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Programmable Transdermal Clonidine Delivery through Voltage Gated Carbon Nanotube Membranes

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Abstract

Oral dosage forms and traditional transdermal patches are inadequate for complex clonidine therapy dosing schemes, because of the variable dose/flux requirement for the treatment of opioid withdrawal symptoms. The purpose of this study was to evaluate the *in vitro* transdermal flux changes of clonidine in response to alterations in carbon nanotube (CNT) delivery rates by applying various electrical bias. Additional skin diffusion studies were carried out to demonstrate the therapeutic feasibility of the system.

This study demonstrated that application of a small electrical bias (-600mV) to the CNT membrane on the skin resulted in a 4.7-fold increase in clonidine flux as compared to no bias (0mV) application. The high and low clonidine flux values were very close to the desired variable flux of clonidine for the treatment of opioid withdrawal symptoms. Therapeutic feasibility studies demonstrated that CNT membrane served as the rate limiting step to clonidine diffusion and lag and transition times were suitable for the clonidine therapy. Skin elimination studies revealed that clonidine depletion from the skin would not negatively affect clonidine therapy. Overall, this study showed that clonidine administration difficulties associated with the treatment of opiate withdrawal symptoms can be reduced with the programmable CNT membrane transdermal system.

Keywords

Transdermal delivery; Carbon nanotube membrane; Programmable drug delivery; Drug addiction; Clonidine; Diffusion; skin permeation

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1. Introduction

Opioids induce a euphoric state or relieve distress with chronic exposure, resulting in adaptive changes in the central nervous system leading to dependence and addiction¹. Heroin is the most widely abused and fastest acting of the opioid family. In fact, nearly 3.7 million Americans report having used heroin in their lifetime, with over 281,000 treated in clinics annually for opioid addiction². Unfortunately, only 50% of treatment seeking individuals complete the clinic stay and detoxification³. Lack of compliance in the first couple days of treatment can be attributed to the harsh withdrawal effects a heroin-dependent person experiences, including lacrimation, rhinorrhea, piloerection, gastrointestinal cramping, nausea, vomiting, yawning, sneezing, muscle pain, tachycardia, and hypertension⁴. Clonidine has been shown to mitigate these noradrenergic symptoms and thereby aid in opioid abstinence⁵⁻⁸. Unfortunately, dosing schemes are complex and require multiple oral doses throughout the clinic stay³. Transdermal clonidine systems are commercially available and have been applied to heroin withdrawal treatment⁹⁻¹⁴. While currently available transdermal clonidine delivers the drug at a constant rate over a week¹⁵⁻¹⁸ and avoids some of the problems associated with oral dosing, such as gastrointestinal complications and subsequent inadequate dosing, the required variable plasma concentrations of drug during the week long withdrawal treatment period are currently complicated when using traditional transdermal patches. There is a need for a single transdermal patch that can have variable clonidine delivery rates in a compact programmable device.

A new class of conductive permeable membranes based on carbon nanotubes has recently been developed^{19, 20} with dramatic flow properties²¹ that can result in very power efficient pumping with chemical modification²². A 4 fold reduction of flux of a large dye molecule through carbon nanotube (CNT) membranes by the application of an electrical bias has been previously demonstrated by Majumder *et al*²³. However, more recently it was found that the phenomena of electroosmosis and electrophoresis can pump both neutral and charged drugs at 20 fold flux enhancement compared to diffusion with a 40 fold reduction in power compared to conventional membrane materials. This increase in power efficiency would allow a standard watch battery to continuously transdermally deliver high doses of nicotine for 12 days²⁴. In the case of opioid withdrawal treatment, these systems could be programmed and tailored to a specific individual's needs, and furthermore eliminate the difficulties of multi-dose administration associated with patient compliance. Pulsatile delivery has been shown to be achieved across the skin using methods to enhance permeability, such as iontophoresis²⁵, however, the CNT membrane transdermal patch is novel in that it does not use skin enhancement methods with irritation side effects and high power requirements. In this case drugs are to be delivered at a variable rate to the stratum corneum.

This study shows that the incorporation of programmable CNT membranes with a transdermal formulation of clonidine was successful in varying delivery rates of a known variable clonidine treatment regimen for opioid withdrawal. This affords the opportunity to manipulate the flux, and thereby dose of a specific drug to a patient during their rehab stay or in outpatient settings of relapse with counseling. Utilizing programmable membranes

such as these in the drug delivery field would open an avenue for highly effective, one time application patches for therapies that require variable rate delivery and the potential for remote programming in outpatient relapse care with associated telephone or internet counseling. In this study, it was important to demonstrate the therapeutic feasibility of delivering successive variable rates of clonidine through human skin. Additional studies of gel formulation and polymer/adhesive permeation were also conducted for future prototype development goals. The first aim of the present study was to investigate clonidine flux through the skin, and to assess whether skin responds to successive changes in the flux without excessive lag times or depot effects. The second aim was to focus on the drug permeation from solution versus gel formulations, of which the latter is required for reservoir patch drug systems. The third aim was an investigation of high permeability polymer membrane and PSA systems, which will be required for a commercial prototype patch utilizing the CNT membrane. The fourth aim was to investigate the elimination characteristics of clonidine from the skin as this depot of drug will still be delivered to systemic circulation upon deactivation or complete removal of the patch. The fifth and final aim of this study was to show controllable delivery of the drug through the CNT membrane alone and through the CNT membrane/skin system *in vitro* as a function of applied bias.

2. Materials and methods

2.1. Materials

Clonidine HCl and acetonitrile (ACN) were purchased from VWR International, LLC (West Chester, PA). Clonidine HCl was converted to clonidine base. Potassium phosphate monobasic, dibasic potassium phosphate, sodium chloride, ammonium bicarbonate, ammonium hydroxide, and hydrochloric acid (HCl), was purchased from Fisher Scientific Inc (Fairlawn, NJ). Hydroxyethylcellulose (HEC; Natrosol®) was used as the gelling agent for donor solutions as well as for contact gel between the skin and CNT membrane and was obtained from Hercules Inc (Wilmington, DE). Deionized distilled water was generated from the Barnstead Nanopure Diamond™ system (Dubuque, IA).

2.2. Fabrication and functionalization of CNT membranes

Multi-walled carbon nanotubes (MWCNTs) with an average core diameter of ~7 nm were fabricated on quartz substrate via a chemical vapor deposition (CVD) approach using ferrocene/xylene as the feeding gas¹⁹. The length of MWCNTs ranges from 100 to 150 microns as measured by DekTak Profilometer. In the next step, MWCNTs were mixed thoroughly with Epon 862 epoxy resin (Miller Stephenson Chem. Co.), surfactant Triton-X 100 (Sigma Aldrich), catalyst 1-Cyanoethyl-2-ethyl-4-methylimidazole (2E4MZ-CN, Shikoku Chemical, Japan) and hardener methylhexahydrophthalic anhydride (MHHPA, Broadview Tech. Inc.) using a Thinky™ Mixer. The appropriately-cured CNTs-Epoxy composite was cut into ~5 μm thick membranes using a microtome equipped with a glass knife. The residual epoxy on the tips of CNT was removed via a following H₂O plasma oxidation. Double-walled CNT (~2 nm core diameter, Cheaptubes, Inc) membranes were prepared using a similar method as described above. Porosities of both MWCNT and DWCNT membranes were screened by ion diffusion flux. Individual membrane samples were chosen with highest porosity (~.01%) and within 20% variation. The as-cut CNT

membranes were functionalized using a 2-step process. In the first step, CNT were grafted with benzoic acid through electrochemically reducing 5 mM 4-carboxy phenyl diazonium tetrafluoroborate in 0.1 M HCl and 0.1 M KCl electrolyte at -0.6 V for 4 minutes²³. The diazonium compound was synthesized following a method reported by Belanger et al²⁶. The benzoic acid was then coupled to Direct Blue 71 dye via a carbodiimide coupling reaction. Specifically, 10 mg 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (EDC, 98%, Aldrich) was added to 4 mL of 50 mM Direct Blue 71 dye in 0.1 M 2-N-morpholino ethanesulfonic acid (MES, 99%, Sigma) buffer, and the reaction lasted for 12 h at ambient temperature, after which the membrane was rinsed thoroughly using 0.1 M MES buffer, 0.1 M KCl solution and deionized water in order to remove un-reacted chemicals.

2.3. Human skin preparation

Human skin harvested during abdominoplasty was used for the transdermal clonidine delivery studies and obtained from the Cooperative Human Tissue Network (CHTN). Human tissue use was approved by the University of Kentucky Institutional Review Board. Skin sections were prepared using a Padgett dermatome set to 250µm and then stored at -20°C until use. Stored skin samples were thawed to room temperature at the time of the experiment.

2.4. Clonidine skin studies

A PermeGear flow-through (In-Line, Hellertown, PA, USA) diffusion cell system with a diffusion area of 0.07 cm² was used for all *in vitro* skin diffusion studies. Skin surface was maintained at 32°C with a circulating water bath. Stratum corneum integrity was checked before all experiments using TEWL instrumentation (RG1 Evaporimeter, cyberDERM Inc., Broomall, PA). Donor solutions for all skin diffusion studies were clonidine in water. Donor concentrations were varied based on the study being conducted and are mentioned in the respective sections. The receiver solution was 0.9% saline with 10% ethanol and flowed at a 7ml/h rate. Samples were collected every 3 hours and the diffusion study was conducted for 24 h unless otherwise stated in the sections below. All samples collected were stored at 4°C until analyzed by HPLC.

2.4.1 Determination of maximum flux—Dermatomed human abdominal skin sections were loaded into the diffusion cells, and varying concentrations of clonidine solution (325µg/ml and 950µg/ml) were applied directly to the skin. Frequent sampling of the receiver solution was done for 24 h and samples were analyzed by HPLC.

2.4.2 Flux variation studies—For flux variation study, dermatomed human abdominal skin was loaded into the flow through diffusion apparatus, and a drug solution at a given concentration was applied to the prepared skin area in the diffusion cell and a steady-state flux was obtained over the first 24h dosing period. Next the donor solution was removed and the donor compartment and skin surface was quickly (less than one minute) rinsed with 2ml of water. The skin was then immediately charged with a new donor concentration and the new steady state flux was obtained over the next 24h dosing period. This procedure was repeated until the desired rate changing profile was completed. Clonidine concentrations to show flux variation included 75, 137 and, 205µg/ml. The studies were carried out in 4

diffusion cells (n=4) for each drug concentration utilizing skin from the same donor. Lag times were obtained by extrapolating the steady state portion of the cumulative profiles to the x-axis. Transition times, time it takes to reach the new steady state were ascertained from these experiments.

2.4.3 Liquid versus gel formulation investigation—For an investigation of the effects of gel formulations on flux, each diffusion cell was charged with either 500µl of liquid donor, or a gelled donor of the same concentration after human skin was loaded into the cells. HEC was used as the gelling agent. A 1.5% w/w HEC was chosen based on visual examination and consistency for what could be used in a transdermal patch or applied topically for future *in vivo* studies. A drug concentration of 894µg/ml, which is below the saturation of the drug, was chosen as the investigational formulation.

2.4.4 High permeability polymer and contact adhesive studies—In this study, several polymer and pressure sensitive adhesive (PSA) systems were investigated. Specifically, Medifilm™ 390, 437 and 810 co-polyester from Mylan® Technologies were each investigated with Bio-PSA® 7-4303, a silicone adhesive from Dow Corning. These polymer membranes and adhesive combinations were applied to human skin and clonidine donor solution at a concentration of 777µg/ml was loaded into each diffusion cell.

2.4.5 Skin elimination studies—Dermatomed human abdominal skin was loaded into the flow through diffusion apparatus, and clonidine donor solutions at a concentration of 117, 154, and 400µg/ml (n=4 for each drug concentration) were applied to the skin. The study was allowed to run long enough to reach steady state, when the donor solution was removed and the diffusion cell chamber and skin were briefly washed with water. Receiver solution was sampled every 3 hours during the time to reach steady state, and every hour during the depletion phase. All data was analyzed by HPLC and mathematical modeling of the profiles was conducted using Graphpad Prism® (La Jolla, CA) software. The kinetics of elimination were obtained based on equation 1 where X_0 is the time at which the elimination from the skin begins and expressed in units of time, and Y_0 is the average amount permeated at steady state up to the point at which elimination begins or drug is removed from the skin. The plateau (P) is the Y value at infinite time and the span is the difference between Y_0 and plateau. k is the rate constant and expressed in units of inverse time.

$$Y = P + (Y_0 - P) \times e^{(-k(X - X_0))} \quad 1$$

2.5. CNT membrane studies

All clonidine studies involving CNT membranes on human skin were carried out using the PermeGear flow-through diffusion apparatus as used for skin diffusion studies above except that it was equipped with three electrodes in order to apply bias on CNT membrane (see Figure 1). Electric potential was applied by a eDAQ model EA161 E-corder Potentiostat (eDAQ, Denistone Australia). CNT membrane, Ag/AgCl in saturated KCl solution and platinum wire were used as working, reference and counter electrodes, respectively. The CNT membrane is conductive with a resistivity of ~100 ohm-cm. The area of CNT

membrane was 0.07 cm^2 , and the distance between working and counter electrodes was $\sim 3 \text{ mm}$. For CNT membranes alone (no skin), a 10ml Franz cell with a 3mm diameter orifice was utilized and 0.5 ml aliquots of receiver solution was analyzed for clonidine concentration as a function of time and applied bias. Electric potential was applied by a Potentiostat (Princeton Applied Research, Oak Ridge, TN, USA).

2.5.1. Clonidine delivery through CNT membrane—This study assessed diffusion of clonidine through CNT membrane alone. For the MW CNT membrane, all the experimental setup was as described above except that no skin or gel was used. Donor solution was $255 \mu\text{g/ml}$ clonidine in water. The receiver solution was 0.9% saline with 10% ethanol and a 0.5 ml aliquot was removed (and replaced with receiver solution) every 6 h. A positive 300mV bias was applied for 48h followed by a negative 300mV bias for an additional 48h without dismantling the diffusion setup to show that clonidine flux can be controlled by applying different bias to CNT membrane. Clonidine delivery through DWCNT was carried out in a 3.5 ml Franz cell. The donor solution was $700 \mu\text{g/ml}$ clonidine in 50 mM phosphate buffer, and saline solution containing 10% EtOH was used as the receiver solution. Half a ml of receiver solution was drawn at each time point.

2.5.2. Clonidine delivery through CNT membrane and skin—This study was carried out to assess the diffusion of clonidine through CNT membrane/gel/skin system after application of different bias. The bias sequence for these studies ($n=3$) was 0mV for 24h followed by a negative 600mV bias for the next 24h and finally a 0mV bias for an additional 24h (OFF/ON/OFF). Samples were collected every 4 hours and analyzed by HPLC. The receiver solution was 0.9% saline with 10% EtOH and was used at a flow rate of 1ml/h. The donor solution was 70% saturated ($700 \mu\text{g/ml}$) with clonidine in 50 mM pH 7 phosphate buffer. Ten mg of a gel containing 2% HEC in 0.2 M ammonium bicarbonate/ammonium hydroxide buffer pH 9 was used to provide good contact between the skin and CNT membrane. One additional study ($n=1$) with a different bias sequence was also carried out. In this study, a negative 600mV bias was applied for 24h followed by a 0mV bias for another 24h and again a negative 600mV bias for the next 24h (ON/OFF/ON) under the same experimental conditions as above. It is important to note that the epoxy matrix used to form CNT membranes is impermeable to clonidine and there is an exceedingly small volume in the open CNT pores ($\sim 10^{-5}$ microliters or $2 \times 10^{-8} \mu\text{g}$ of clonidine). Thus we are able to reuse membranes without any measurable artifact and have the benefit of eliminating device to device variation

2.6 Quantitative analysis

All clonidine samples were analyzed by an HPLC consisting of a Waters (Milford, MA) 717plus autosampler, a Waters 1525 Binary HPLC pump, a Waters 2487 dual absorbance detector set at a wavelength of 215nm and utilizing Waters Breeze™ software. Additionally, a Brownlee (Wellesley, MA) C-18 reversed-phase Spheri-5 μm column ($220 \times 4.6 \text{ mm}$) with a C-18 reversed phase $7\mu\text{m}$ guard column ($15 \times 3.2 \text{ mm}$) by Perkin Elmer® was used. The mobile phase consisted of 50mM ammonium bicarbonate adjusted to a pH of 9.5 with NH_4OH (with 5 %w/w ACN):ACN (60:40) and flow rate of 1.0ml/min. The injection volume was 35 μl , runtime of 6 minutes, and the retention time of clonidine was 4.8 minutes.

To detect the low clonidine concentrations, all samples were required to be concentrated by nitrogen (N_2) evaporation of a 0.5ml of sample to dryness, and then reconstituted in 100 μ L of ACN for a five-fold concentration. Calibration plots were prepared using clonidine standards with the final concentrations over a range of 25-1500 ng/ml. The correlation coefficient (r^2) obtained was 0.99 for standard curves. The lower limit of quantification (LLOQ) was 10 ng/ml.

2.7. Data analysis

The cumulative quantity of drug collected in the receiver compartment was plotted as a function of time. The flux value for a given experiment was obtained from the slope of the steady state portion of the cumulative amount of drug permeated plotted over time.

Statistical analysis of the *in vitro* data was performed by one-way ANOVA followed by Tukey's post-hoc analysis using SigmaStat (SPSS Inc., Chicago, IL). The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Prototype patch design

A schematic of the prototype commercial patch design containing CNT membrane can be seen in Figure 2.

This design consists of an occlusive patch cover, a gelled drug reservoir that sits atop the CNT membrane, followed by a contact polymer/adhesive layer allowing application to the skin. This layer consists of a high permeability polymer and a PSA. The polymer layer has no rate controlling properties in slowing the operation of the transdermal device. It merely provides close skin contact and structural support for the CNT membrane in addition to protecting the skin surface from any inadvertent irritation from the CNT membrane or applied electrical bias. This gives the CNT system an added advantage over iontophoresis in that no irritation will result from current flowing through the skin. The bias required for control of the flux will be applied only to the membrane surface through a series of electrodes attached to a watch battery within the patch device.

As explained earlier, the experimental setup for the study of clonidine delivery through CNT membrane/skin system in the current research utilized a PermeGear® flow-through diffusion apparatus shown in Figure 1. The contact polymer/adhesive component of the commercial patch was replaced with a contact gel (2% w/w HEC in ammonium bicarbonate buffer pH 9) in this study so that the membrane could be removed from the skin without damage and be reused for further studies. Additionally, liquid donor solutions were used in the experimental setup in order to monitor and prevent drug depletion for the prolonged studies. It is shown below in detail that gelled formulations do not significantly ($P > 0.05$) affect clonidine drug flux through the skin as compared to liquid formulations.

3.2 Clonidine skin studies

3.2.1 Determination of maximum flux—It is important to ascertain whether or not the clonidine flux achieved when a negative bias is applied during the ON state of the CNT is in

fact deliverable through the skin. The required flux values to be delivered during the ON state are those needed for prevention of withdrawal symptoms based on the oral or buccal bioavailability and desired plasma concentrations of current medications for therapy. The required ON and OFF flux values were determined as follows. For a traditional five-day opioid withdrawal symptom treatment regimen using clonidine, the first and fourth days require a total of 0.2mg per day of oral clonidine, days two and three a total of 0.3mg per day, and the fifth day 0.1mg of oral clonidine³. Based on a patch size of 10cm², 100% bioavailability²⁷, and each day's oral dose over a 24 hour period, effective transdermal flux values of 3.5, 5.4, and 1.8 nmoles/cm²/h are required (Table 1).

The maximum desired flux for opiate withdrawal symptom treatment was obtained by utilizing a 325µg/ml clonidine solution yielding a flux of 5.3±0.16 nmoles/cm²/h. This concentration is well below the saturation of clonidine (1mg/ml). The transdermal flux obtained from a 950µg/ml concentration was 33.7±3.03 nmoles/cm²/h. Thus, the maximum level of passive permeation for a 95% saturated drug solution through the skin was far beyond the therapeutic delivery rate requirements for the standard 5-day drug rehab oral regimen shown in Table 1. Obtaining flux beyond the therapeutic requirements demonstrated that the membrane would act as the rate limiting step upon incorporation into the system, a characteristic essential to the membrane's success as a variable transdermal patch component.

3.2.2 Flux variation studies—This work first required determining that a change in transdermal flux could in fact be observed through human skin. In other words, should the drug experience a large enough depot effect in the skin that observable flux transitions were not feasible, it would not be possible to develop a variable dosing transdermal device without skin enhancer technology and their associated skin irritation problems. Clonidine flux changes through human skin are shown in Figure 3a. It is evident from this figure that significantly different flux was obtained through the same skin area throughout the five day treatment period when differing donor concentrations were placed on the skin in a consecutive fashion. The desired profile for clonidine administration of a moderate dose for the first day, to an increase in delivery rate for the second and third days, and then a tapering off to the end of the therapy was obtained. All flux values were significantly different ($p<0.05$, $n=3$, t-test, SigmaStat) than the flux from the dose administered prior to it. Figure 3b shows that the flux in Figure 3a is proportional to donor concentration, thus clonidine provides a standard transdermal system for simple programmed dosing. The time it takes to reach steady state delivery and the time it takes to reach a new steady state after the application of a new dose are termed lag and transition times, respectively and are displayed in Table 2. The lag time for clonidine was 1.64 ± 1.42 h and the transition time from day 1 to day 2 was 6.3 ± 0.5 , from day 3 to 4 was 7.3 ± 1.5 , and day 4 to 5 was 8.8 ± 2.1 h. Although these transition times are several hours, it actually will prove advantageous for clonidine therapy, as the five day treatment calls for tapering doses, not immediate change in dose. Together this data proves that variable rate delivery was possible through human skin with flux proportional to donor concentration above the skin and acceptable lag and transition times. Therefore it is determined to be therapeutically feasible to continue

developing a programmable clonidine drug delivery system, in this case using CNT membranes.

3.2.3 Liquid versus gel formulation investigation—A reservoir transdermal patch system requires the use of a gelled solution of the drug. For this reason, we found it important to confirm that the gelling agent, HEC, as well as the physically gelled matrix of the donor solution would not inhibit diffusion as compared to that of a liquid donor. Table 3 shows the results of these studies with a donor concentration of 894 ug/ml. As can be seen, there is no statistically significant difference ($p > 0.05$) between clonidine permeation through skin based on a liquid or gelled drug donor.

3.2.4 High permeability polymer and contact adhesive studies—As shown in Figure 2, a prototype marketed patch system incorporating CNT membrane will make use of a high permeability polymer and contact adhesive. For this reason, several polymer/PSA systems were investigated to find the least inhibitive pairing for clonidine permeation. All polymers were 1 mil thick. The results of this study using a donor solution of 770 $\mu\text{g/ml}$ can be seen in Table 4. These selections represent a sampling of the different types of membranes and adhesives commercially available. Clearly the combination of Medifilm™ 390, co-polyester from Mylan® Technologies, and Bio-PSA® 7-4303 a silicone adhesive, from Dow Corning, was found to be the least resistant to diffusion and the same flux could be obtained as that through the skin alone. Accounting for the donor concentration difference, the skin alone flux would be 30 $\text{nmol/cm}^2/\text{hr}$, which is very close to the best polymer/skin case of 24 nmol/hr/cm^2 . Hence, this combination of polymer/PSA system can be chosen for the future patch design. It is important to note that this delivery device design requires that the CNT membrane be the rate limiting step and that diffusion through the polymer barrier/adhesive and skin be faster than the CNT membrane pumping rate. In the case of increasing pH for gel contact, the diffusion in the gel would be nearly identical to bulk solution diffusion but there would be faster transport through skin at the higher pH, further helping this system keep the CNT membrane as the rate limiting step.

3.2.5 Skin elimination studies—Experiments to investigate the kinetics of clonidine elimination from the skin were conducted as the drug that depots in the skin during the patch activation will still be delivered to the body after deactivation or removal of the system. This would limit the effectiveness of a device switching to a low dosing stage of treatment. Clonidine elimination was tested at three different concentrations and the profiles of each are shown in Figure 4. The kinetic parameters, summarized in Table 5, were obtained based on equation 1. The rate constants for each concentration were not statistically different ($p > 0.05$, One-way ANOVA, SigmaStat) and therefore elimination of clonidine from the skin was a first-order process. The half-life was on the order of 1hr and transition times were consistent with dosing increase/decrease of Figure 3. Though complete elimination from the skin takes at least six hours, this should not affect opioid withdrawal symptom treatment as it does not require pulsatile delivery. Furthermore, the treatment plan calls for a tapering dose on the final days of the therapy and a slow skin elimination of clonidine will aid in this after the system is removed.

3.3. CNT membrane studies

3.3.1 Clonidine delivery through CNT membrane—To demonstrate that the clonidine flux change through a CNT membrane is sufficient with the application of an electrical bias, permeation studies were conducted on a MWCNT and DWCNT membranes alone. Figure 5a shows the change in flux of clonidine through the MWCNT membrane when the applied bias (CNT membrane as working electrode) was switched from a positive 300mV to a negative 300mV. The positive bias state is when the membrane is deactivated and, depending on the therapy, delivers no drug or, in the case of clonidine therapy, is operating at its first low steady state level. The negative bias pumps the drug through the CNT membrane at a high steady state level by a combination of electroosmosis and electrophoresis²⁸. When the donor solution was changed to pH 10 from pH 6, the ‘on-state’ flux was reduced by a factor of 2.9, presumably from the loss of the electrophoresis component of the protonated clonidine. To improve electroosmotic flow and pumping efficiency an anionic dye was grafted to the entrance of CNT pores. This allowed preferential flow of cations with the electric field generating electroosmotic flow.

As shown in the figure, clonidine low and high flux values through the CNT membrane alone were 3.48 and 50.4 nmoles/cm²/h, respectively. The low flux 3.48 nmoles/cm²/h matches the desired clonidine delivery rate on the first day of treatment (Table 1). However, the high/low clonidine flux ratio (ON/OFF ratio, 14.5) far exceeded the required flux for clonidine therapy and can be tuned with the application of lower negative bias or reduction in patch size. It should be noted that the flux ratio can be expected to be lower in a skin/gel/CNT membrane system having two extra diffusional barriers compared to the CNT membrane alone and can be modeled as a diffusion in series system²⁴. Nevertheless, this study proved that variable clonidine delivery rates can be achieved through the CNT membrane above therapeutically useful levels.

Figure 5b shows the change in flux of clonidine through the DWCNT membrane when the applied bias was switched from a positive 600mV to OFF and then to a positive 600mV again. It can be clearly seen that the flux of clonidine after a positive 600 mV bias was increased, decreased after 0V, and increased again after application of 600 mV bias.

It is important to note that the transition time to reach the new flux upon negative bias application was less than thirty minutes proving that the membrane responds quickly or nearly instantaneously to a bias change and should not add greatly to the transition time seen in the previous skin studies. Furthermore, the negative bias flux is nearly thirty times greater than the desired unbiased flux (1.7nmoles/cm²/h) for clonidine therapy. This enhanced pumping is due to electroosmosis effects in conjunction with electrophoresis of cationic clonidine. The electroosmotic driving of drug molecules through the membrane could be highly advantageous in future work in that drug delivery to the skin greater than originally hypothesized with simple closing of CNTs with bias²³ allowing more compact devices.

3.3.2 Clonidine delivery through CNT membrane and skin—The permeation profile of clonidine through the CNT membrane, gel contact layer and skin with bias application is presented in Figure 6 and the corresponding flux, and ON/OFF flux ratios are summarized in Table 6. In this case the smaller DWCNTs were used due to more power

efficient electroosmotic pumping²⁴ and higher porosity. Figure 6A shows a representative figure of the flux changes when 0mV, -600mV and 0mV bias was applied in series every 24 h. The negative bias of -600mV was chosen to provide the target flux, and minimize the transition or lag time to reach the new steady state flux once the system has been switched on. The average high and low flux values obtained from 3 studies were 5.1 ± 2.4 and 1.1 ± 0.6 nmoles/cm²/h, respectively, when the bias was switched from 0mV to -600mV. These values are very close to the required lowest and highest therapeutic flux of clonidine for the treatment of opioid withdrawal symptoms (Table 1). The average ON/OFF flux ratio obtained for this set of bias studies was 4.69 ± 0.87 , which slightly exceeded the demand of 3.0 for the clonidine therapy. The flux values and ON/OFF ratio obtained when -600 mV was switched back again to 0mV was also very consistent with the desired values. One additional study was conducted with different bias sequence (ON/OFF/ON) to make sure that the obtained change in flux was not an artifact. As shown in Figure 6B and Table 6, the flux responded to a change in bias from -600mV to 0mV and again to -600mV. These studies proved that the CNT membrane transdermal device would provide programmable alternate flux values of clonidine for opioid withdrawal symptom treatment. The increased flux of clonidine through the CNT membrane/skin system under electric field is primarily due to electrophoresis through the rate limiting CNT membrane since clonidine would be 90% charged at pH 7 of the donor solution. The gel was buffered at pH 9 to neutralize clonidine after passage through CNT membrane and ensure the high skin permeability that was previously observed. No quantifiable damage to skin at pH 9 was seen after 5 days and the ability to have steady state off rates at beginning and end of the series for figure 6 A & B is further evidence of skin barrier retention. However, we did find that going to pH 9.6 resulted in quantifiable damage to skin and that condition was not used in this study.

Since CNT membranes have different degrees of porosity due to blocking by the catalyst particle during the fabrication process, the flux differences between the membrane samples is the largest source of variation in our measurements. By choosing a membrane with unbiased flux close to the lowest therapeutic delivery rate of clonidine would help achieve the desired target flux. This production process can be automated by measuring ionic current through CNT membranes before incorporation into devices. The necessary ON/OFF flux ratio can be simply achieved by tuning the bias to be applied during the on-state. It should be noted that both MWCNT and DWCNT membranes show electrophoretic and electroosmotic pumping with the smaller DWCNTs showing higher on off ratios. In both cases the on/off ratio and maximum flux rates exceed what is needed for therapy and are used in this study. The power efficiency of electrophoretic/electroosmosis through the CNT membrane is 40 fold improved compared to other membrane materials. This allows watch batteries to last 12 days at continuous pumping, which is an important consideration for compact low power devices

The size of the patch required for a therapeutic flux of clonidine (Table 1) is 10 cm². This estimation is assuming that only one CNT disk is to be utilized. One can change the size of the patch by utilizing multiple CNT membranes in the new CNT patch system and it is in our future plan to fabricate flexible, larger, and more permeable CNT membranes for commercial patch design. Another way to decrease the required patch size would be to

increase the delivery rate through the CNT membrane and skin by manipulating the pH of the clonidine formulation and the contact gel, respectively.

4. Conclusions

This study successfully demonstrated the programmed transdermal delivery of therapeutically useful flux values of clonidine using switchable CNT membranes for the treatment of opioid withdrawal symptoms. Therapeutic feasibility studies showed that CNT membrane was the rate limiting step in the delivery of clonidine. The ability to alter flux through the skin was a critical conclusion in these therapeutic feasibility studies and it was demonstrated that the skin permits an observable change in flux for clonidine with different donor concentrations at the skin surface. Lag and transition times though longer for clonidine, will not inhibit proper therapy, and are suitable for the described therapy. Skin elimination studies revealed that clonidine was depleted from the skin by first order kinetics, and that the rate obtained would not negatively affect opiate withdrawal therapy. Furthermore, for prototype development, it was shown that using a gel formulation will not significantly inhibit diffusion of drug through the skin and that Medifilm® 390 and the silicone adhesive BIO-PSA 7-4303 system had the least influence on transdermal diffusion. In other words, diffusion through CNT membrane, PSA and skin were comparable to flux through the skin only at the same concentration. A low-power, compact, and programmable clonidine delivery device can have important application in opioid withdrawal treatment with single use application for improved patient compliance. The potential for remote programming can aid with relapse treatment or counseling based therapy.

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Figure 1. Schematic of modified PermeGear In-Line diffusion cell for transdermal flux analysis modified to apply voltage to CNT membrane for variable drug delivery rate. This schematic is modified from www.permegeard.com (Use permission pending)

Figure 2. Schematic of future transdermal patch system. Bias to the membrane can be applied by electrodes and a small watch battery attached to the CNT membrane with a counter electrode in the gelled drug solution.

Figure 3.

a. Flux variation for clonidine. Concentrations applied are indicated atop each flux bar. All dose fluxes ($n=3$ skin samples from the same donor) were significantly different than the dose administered prior to it except day two and three as expected ($P<0.05$, Student's t -test, SigmaStat). **b.** Flux as a function of donor concentration.

Figure 4.

Clonidine depletion from the skin. Various concentrations of clonidine were applied for approximately 24h to achieve steady state and then removed. Sampling was continued for an additional 16h to obtain the depletion graphs of (a) 400µg/ml (b) 154µg/ml (c) 117µg/ml. n=4 samples from the same donor for each concentration applied.

Figure 5.

- a.** A representative permeation profile for applied bias to CNT membrane. A positive bias (300mV) was applied for 48h and then a negative bias was applied immediately without dismantling the apparatus for an additional 48h.
- b.** Cumulative clonidine release from DWCNT membrane after applying various biases (600mV/0mV/600mV).

Figure 6.

A representative permeation profile of clonidine through CNT membrane and skin after application of different bias sequences. A. A 0mV, a negative bias (-600mV) and finally a 0mV bias was applied each for 24 h (OFF/ON/OFF). B. A negative bias (-600mV), 0mV, and finally -600mV bias was applied each for 24 h (ON/OFF/ON).

Table 1

5-day oral clonidine dosing regimen for treatment of opioid withdrawal symptoms and the corresponding required transdermal flux

Day	Oral Dose (24h) *	Transdermal Flux Required** (nmoles/cm ² /h)	Required highest to lowest Flux ratio (ON/OFF ratio)
1	0.2mg	3.5	
2	0.3mg	5.4	3.0
3	0.3mg	5.4	
4	0.2mg	3.5	
5	0.1mg	1.8	

* Oral doses are multiple 0.1mg tablets (Day 1 two oral tablets administered, day 2 three oral tablets, etc.)

** Transdermal flux based on 100% oral bioavailability, 10cm² patch, and each day's oral dose to be distributed transdermally over 24h.

Table 2

Lag and transition times for clonidine

Day	1	2	3	4	5
Transition Time (h)	1.64 ± 1.42	6.3 ± 0.5	N/A	7.3 ± 1.5	8.8 ± 2.1

n=3 skin samples from the same donor.

Table 3

Liquid and gel formulation comparison for clonidine

Concentration	894 µg/ml	
	Liquid	Gel [*]
Average Flux (nmol/cm ² /h)	35 ± 3.0	34 ± 8.3

* 1.5% w/w of HEC was utilized. (n = 4 skin samples from the same donor, P>0.05, student's t-test)

Table 4

Clonidine diffusion through high permeability polymer and PSA applied to skin

Medifilm™/PSA	390/ 7-4303	437/ 7-4303	810/ 7-4303
Flux (nmoles/cm ² /hr)	24.2 ± 1.71	13.8 ± 3.58	4.82 ± 2.49

n=3 skin samples from same donor per polymer/PSA investigated

Table 5

Modeling parameters for skin elimination after various clonidine concentration exposure

Concentration ($\mu\text{g/ml}$)	400	154	117
X_0 (h)	17.88 ± 0.19	23.70 ± 0.36	23.74 ± 0.28
Y_0 (μmol)	0.1011 ± 0.0073	0.00718 ± 0.00048	0.0070 ± 0.0005
P (μmol)	$0.00038 \pm .00048$	0.000715 ± 0.00040	0.00103 ± 0.00033
k (h^{-1})	0.871 ± 0.339	0.509 ± 0.165	0.774 ± 0.340
Half Life (h)	0.796 ± 0.223	1.361 ± 0.233	0.895 ± 0.273
Span (μmol)	0.1007 ± 0.0088	0.00646 ± 0.00061	$0.00597 \pm .00059$

Model from GraphPad Prism[®] software. n=4 skin samples from the same donor per concentration investigated.

Table 6

Flux values, and ON/OFF flux ratios obtained after applying various bias through CNT membrane and skin

Applied bias*	0mV flux ^c 1st	-600mV flux 1st	0mV flux 2 nd	-600mV flux 2 nd	ON/OFF ratio 1st	ON/OFF ratio 2 nd
OFF/ON/OFF ^a	1.1 ± 0.6	5.1 ± 2.4	1.1 ± 0.3	-	4.69 ± 0.87	4.72 ± 1.51
ON/OFF/ON ^b	-	19.2	2.4	13.6	8	5.6

* ON bias= -600mV; OFF bias=0mV

^a n=3;

^b n=1

^c All flux values are expressed in nmoles/cm²/h.