. After electrical treatment, the area was occluded by coated adhesive tapes to avoid drying of the gel. From the tail vein, 0.2 mL of blood was withdrawn at regular time intervals following administration for

estimation of blood glucose. The treatment groups included the following:

Control [diabetic control; intravenous (i.v.) normal saline, $n = 6$]
Insulin [subcutaneous (s.c.) injection of insulin 30 IU/kg body weight, $n = 6$]
NP [intravenous (i.v.) injection of loaded nanoparticles (~50 mg) equivalent to 30 IU insulin, $n = 8$]
Insulin [electroporation (electro) 30 IU insulin in 2% CMC gel, $n = 8$]
NP electro [electroporation of nanoparticles equivalent to 30 IU insulin-loaded nanoparticles (~50 mg) in 2% CMC gel, $n = 8$]

To counter the loss of fluid, the animals were injected with 0.5 mL normal saline (i.v.) every 24 hours during the experiment. All results expressed are a mean of at least six animals. Blood glucose was determined using Ascensia kit (Bayer Healthcare, Mumbai, India; range = 10–550 mg/dL). Serum insulin concentration was determined by enzyme-linked-immunosorbent assay method (Human Insulin ELISA kit, Mercodia, Uppsala, Sweden) as described earlier under '*In vitro skin permeation and deposition*'.

Results and discussion

Phospholipid vesicles such as liposomes and transferosomes are known to be good candidates for enhancing transdermal permeation during passive as well as electrically assisted drug delivery[23–26]. This study is an approach to explore whether structurally similar polymeric vesicles or polymerosomes of nanosize can also have the potential to enhance transdermal permeation of molecules alone or in combination with electroporation. In a previous study by our group, we have shown the possibility of developing polymerosomes for topical delivery of therapeutics[17]. The movement of these vesicles in the epidermal layers was found to be slow. It is therefore expected that on application of external electrical assistive forces such as high-voltage pulses (electroporation) the permeation of these nanovesicles can be accelerated.

Characterization of polymer vesicles

Block copolymer (M_n ~ 6900 and polydispersity = 1.276 determined by ^{1}H-NMR and gel permeation chromatography, respectively) was used in the preparation of polymerosomes by double emulsion method. The nanosystems had an average diameter of 85 ± 9.4 nm with ζ-potential of –37 mV and insulin entrapment efficiency of 32.9 ± 7.6%[20]. Figure 2 shows the transmission electron micrograph of the redispersed vesicles in aqueous media.

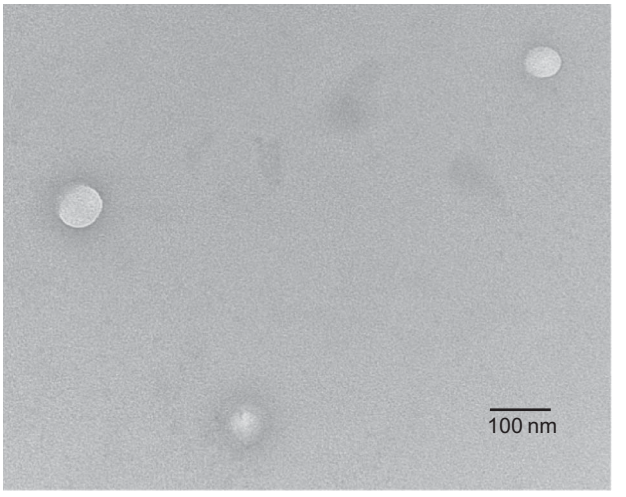

Figure 2. Transmission electron micrograph of blank PCL and PEG copolymer nanoparticles.

Effect of electroporation protocols on insulin permeation

Rat abdominal skin is often not considered to be a suitable model for transdermal studies because of the obvious difference in chemical composition, nature, and thickness of epidermal layers and follicular density. However, sizeable numbers of reports mention the use of murine models, namely, mice and rats, because of well-rehearsed arguments: they are small, easy to work with, and inexpensive to keep; their generation time is short; and they have large litters that can allow controlled breeding programs[27–29]. In this study, the effects of applied voltage, pulse length, and number of pulses on the electroporative transport of insulin through rat skin were studied. Figure 3 shows the permeation profile of insulin when subjected to electroporative pulses followed by passive diffusion. Diffusion of insulin on passive application was negligible (0.005 ± 0.001 μg/cm^2). Upon electroporation, the insulin flux was seen to change linearly with the applied voltage from 50 to 200 V ($R^2 = 0.9514$, $P < 0.05$, Figure 3A). The maximum flux of 0.224 μg/cm^2 h was noted with 200 V. Similar findings have been reported by Sharma et al.[30] and Medi and Singh[31] for the delivery of terazosin hydrochloride and human parathormone, respectively. The increase in permeability observed after high-voltage pulsing has been related to a general perturbation of the SC–lipid structure because of localized heating and formation of local transport regions[32,33]. It has also been noted that the rate of permeation remained elevated for several

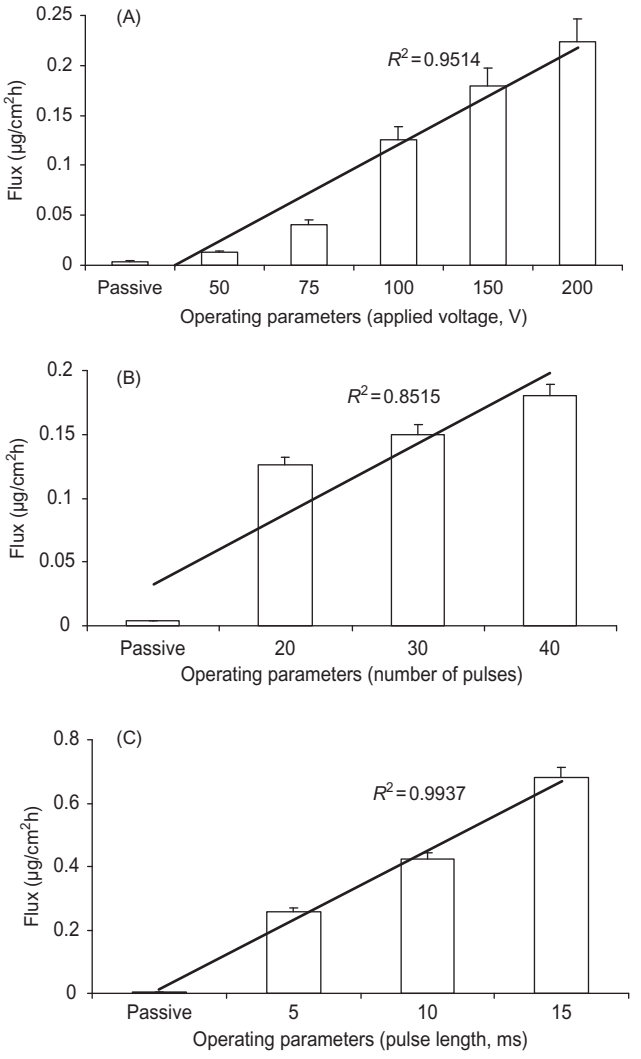

Figure 3. (A) Effect of pulse voltage, (B) number of pulses, and (C) pulse length on electroporative insulin flux across rat skin. A good linear trend was observed with pulse voltage (R^2 = 0.9514) and pulse length (R^2 = 0.9937); n = 4 for all experiments.

hours postelectroporation, suggesting the creation of a drug reservoir within the skin and/or a persistent change in skin permeability because of altered skin structure[34].

Enhancement in insulin permeation was also observed on increasing the number of pulses applied (R^2 = 0.8515, $P > 0.05$; Figure 3B) when 1-ms pulses of 100 V each were applied. This observation is consistent with the reports of Prausnitz et al.[35] and Vanbever et al.[36], in which increasing the pulse numbers is correlated to increase in the sizes of the formed pores[35,36]. However, no peaks were observed as reported by Sung et al.[37] for the transport of nalbuphine and its prodrugs[37]. Pulse duration or pulse length also showed a linear trend (R^2 = 0.9937, $P < 0.05$, Figure 3C). The maximum flux was obtained with pulse length of 15 ms at 10 pulses of 100 V applied voltage (0.86 µg/cm^2 h). The linearity in the trend is in concordance with the previous reports, which demonstrate that longer pulse durations cause formation of pores with larger sizes, namely, increase in the size of the local transport regions or causing merging of the local dissipation regions to form larger regions containing local transport regions, thus enhancing the transdermal flux[35–38].

Histological assessment

No visible changes were observed in the skin immediately after electroporation in all the protocols. Figure 4 shows the light micrographs of control and electroporation-treated full-thickness rat skin (10 pulses of 100 V and 15 ms length). However, the SC was seen to be detached from the underlying layers (stratum malpighii) in all electroporated samples. Epidermis in control shows a rippled course, strictly adherent to the dermis below; furthermore, it frequently presents, emerging from the dermis, hair follicles and sebaceous glands. Table 1 summarizes the findings of the histological alterations upon various treatment protocols. Increase in the applied voltage above 100 V caused a 'pitted' appearance and sloughing off the SC. This observation is consistent with that of Medi and Singh[31,39] wherein the histological changes of voltage ranging from 100 to 300 V were studied on porcine skin. Thickening was noted postelectroporation in samples treated with 200 V and on increasing the pulse length. Application of electroporation showed widening of the openings of the hair follicles and a highly ruffled appearance of the epidermis in all the cases. Swelling of the follicle bulb was noted at lower voltages of 50 and 75 V, suggesting the appendageal routes to also play some role in enhancing electroporative permeation. Disruption of dermis and cell–cell separation was observed with increase in number as well as duration of pulses. However, the extent of appendageal degeneration was less pronounced with alteration in pulse number and duration. Furthermore, for the clinical application of drug delivery by electroporation, it would be better to decrease the number of pulses, to shorten the treatment, and to reduce the number of electrically induced muscle contractions.

Recovery in skin structure was studied at 24 and 48 hours for samples subjected to 10 pulses of 100 V and 15 ms length (Figure 4). Whereas the samples showed a widened opening of the hair follicles at 24 hours, the areas were packed by the 48th hour. It is to be noted that electroporation protocol hereafter refers to application of 10 pulses of 100 V and 15 ms length.

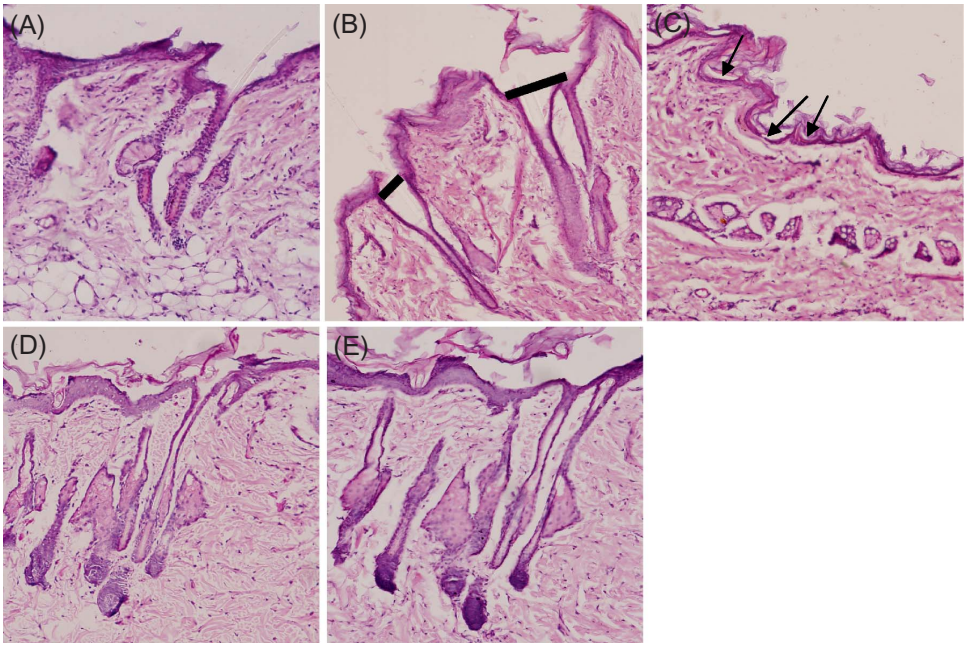

Figure 4. (A) Histological examination of untreated rat abdominal skin. Hair follicles showed separation and increase in pore size as marked. (B) The hair bulbs were also dilated. (C) The stratum corneum (SC) surface showed formation of 'craters' or pits with stripping SC layers. Extent of recovery of treated skin at (D) 24 and (E) 48 hours postelectroporation.

Table 1. Study of instantaneous effect of operating electroporation voltage (V), number of pulses (n), and pulse length (l) on the histology of rat skin.

	Histological changes			
	S.c. shedding	Cell separation	Appendageal degeneration	Acanthosis
Control	–	–	–	–
Effect of operating voltage (n = 10, l = 1 ms)				
50 V	+	+	+	+
75 V	++	++	++	+
100 V[a]	++	++	++	+
150 V[a]	+++	+++	++	–
200 V[a,b]	+++	+++	+++	–
Effect of number of pulses (V = 100 V, l = 1 ms)				
20[a]	+++	++	–	–
30[a,c]	+++	+++	+	–
40[a,b,c]	+++	+++	+	–
Effect of pulse length (V = 100 V, n = 10)				
5 ms[a,c]	+++	+++	+	–
10 ms[a,b,c]	+++	+++	+	–
15 ms[a,b,c]	+++	+++	+	–

+ → +++ lowest to highest change. [a]Complete removal of s.c. [b]Formation of pits. [c]Disruption of dermis.

Electroporation of solution versus nanoparticles

Figure 5 shows the comparison of permeation profile of vesicles on passive treatment and electroporation. Passive application of polymerosomes resulted in 1.7-fold increase in cumulative insulin permeated as compared to passive insulin solution. Passive treatment with NR-loaded vesicles has shown extensive fluorescence in cadaver epidermis at a depth of 45–50 µm. Earlier studies have shown time-dependent increase in depth of penetration[17]. Thus, the lodging of these poly-

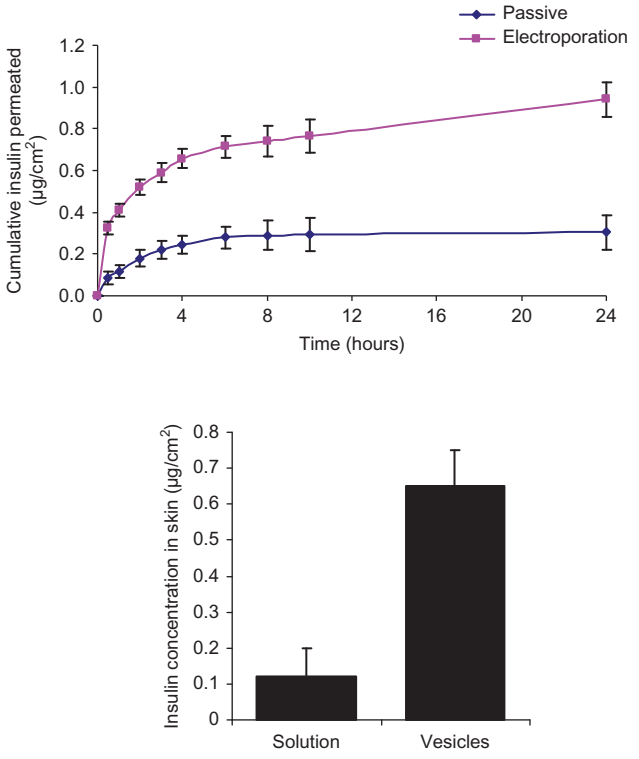

Figure 5. Top: Comparison of passive and electroporative application of loaded vesicles on the permeation of insulin across rat skin. Bottom figure shows the quantities of insulin retained in the skin after electroporation of solution and vesicles after 24 hours.

merosomes in the epidermal layers can be responsible for insulin permeation even in the passive state. Application of electroporation, however, resulted in approximately 6.5 times increase in cumulative amount of insulin permeated over passive vesicles (flux = 0.3241 ± 0.092 µg/cm^2 h). Interestingly, whereas electroporation of nanocarriers showed much lower insulin content in the receptor chamber as compared to insulin solution (0.598 ± 0.14 versus 1.53 ± 0.29 µg/cm^2 at t = 4 hours, P = 0.03), the skin content was nearly 4 times higher than the latter. Such observations have been noted by Essa et al.[25] wherein the phospholipids in liposomes were reported to act as retardants. The sustained permeation profile of insulin upon electroporation also proves the intactness of the vesicles upon pulsing.

Assessment of permeation by CLSM

Application of polymerosomes on cadaver epidermal membrane has been shown to result in accumulation of the vesicles in low-resistance 'intercluster' pathways such as *rete* and in hair follicles. The depth of penetration was noted to be dependent on the duration of contact and reached a maximum of about 60 µm in 24 hours[17]. Vertical sections of electroporated samples showed highlighted epidermis and hair follicles, suggesting accumulation of the polymerosomes in these areas. Surface view of the same samples illustrated distinct separation of corneocyte islands upon electrical treatment (Figure 6). Distinct channels of bright fluorescence were observed on *xz* sectioning reinforcing the formation of 'new pathways' with high-voltage pulses. The presence of vesicles (red) was traceable throughout the complete depth of the tissue.

Pharmacokinetic evaluation

Figure 7 shows the reduction in %BGL upon various modes of insulin administration. Briefly, 1 mg of insulin or an equivalent amount of loaded polymer vesicles (~50 mg) was suspended in 2% CMC gel and placed on shaved and cleaned backs of anesthetized animals before pulsing. For comparison, s.c. insulin- and i.v. insulin-loaded nanoparticles were administered in separate sets of diabetic animals.

S.c. administration of insulin showed a lowering in %BGL within 1 hour of administration by nearly 92% of the original values. The therapeutic activity was maintained for a period of 4–6 hours only. Thereafter, the glucose levels returned back to the original state. On the other hand, electroporation of insulin showed a lowering in %BGL by 70% within 1 hour of pulsing. This decline was maintained for a duration of nearly 2 hours. Thereafter, a trend toward recovery was noted till t = 12 hours (%BGL = 55.2 ± 8.9, 65.5 ± 10.0 mIU/mL). The blood glucose was seen to be maintained in the range of 50–80% from the 12th to 24th hour (~30.0 ± 7.4 mIU/mL). This observation clearly shows that electroporation results in transient changes in the skin structure, thereby enhancing the movement of the drug from the application site through the disturbed architecture. The results indicate that electroporation delivers higher amount of the drug as compared to injectable systems. Because electroporation lasts for a very small duration,

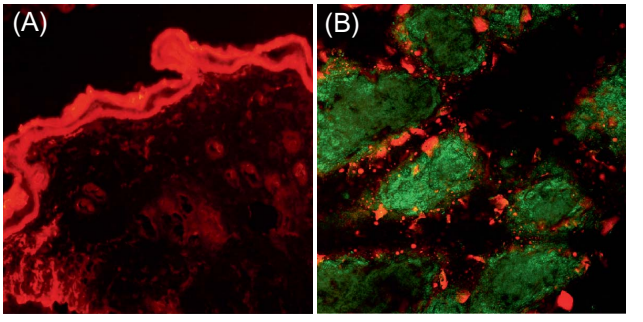

Figure 6. (A) Vertical section of the electroporated skin shows bright red fluorescence in the epidermal layers and marked hair follicles. (B) *xz* sectioning of the epidermis depicts marked separation of groups of corneocyte cells with accumulation of labeled vesicles in the cavities around them.

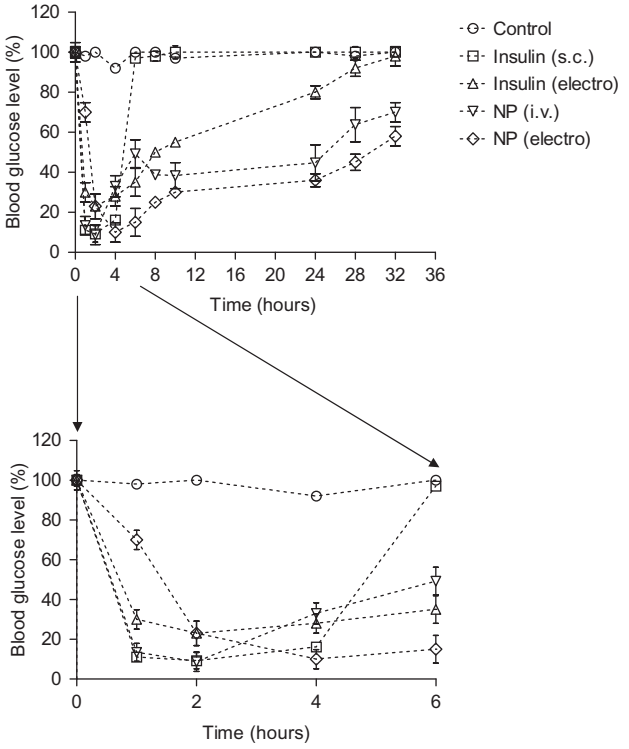

Figure 7. Reduction in blood glucose levels (%) in diabetic Wistar rats on injectable and electroporative administration of insulin and insulin-loaded nanoparticles [Control, saline i.v.; insulin (s.c.), subcutaneous insulin; NP (i.v.), i.v. administration of insulin-loaded nanoparticles; insulin (electro), electroporative delivery; NP (electro), electroporation of insulin-loaded nanoparticles]. Inset shows initial hypoglycemia posttreatment till t = 6 hours. All results expressed are a mean of 6–8 animals.

typically less than 1 minute, the prolonged duration of therapeutic activity also suggests the formation of a depot within the skin layers from which the drug is released into the systemic circulation.

Parenteral administration of insulin-loaded polymer vesicles resulted in a maximum reduction of 13.45% in %BGL (22.86 ± 4.42 mIU/mL) within 1 hour of administration. Lowered glucose levels in the range of 50–70% were maintained thereafter for nearly 36 hours. Electroporation of the carriers mimicked the blood glucose profile similar to that of the i.v. injection. However, a slight delay was observed at the first hour postadministration with only 25% of reduction in blood glucose (15.9 ± 9.1 mIU/mL) in comparison to electroporation (~70%, 40.0 ± 10.0 mIU/mL). This observation suggests that the movement of vesicular systems across the skin layers is not rapid and therefore does not reach the blood circulation instantaneously as is the case with i.v. administration. Once in circulation, the therapeutic potential of the nanocarriers mimicked that of the injected systems[20]. The maximum lowering in %BGL was found to be 14.2 ± 9.0% (37.9 ± 13.2 mIU/mL) of the original value at t = 4 hours. BGLs were seen to fluctuate between 30% and 60% of their original values from 8 to 32 hours corresponding to serum insulin levels of 30.2–41.4 mIU/mL. At the 36th hour, the BGL was noted at 82.3 ± 15.0% of the original value (20.1 ± 7.5 mIU/mL). Electroporation of nanoparticles was therefore seen to prolong the delivery of insulin with effective lowering of BGLs till a period of 36 hours in comparison to 24 hours by electroporation. The serum insulin levels were also maintained for the same duration. This can lead to reduction in the long-term complications encountered by fluctuating insulin concentrations in diabetic cases. Also, this strategy can be patient compliant as it circumvents the trauma associated with self-injections as well as reducing the frequency of administration. Overall, the study proves the efficiency of electroporation of nanosystems as an alternative to injectable administration and the utility of block copolymer vesicles as transdermal carriers.

Conclusion

The quest for achieving normoglycemic levels in diabetic cases with reduced frequency of administration has led to the development of newer insulin analogs and delivery technologies. In this study, the ability of polymer vesicles for electroporative delivery of insulin was investigated. Enhancement in the insulin deposition in skin and flux was noted upon pulsing of insulin-loaded nanoparticles in vitro. CLSM analysis demonstrated the presence of nanoparticles in the deeper layers of epidermis. In vivo comparison of electroporation of insulin solution and nanoparticles showed a peak action at 1 and 2 hours, respectively, with prolonged hypoglycemic levels for up to 24 and 36 hours. Electroporation of nanoparticles can therefore be considered comparable to injectable administration and can serve as a suitable alternative. Further studies related to degradation of nanoparticles in skin and any related toxicity are in progress.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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