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Programmable carbon nanotube membrane-based transdermal nicotine delivery with microdialysis validation assay

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Abstract

To evaluate the performance of switchable carbon nanotubes (CNT) membrane devices for transdermal nicotine delivery, we have developed an *in-vitro* microdialysis method that allow us to detect variable transdermal fluxes of nicotine through CNT devices and can be applied directly to *in-vivo* studies. Microdialysis membranes were placed beneath the porcine skin and its nicotine levels increased 6-8 times when the CNT membrane on skin was turned from OFF to ON state by application of bias. Fluxes in the ON state were approximately 3 times that of commercial nicotine patches and switching times were less than two hours, thus suggesting the improved therapeutic potential of our device. Blue tooth enabled CNT devices that can be programmed by smartphone and coupled with remote counseling application for enhanced smoking cessation treatments.

Key words: Microdialysis; CNT membrane; Nicotine; Smoking cessation; Remote counseling

Addiction treatment is a particularly difficult challenge due to the combination of complex brain physiology and psychiatric behavioral cues involved. To address brain physiology in smoking cessation treatment, nicotine replacement therapy (NRT) uses transdermal patches for steady background levels for reducing withdrawal symptoms and relapse, and nicotine gum for dosing spikes for craving events has had limited success rate of 18–24% after 1year[1,2] and an overall 90% long term failure rate.[3] The major disadvantages of currently available transdermal patches include non-variable nicotine delivery rate to respond relapse cues,[4] and underdosing for heavy smokers.[5–7] Attempts to increase patch loading result in nicotine toxicity especially in heavy smokers with psychiatric comorbidity in response to continued smoking.[6] Nicotine gum to deliver nicotine dose spikes is generally self-dosed and perpetuates nicotine addiction.

Psychological counseling along with NRT is the most effective (~40% success rate without 2-year relapse) but most costly in terms of professional staffing and patient time away from work.[8,9] Less expensive remote counseling based on phone/Internet can be used in conjunction with NRT with somewhat encouraging results (~14.9% phone and 18–23% Internet counseling success rates at 6 months).[9–12]

A significant improvement to smoking cessation therapy, would be to remotely program nicotine dosing levels and nicotine spikes with input from counselor and patient through a smart phone platform along with a smart phone application (App) for remote counseling. Although iontophoresis transdermal drug delivery has the capability to provide variable and programmable delivery rates, it requires strong current across the skin, which causes skin irritation,[13] need bulky external power source[14] and polarization within the skin reduces the current across skin over time affecting dosing.[15,16] A new technology based on switchable carbon nanotubes (CNT) membrane can provide variable nicotine dosing (in therapeutic range) by using a small bias of compact watch battery. Due to dramatic nanofluidic properties[17] and highly efficient electroosmotic pumping,[18] watch battery devices can readily have operational pumping time of up to 10 days.[19] Unlike iontophoresis, applied bias to the CNT membrane is contained within the device and controls the drug

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transport rate to the skin surface at programmed dosing level. A smart phone counseling program coupled to a bluetooth controlled voltage source on the CNT membrane device can release nicotine at variable rates giving a promising approach for smoking cessation treatment (Figure 1).

Previously, we have demonstrated that it is possible to obtain programmable transdermal drug delivery of nicotine at therapeutic levels through *in-vitro* human skin and *in-vivo* with hairless guinea pigs by using CNT membrane based device.[19,20] The previous *in-vivo* study did not show the true potential of this device as the ON/OFF nicotine flux ratio (~2 times) did not meet the desired nicotine target (3.6 times) corresponding to large and small patch sizes used at beginning and end of NRT program. This *in-vivo* study had minimal temporal resolution of cotinine measurements due to limitations of blood sampling intervals required for animal's well-being.[20] Also nicotine levels were not directly measured due to the rapid conversion of nicotine to cotinine making it difficult to analyze multiple ON/OFF cycles since cotinine clearance kinetics in hairless guinea pigs are not accurately known. For switchable transdermal devices *in-vitro* experimental designs that accurately quantify multiple ON/OFF cycles and directly measure nicotine delivery across the epidermis are needed.

Microdialysis membrane probe implanted in dermis can collect drug diffusing from the patch into dermis of skin in a continuous manner without blood sampling. This is a well-established technique and has been used in dermal pharmacokinetic studies of many drugs in human and animals.[21–23] Various studies have successfully quantified transdermal patch nicotine delivery using microdialysis membrane in animals[24] and human volunteers.[22,25] Critically this technique can provide direct measurement of nicotine skin permeation with high temporal resolution and overcome the uncertainty associated with metabolite clearance kinetics. Demonstrated here is an efficient *in-vitro* microdialysis technique to measure variable therapeutic transdermal fluxes from a switchable device that can be directly applied to *in-vivo* studies. CNT membranes are also shown to have improved ON/OFF ratios and can exceed fluxes given by commercial nicotine (Nicoderm) patches.

Methods

Fabrication and characterization of double-walled carbon nanotubes (DWCNT) membranes

DWCNT with average inner diameter of 1.3-2 nm and length of 50 μm were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). A JEOL 2010F Transmission Electron Microscope (TEM) was used to see the diameter of as-purchased DWCNT. DWCNT membranes were fabricated as reported previously.[19,26] Briefly after a sonication/dispersion step, 2.5 wt% DWCNTs were mixed into Epon 862 epoxy resin (Miller-Stephenson Chemical Co., IL, USA), hardener methyl hexahydrophthalic anhydride (MHHPA, Broadview Tech. Inc., NJ, USA) and 0.1 g surfactant Triton-X 100 (Sigma, St. Louis, MO, USA) using a Thinky™ (Tokyo, Japan) centrifugal shear mixer. As-prepared CNT–epoxy composite was cured at 85 °C before being cut into CNT membranes (5 μm thick) using a microtome equipped with a glass blade. These as-made membranes were characterized for thickness by S-4300 HITACHI Scanning Electron Microscope (SEM). The membranes (~0.6 × 0.6 cm^2) were glued over 3 mm diameter hole in polycarbonate plate (1 mm thick) covered with nylon mesh (0.8 × 0.8 cm^2), which act as a mechanical support. Membrane area was 0.07 cm^2. The membranes were screened for ionic current separating two sides of a U-tube cell each with 0.1 M KCl and Ag/AgCl electrode (*In Vitro* Metric, E215P, Healdsburg, *CA*, USA) as working and connected reference/counter electrode (R.E/C.E.). A constant potential of −0.6 V was provided by e-corder 410 (E-daq potentiostat, Colorado Springs CO, USA) and current was monitored. Only CNT membranes with screening current between −6 and −8 μA (−0.6 V) were used in further studies. Each side of successfully screened membrane was treated using water plasma oxidation for 1 min to add carboxylate functionality for chemical modification.

Porosity of DWCNT membranes was determined from the steady state $Ru(bi\text{-}Pyr)_3Cl_2$ (5 mM feed) flux through the plasma oxidized membrane from using Ficks law of diffusion.[27] The permeate was measured after 24 h of diffusion experiments using a UV–Vis spectrophotometer (BioTek Synergy H1 Hybrid reader, BioTek Instruments, Inc., Vermont, USA) and calculated at 286 nm.

To functionalize as-prepared DWCNT membranes they were first flow grafted with benzoic acid using 4-carboxy phenyl diazonium tetrafluoroborate synthesized in our laboratory using the literature reported method,[28] which was then coupled to Direct Blue dye 71 using carbodiimide chemistry as reported previously.[29] A 30 nm and 5 nm Au/Pd films were sputtered deposited on the edge of the CNT membrane area and on the CNT membrane, respectively to give electrical contact for applying biases and to act as effective working electrode. For ionic rectification measurements the Au/Pd coated DWCNT membranes were used as a working electrode and was placed in U-tube filled with 10 mM potassium ferricyanide on donor side and deionized water on permeate side.[30] The reference/counter electrode (R.E/C.E.) was Ag/AgCl electrode. Linear scan was ranged from −0.60 to +0.60 V at the scan rate of 50 mV/s.

In-vitro determination of nicotine fluxes by microdialysis membrane probe

Chemicals and assay

(−)-Nicotine was purchased from Sigma (St. Louis, MO, USA). Nicotine patches (Nicoderm CQ, 21 mg/24 h) were purchased from local retailor. HPLC grade acetonitrile and methanol were obtained from Fisher Scientific (Fairlawn, NJ, USA). All standards and solutions were prepared using purified water obtained from Barnstead Nanopure System. All other chemicals were reagent grade or better and used as received.

The concentration of nicotine in dialysate and flow cell samples was determined using Shimadzu high performance liquid chromatography (HPLC).[31] In brief, HPLC was performed at flow rate of 1.5 ml/min using a C_{18} column and a detection wavelength of 254 nm. The mobile phase was water:methanol:acetate buffer (0.1 M, pH 4):acetonitrile