Novel Method for Topical and Transdermal Delivery of Nanomaterials

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ABSTRACT

Nanoscale drug delivery systems including, but not limited to nanoparticles, liposomes, nanotubes, quantum dots, could potentially revolutionize modern drug delivery systems. Most nanomaterials are significantly large in size, which prevents their topical delivery without artificially enhancing skin permeation. The only method to deliver these novel nanomaterials is via injection, which limits the distribution in skin and may enhance their propensity to agglomerate. Over the last decade, cold electrical plasmas have been widely studied by various groups across the world for clinical and biomedical applications beyond sterilization. It has been widely demonstrated that cold plasmas can be safely applied directly to living cells and tissue, thereby enabling various beneficial effects including wound healing [1], cell transfection [2], and cell proliferation [3], but the focus was not related to enhancement of skin permeation for transdermal drug delivery. The objective of this work was to determine the feasibility of non-thermal dielectric barrier discharge (DBD) plasma to enhance skin permeation and transdermal drug delivery especially of nanomaterials including nanoparticles and liposomes without causing any thermal damage. We investigated the ability of non-thermal plasma to drive such large molecules across the stratum corneum to deeper layers of the skin in a controlled manner.

Keywords: Non-thermal plasma, DBD, Nanomaterials, Topical Drug-delivery, Liposomes

1 INTRODUCTION

Nanomaterials have wide applications in pharmaceutical sciences including their use in drug delivery as well as diagnostic imaging, and biosening. Nanomaterials are attractive because of their large surface to volume ratio that helps to bind, adsorb, and deliver other compounds such as drugs, probes, and proteins together. The nanosize device systems can eventually reach in generally inaccessible areas such as tumor cells and inflamed tissues due to their enhanced permeability. Further nanomaterials on chips, nanorobotics, and magnetic nanoparticles attached to specific antibodies are new dimensions of their use in drug delivery. Nanomaterials can enable development of new drug-delivery systems and reformulation of existing drugs to enhance the effectiveness, patent protection, patient-compliance, safety and decreasing the cost of health care [4]. Most nanomaterials are significantly large in size, which prevents their topical delivery without artificially enhancing skin permeation. The only method to deliver these novel nanomaterials is via injection, which limits the distribution in skin and may enhance their propensity to agglomerate.

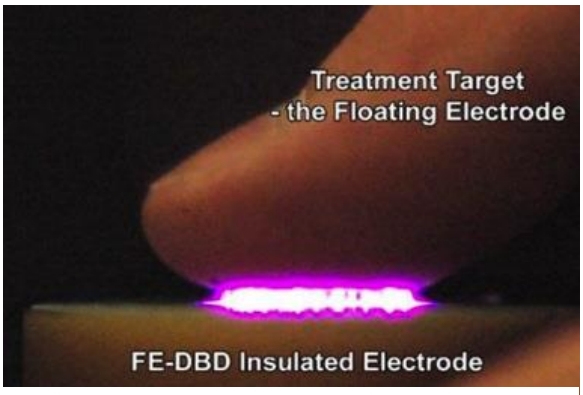

Figure 1: Cold plasma can be safely applied to skin and tissue.

Transdermal drug delivery has many advantages over other traditional methods for drug administration. It can be applied in a localized, non-invasive way, and has the potential for sustained and controlled release of drugs, and other molecules [5, 6]. In addition, transdermal drug delivery avoids first-pass metabolism which reduces the concentration of a drug before it reaches the circulatory system. Percutaneous absorption minimizes the risk of irritation of the gastrointestinal tract, reduces pain and minimizes complication associated with intradermal or intramuscular injections. However, only a small percentage of topically applied compounds can be delivered transdermally due to the skin barrier properties, namely the highly lipophilic stratum corneum (SC). As a result only molecules with a molecular weight of less than 500 Da can be administered percutaneously [7]. In addition, transport of most drugs across the skin is very slow as lag times to reach steady-state flux could be hours. Achieving a therapeutically effective drug level through transdermal delivery is therefore difficult without artificially enhancing skin permeation. Many passive (patches, oils, creams) and active (iontophoresis and electroporation) methods of enhancing skin permeation have been attempted, but have failed for various reasons including limitation on drug formulation, skin damage, pain, patient discomfort, electric shock, skin irritation and involuntary muscle contractions. Efficient drug delivery through the skin barrier still remains a challenge in medicine and dermatology, although topical

treatment is most preferred route of administration by physicians and clinicians.

In this paper, we demonstrate for the first time the use of ambient air-based non-thermal atmospheric pressure DBD plasma (shown in **Figure 1**) for enhancing transdermal delivery of various molecules including nanoparticles, liposomes, and proteins across *ex vivo* porcine skin. We present non-thermal plasma as an alternative technology for non-contact, non-invasive, needle-free and potentially cost effective application that would revolutionize transdermal drug delivery.

2 METHODS

2.1 Materials

Green (fluorescein) or red (Texas red) fluorescently labelled lysine fixable dextrans (Life Technologies PA, USA) of different molecular weights (3,000 – 70,000 Da) were dissolved in water at a concentration of 5 mg/ml and applied directly to porcine skin after plasma treatment. Fluorescently tagged silicon oxide nanoparticles (2.5% w/v) having an average diameter of 50 nm were procured from Corpuscular, Inc., NY, USA. DOPC/CHOL/mPEG-DSPE liposomes labelled with Fluorescein DHPE and having a diameter of 100 nm were obtained from FormuMax Scientific, CA, USA and applied to ex-vivo porcine skin at a concentration of 1 mg/ml. Albumin (66 kDa) and Human Immunoglobulin G (IgG, 115 kDa) were obtained from Sigma-Aldrich, MO, USA and tagged with fluorescein isothiocyanate (FITC) using a commercially available protein labelling kit (Sigma-Aldrich, MO, USA). The proteins were applied to ex-vivo porcine skin at a concentration of 1 mg/ml.

2.2 Tissue Samples

The skin permeation experiments were carried out on full thickness *ex vivo* porcine skin obtained from the abdomen of 4-6 mo old Yucatan minipigs (Lampire Biological Products, PA, USA). Porcine skin is considered a surrogate to human skin with respect to morphology and function. Prior to plasma treatment the skin was shaved, washed under running water, pat-dried with paper towels, cut in to 25.4 mm x 25.4 mm squares and hydrated for 30 min to 1 hour in a humidified container to maintain the skin in its native state.

2.3 Plasma Treatment

DBD plasma was applied to porcine skin using an experimental setup shown schematically illustrated in **Figure 2.** Plasma was generated by applying alternating polarity pulsed (50 Hz –3.5 kHz) voltage of 20 kV magnitude (peak to peak), pulse duration ranging from 1 – 10 µs and a rise time of 5 V/ns between the high voltage electrode and the skin using a microsecond pulsed power supply (Advanced Plasma Solutions, PA, USA). One mm thick fused quartz glass was used as an insulating dielectric barrier covering the 1-inch diameter copper electrode. The discharge gap between the bottom of the quartz and the treated skin surface was fixed at 2 mm. Samples were treated for a duration ranging from 15 s – 120 s. The electrical plasma parameters were chosen such that they do not lead to visual and microscopic damage to the skin after treatment. All formulations were allowed to sit on the treated skin for 1 h after plasma treatment prior to obtaining biopsies for further analysis. Control samples were not plasma treated prior to application of the molecule of interest. Before obtaining biopsies, excess solution from the surface of the skin was dabbed with a paper towel.

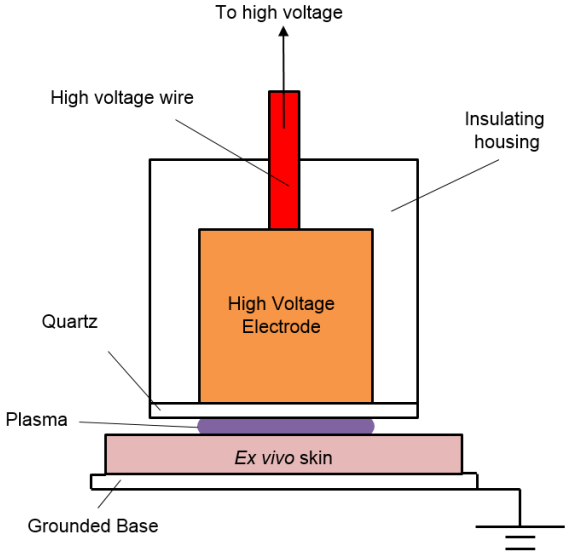

Figure 2: Schematic of dielectric barrier discharge (DBD) plasma for skin or tissue treatment

2.4 Electroporation

Electroporation pulses were applied to *ex vivo* porcine skin using a two-array needle electrode that was in contact with skin. The distance between the electrodes was 5 mm. *In vitro* electroporation parameters were: 100 V/mm, 150 ms pulse duration, 16 pulses with reversal of polarity after 8 pulses at a frequency of 1 Hz, given by a BTX 820 square wave generator (Harvard Apparatus, MA, USA)

2.5 Skin processing and analysis

Biopsies (10 mm) were obtained from the center of the plasma treated area 2 hours after treatment. They were immediately preserved in 10% neutral buffered formalin and shipped to an external lab for histological analysis (Histotox Labs, CO, USA). Biopsies were prepared for histological analysis using cryostat sectioning. 10 µm slices were obtained perpendicular to the surface of the skin and mounted on glass slides. Skin sections were then evaluated for morphological changes and depth of permeation using a fluorescently enabled inverted microscope (Evos, AMG, PA, USA). Skin morphological changes, depth of

permeation and distribution of the topically applied molecule were also evaluated using a fluorescently enabled multilaser confocal microscope (Vivascope®, Caliber ID, NY).

3 RESULTS

The objective of this work was to determine the feasibility of atmospheric pressure non-thermal DBD plasma to enhance skin permeation and transdermal drug delivery without causing any thermal or structural damage. The ability to drive significantly large molecules including nanoparticles and liposomes across the highly resistive stratum corneum to deeper layers of the skin in a controlled way was investigated.

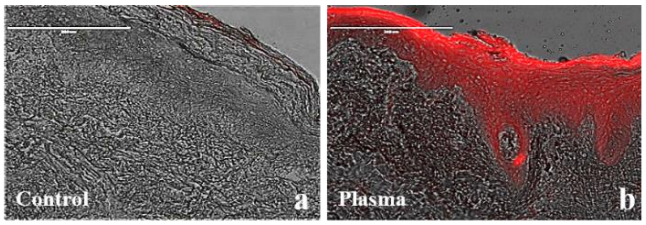

Figure 3: Plasma enabled transdermal delivery of fluorescently tagged dextran molecules. (a) Untreated skin sample with topical application of dextran. (b) Topical application of red dextran molecule after plasma treatment for 1 min. *Scale bar – 200 µm*

Initial studies were conducted with dextran molecules to demonstrate the possibility of enhancing transdermal drug delivery across porcine skin using non-thermal dielectric barrier discharge plasma. Dextrans are hydrophilic and highly water-soluble but their transdermal diffusion is limited. Additionally, they can be used as probe molecules and their presence in deeper layers of the skin can be confirmed via simple fluorescent imaging methods.

As shown in **Figure 3** in comparison to the control sample (untreated), non-thermal DBD plasma treatment for 1 min at a power density of 10 W/cm^2 is able to significantly enhance the transdermal delivery of 3 kDa dextran molecules (1 nm hydrodynamic radius) through intact porcine skin without thermal damage. Results show that the molecule reached the epidermal layer at a depth of approximately 300 µm. The response was very fast, within one hour after treatment. As expected, dextran molecules remain on the surface of the skin sample that was not treated with plasma. These results show that plasma is able to enable overcome the highly resistive SC and enhance permeation of skin to promote transdermal delivery of molecules that are at least six times as large as those molecules that are freely able to diffuse across skin [3]. This can be attributed to the impact of the high electric field that can cause breakdown of the stratum corneum through the formation of new channels through which the molecules can be transported.

The study also focused on enhancing the permeation of drug delivery vehicles including liposomes, micelles, etc. These molecules have recently garnered a lot of attention due to their numerous cosmetological and dermatological applications. The advantage of being able to deliver any type of drug without the need for complex formulation and

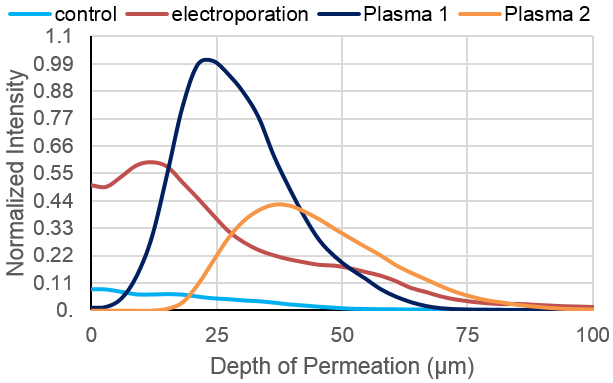

Figure 4: Plasma-enabled transdermal delivery of liposomes.

preventing interaction with skin is promising. Unfortunately, most delivery vehicles are significantly large in size, which prevents topical delivery without artificially enhancing skin permeation. This poses a challenge for plasma-assisted drug delivery: *Can non-thermal plasma enable the safe transport of 100 nm liposomes across ex vivo porcine skin in a reasonable amount of time and at sufficient quantities?*

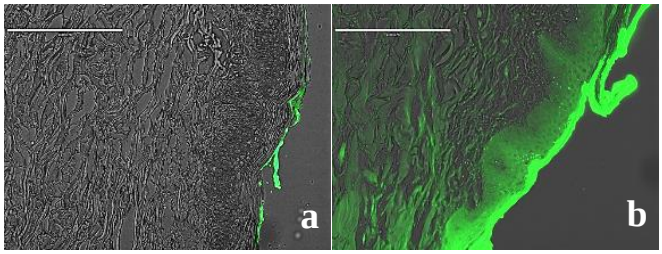

Figure 5: Plasma enabled topical delivery of fluorescently tagged silicon oxide nanoparticles. (a) Untreated skin sample with topical application of nanoparticles (b) Topical application of nanoparticles after plasma treatment for 1 min. *Scale bar – 200 µm*

Additionally, a comparison between the efficacies of plasmaporation to that of electroporation for delivery of liposomes was performed. Electroporation is a method that has been widely studied for enhancing skin permeation. 100 nm liposomes at a concentration of 1 mg/ml were applied directly to porcine skin immediately after plasma treatment or electroporation. The depth of permeation was determined using fluorescently enabled confocal imaging 2 hr after skin treatment. Also, the concentration of liposomes was determined by measuring the intensity of fluorescence at different depths using ImageJ (NIH) image analysis software. The normalized intensity values of fluorescence

versus depth of permeation were plotted for control and treated samples. As shown in **Figure 4**, in contrast to electroporation and control samples, where majority of the intensity (consequently the concentration) is detected above 20 µm in depth (stratum corneum), plasma treated samples show a high concentration of liposomes below 20 µm (epidermis) in depth. However, the plasma processing parameters seem to affect the concentration and delivery depth of the applied molecules. This demonstrates that non-thermal DBD plasma enables the transport of 100 nm liposomes transdermally by enhancing skin permeation without the side effects associated with electroporation. Furthermore, greater concentration of the liposomes was delivered by plasma to a greater depth in the skin than electroporation.

In addition to liposomes we also tested the efficacy of non-thermal plasma to delivery 50 nm diameter fluorescently tagged silicon oxide nanoparticles. 100 µl of Silicon oxide nanoparticles, 50 nm in diameter was applied directly to porcine skin at a concentration of 2.5 % w/v immediately after plasma treatment. The depth of permeation was determined by visualizing skin biopsy samples processed via cryostat sectioning using a fluorescently enabled microscope. As shown in **Figure 5** in comparison to the control sample (untreated), non-thermal DBD plasma treatment for 1 min at a power density of 10 W/cm^2 is able to significantly enhance the transdermal delivery of silicon oxide nanoparticles through intact porcine skin without thermal damage. Results show that the nanoparticles were delivered in to the epidermis a depth of approximately 100 µm. The response was very fast, within two hours after treatment. As expected, nanoparticles remain on the surface of the skin sample that was not treated with plasma. These results show that plasma is able to deliver significantly large molecules topically in a relatively short amount of time.

Further experiments have also revealed that 1-min cold plasma exposure enables the transdermal delivery of large dextran molecules: i)10 kDa in size (8 nm hydrodynamic radius) to a depth of 600 µm and ii) 70 kDa in size (49 nm hydrodynamic radius) up to a depth of 150 µm within one hour (data not shown). Authors have also found that under the process conditions described above fluorescently tagged albumin (66 kDa) and human immunoglobulin, IgG (115 kDa) proteins can be delivered to the dermis at a depth of 200 µm within one hour (data not shown).

We hypothesize that non-thermal plasma enhances transdermal drug delivery via the formation of temporary pores in the skin via a process we term 'plasmaporation'. Similar to electroporation, the electric field applied to generate the plasma creates a voltage drop across the skin and most of the drop occurs across the highly resistive stratum corneum (SC). This voltage distribution causes electrical breakdown of the SC. If the applied voltage exceeds a threshold of 75 to 100 V, aqueous micro channels or '*local transport regions*' are created through the breakdown sites of the SC. In modelling studies by *Kushner, et al.,* it has been demonstrated that DBD plasmas applied directly to skin lead to the generation of electric fields with a magnitude of about 100 kV cm^{-1} on the surface and underlying layers of the skin, which far exceeds the threshold voltage needed for electroporation [9]. Further, we have determined that the process of plasmaporation is temporary at certain plasma conditions. For instance, no permeation is observed if the molecule of interest is applied more than 10 min after plasma treatment.

4 CONCLUSIONS

We have demonstrated that non-thermal DBD plasma enables the transdermal delivery of significantly large molecules including lipsomes and nanoparticles across porcine skin in less than a minute without causing any skin damage. Dextran molecules, nanoparticles, liposomes and proteins were delivered to considerable depths in to the skin) which has not been reported elsewhere. We hypothesize that the underlying mechanism of plasma-enabled transdermal delivery is the formation of aqueous pores in the stratum corneum via a reversible 'plasmaporation' process. The depth of permeation and drug concentration can be controlled by controlling various electrical plasma parameters. Non-thermal plasma-enabled skin poration provides a non-invasive, safe means for transdermal delivery and cellular uptake of molecules, drugs and vaccines at room temperature and atmospheric pressure without the pain, skin irritation and other side effects associated with electroporation and other methods. As the application of the method does not require disposable electrodes or needles, the need for disposal of biohazardous waste and illicit reuse of biohazardous consumables is eliminated. An additional benefit of using non-thermal plasma is that of concurrent skin sterilization and plasmaporation.

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