An *ex-vivo* and *in-vivo* performance of a switchable carbon nanotube membrane device for transdermal nicotine delivery

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ABSTRACT

Switchable carbon nanotube (CNT) membrane devices have been developed for transdermal nicotine delivery that can be programmed to deliver variable doses of nicotine timed to the complex patient needs during addiction treatment. The performance of switchable devices for nicotine fluxes was evaluated *ex-vivo* across porcine skin on flow cell geometry and *in-vivo* in skin of hairless guinea pig (HGP). A microdialysis membrane probe was placed beneath porcine skin and implanted in skin of HGP to directly detect nicotine fluxes through skin barriers. When the CNT membrane on skin was turned from OFF (0V) to ON (-1.5V) state by application of bias, nicotine levels were increased in microdialysis membrane probe 8-9 times across porcine skin and 5-7 times in skin of HGP, respectively, thus demonstrating the switching potential of device. These results enable smartphone-controlled, battery operated transdermal delivery devices that can be coupled to remote counseling apps for personalized smoking cessation therapy.
Keywords: CNT membrane, nicotine, smoking cessation, medical device, microdialysis

1 INTRODUCTION

Transdermal nicotine patches and nicotine gums therapy along with psychological counseling support is most widely used therapy for smoking cessation. Nicotine patch that deliver drug transdermally, through the skin, typically provide a low steady dose of drug throughout the day to prevent withdrawal symptoms, and nicotine gums provide a high dose nicotine for acute craving relief. Psychological counseling helps smokers to develop skills to fight cravings. However, nicotine replacement therapy (NRT) along with behavioral counseling has low success rate of 25% for 2 year without relapse [1].The major disadvantages of pharmacotherapy include patient's non-compliance, under-dosing for heavy smokers, and therapy is often decoupled from behavioral counseling [2, 3]. Furthermore, behavioral counseling is expensive in terms of professional staffing and patient time away from work.

An ideal therapy would use a smartphone programmable device to deliver variable nicotine fluxes/doses timed to patients cravings interactively determined by a remote behavioral counseling application (app). A new energy efficient pumping technology based on carbon nanotubes (CNT) membranes [4, 5] can provide variable dosing of nicotine by using a small bias of compact watch battery that can pump for ~4 days. A smartphone counseling program coupled to a Bluetooth controlled voltage source on CNT membrane can release nicotine at variable rates timed to patient's needs, resulting in a personalized approach for smoking cessation (Figure 1).

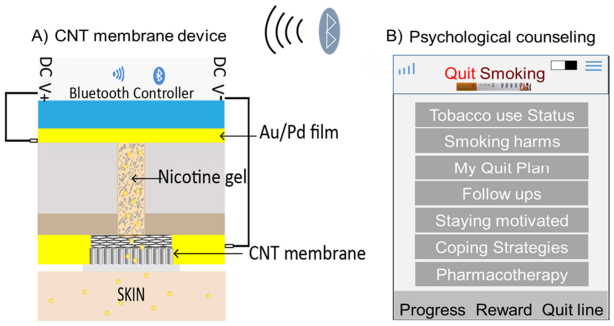

Figure 1: Smartphone based control of nicotine delivery device and example App menu for remote counseling, providing highly personalized smoking cessation treatment.

Herein, we show the performance of the switchable CNT membrane for variable nicotine delivery, at required therapeutic levels, through porcine skin *ex-vivo* and *in-vivo* with hairless guinea pigs. A microdialysis membrane probe was used to directly measure nicotine in animal-skin *in-vivo* since in blood stream nicotine is rapidly converted to cotinine metabolite, making switching events difficult to quantify because of unknown clearance kinetics.

Microdialysis membrane probes were placed beneath porcine skin *ex-vivo* and implanted in skin of hairless guinea pig *in-vivo*. Nicotine levels in microdialysis membrane probe increased 8-9 times *ex-vivo* and 5-7 times *in-vivo* when the CNT membrane on skin was turned from OFF to ON state by application of bias, thus demonstrating the capability of microdialysis for switching fluxes. The ON state nicotine level in microdialysis membrane probe implanted in skin of hairless guinea pig was ~ 4 times than commercial nicotine patch while OFF state was ~1 times. This meets the therapeutic requirements of nicotine gum and nicotine patch doses (gum dose rate ~3.6 times patch).

2 CNT MEMBRANE DEVICE FABRICATION

Double Walled CNT (DWCNT, ~2nm diameter) membranes were fabricated using a microtome-cutting

method as reported previously [4]. The as-made carbon nanotube membranes consisted of nanotubes randomly mixed in epoxy matrix and microtomed to thin disks allowing a significant percentage of CNTs spaning the membrane thickness. The CNT membrane was 5µm thick, 5-6 mm in diameter and mounted on a 3 mm diameter hole in polycarbonate support (0.07 cm^2). To obtain efficient electroosmosis pumping, DWCNT membranes were functionalized at pore entrances with negative charge dye (Direct blue-71) molecules. The Au/pd was sputtered deposited on the bottom of functionalized CNT membrane to make a conducting working electrode.

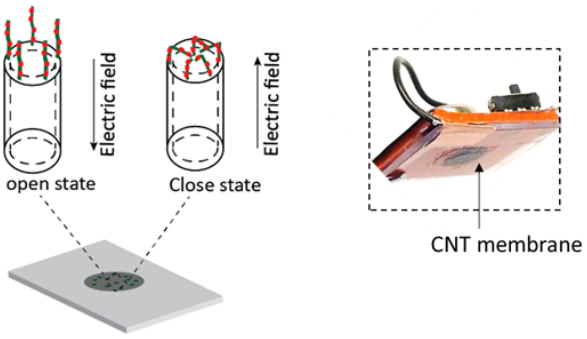

Figure 2. A) Schematic of a functionalized carbon nanotubes membrane (Dye-CNTmembrane). CNT membrane shows a nanotube open state at negative bias and a nanotube close state at positive electrical bias; B) Photograph of CNT membrane device.

Figure 2A shows the schematic of CNT membrane pores functionalized with anionic charged dye molecules. With strong anion functionalization at CNT pore entrances, tethered anionic group blocked the pores at positive bias to CNT membrane while at negative bias pores was opened for cation transport and electroosmotic flow [5] with 100 fold power efficiency improvement compared to conventional membrane materials [6].

Figure 2B shows a picture of the switchable device (diagrammed in Figure 1A) used for *in-vivo* transdermal delivery of nicotine in hairless guinea pigs. The negative electrode of the coin-cell battery is connected to the bottom of DWCNT membrane to provide -1.5V bias while positive counter electrode is touching nicotine donor solution, containing voltage drop within the device.

3 *IN-VITRO* DETERMINATION OF SWITCHABLE NICOTINE FLUXES THROUGH CNT MEMBRANE

Initially CNT membranes screened in a flow cell (Figure 3) apparatus for low (OFF, 0V bias) and high (ON, -1V bias) nicotine fluxes equivalent to nicotine patch and nicotine gum fluxes. The therapeutic low and high fluxes requirements for smoking cessation are 0.1-0.3 µmole/cm^2/h and 0.9-1.1 µmoles/cm^2/h respectively, using a patch size of 22.5cm^2 [4].

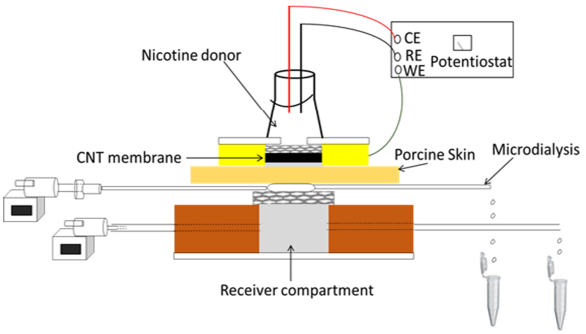

Figure 3. Schematic representation of skin-experimental apparatus (Flow cell) for nicotine permeation studies.

For *in-vitro* studies, nicotine through CNT membrane is passed directly into the receiver compartment of flow cell while for *ex-vivo*, a skin barrier and microdialysis membrane probe were placed between CNT membrane and flow cell. In brief, the infusion pump delivered the phosphate buffered saline (PBS, pH 7.4) to flow cell though polyethylene inlet tubing at a flow rate of 4 µl/min. An outlet tubing from flow cell was connected to fraction collector to collect samples. A CNT membrane was placed on the receiver compartment of flow cell and nicotine donor solution (713 mM, pH 8.0) was kept above CNT membrane. The donor solution had Pt wire as the counter electrode and Ag/AgCl as reference electrode while the conducting bottom contact of CNT membrane was used as the working electrode. The potentiostat was used to deliver bias to CNT membrane. Flow cell samples were continuously collected after every one hour interval for 6h OFF (no bias) and 6h ON (-1V bias) periods of device. Concentration of nicotine in the samples was measured by UV-Vis Spectrophotometer.

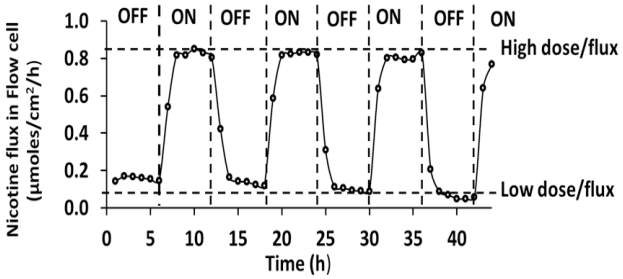

Figure 4: *In-vitro* nicotine fluxes through switching CNT membrane in Flow cell without (OFF)/with (ON) a -1V applied bias.

Figure 4 shows a representative plot of nicotine low and high fluxes through CNT membrane as measured in flow cell without (0V)/with bias (-1V) for three repeated OFF-ON cycles as a function of time. The steady state nicotine fluxes through the CNT membrane in flow cell were ~ 0.15

μmoles/cm^2/h during OFF and ~0.83 μmole/cm^2/h during ON periods. The ON/OFF nicotine flux ratio was greater than 5, suggesting the switching potential of our device that coincide with therapeutic requirement for smoking cessation treatment.

Using typical pumping voltages of -1V and 171.4 μA/cm^2, the power consumption of the CNT membrane is highly favorable for compact devices. For a 22.5cm^2 (commercial available patch size) nicotine transdermal patch made up of a CNT membrane, a coin cell battery (i.e CR2430) with a capacity of 285mWh can be pumping nicotine continuously for 4 days.

4 NICOTINE FLUXES THROUGH CNT MEMBRANE ACROSS PORCINE SKIN (*EX-VIVO*)

The key goal of an *ex-vivo* study is to show that microdialayis membrane probe is an appropriate method to demonstrate switchable transdermal nicotine delivery using CNT membrane and thus can be applied directly to *in-vivo* studies. An efficient *ex-vivo* microdialysis membrane assay for a switchable CNT membrane device enable us to measure direct nicotine in skin of animal and solves the analysis problems associated with unknown clearance kinetic of cotinine (a metabolite of nicotine) in animal-blood.

Figure 3 shows the schematic of the flow cell geometry described in previous section and as earlier [7] to measure nicotine fluxes through CNT membrane across porcine skin in microdialysis membrane probe. A 10 mm long uncoated portion of microdialysis membrane probe was placed on the receptor compartment of flow cell via nylon mesh support. A section of porcine skin (200-400 μm thick) was placed directly on the top of microdialysis membrane. A CNT membrane was placed on skin just above the microdialysis membrane window and nicotine donor solution was placed on the top of CNT membrane. To improve the contact between CNT membrane and skin, a 2% hydroxyethyl cellulose gel was applied on skin. The potentiostat was used to deliver bias to CNT membrane as described in previous section. Concentration of nicotine in the samples was measured by HPLC. Nicotine through CNT membrane had to pass through gel layer on skin and porcine skin barrier before entering the microdialysis membrane probe and saline reservoir compartment of flow cell.

Figures 5A and 5B shows CNT membrane pumping fluxes across skin barrier as measured by microdialysis membrane probe and flow cell and were plotted as a function of time. Two OFF (0-12h and 12-24h) and one ON periods (12-24h) are shown. The steady state OFF (0V bias) and ON (-1V bias) nicotine fluxes were ~ 0.03 and 0.27 μmoles/cm^2/h, respectively in microdialysis membrane probe (Figure 5A). The flow cell OFF and ON nicotine fluxes were ~0.3 and ~2.4 μmoles/cm^2/h, respectively (Figure 5B). The ON/OFF nicotine flux ratios for CNT devices were ~9 for the microdialysis assay and ~ 8 for flow cell assay. The nicotine low and high fluxes in flow cell can meet the demand for nicotine cessation treatment (0.30 and 1.1 μmoles/cm^2/h). It is important to note that the magnitude of two cumulative curves was different due to small diffusion area (0.08 cm^2) of microdialysis membrane as compared to diffusion area of receiver compartment of flow cell (0.3 cm^2). Microdialysis membrane probe was successful to show switching nicotine fluxes of CNT membrane across porcine skin *ex-vivo* and was applied directly to *in-vivo* study for nicotine estimation.

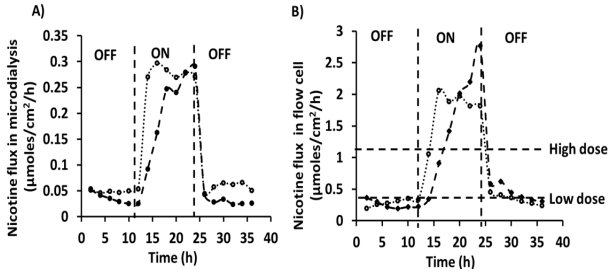

Figure:5 CNT membrane delivered nicotine fluxes across porcine skin in microdialysis membrane probe (A) and Flow cell (B) without (OFF)/with (ON) a -1V applied bias.

5 NICOTINE FLUXES THROUGH CNT MEMBRANE DEVICE IN HAIRLESS GUINEA PIG (*IN-VIVO*)

In-vivo performance of CNT membrane devices was measured by microdialysis membrane probes implanted within skin of HGPs. Importantly, a commercially available nicotine patch (Nicoderm) was used as a calibration standard to measure therapeutic doses that enter microdialysis membrane probe *in-vivo.* This allows for a direct *in-vivo* comparison between CNT device performance and therapeutically used commercial nicotine patch. With the removal of nicotine patch, we were also able to direct measure the lag time of the skin for complete nicotine removal. A critical concern for an *in-vivo* study with switchable transdermal device is depot of drug in skin resulting in a lag time of nicotine removal from skin exceeding desired switching time. A rapid removal of nicotine from skin is desirable for switching transdermal delivery system.

A microdialysis membrane probe was implanted into the skin (~600μm depth) of hairless guinea pigs The implanted microdialysis membrane probe inlet was attached with a syringe mounted on a syringe pump. The microdialysis probe outlet tubing was connected to fraction collector to collect samples in 1.7 ml Eppendorf tubes and analyzed for nicotine. The infusion pump delivered the lactate ringers solution at a flow rate of 2 μL min^{-1}. The nicotine patch or device was placed right above the implanted microdialysis membrane probe. CNT membrane was integrated into a compact device with watch battery as a source of bias (Figure 1c).

Figure 6A shows dialysate nicotine collected in implanted microdialysis membrane probe as a function of time when a commercial nicotine patch was placed (Patch-ON, 0-6h) on skin and removed (Patch-OFF, 6-12h) from skin.

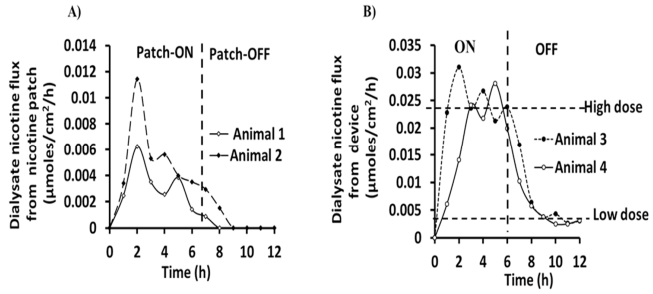

Figure 6: Nicotine fluxes in implanted microdialysis membrane probe after application of nicotine patch (A) and CNT membrane device (B) on skin of hairless guinea pigs.

During patch-ON (0-6h), dialysate nicotine level reached to maximum at 2 h, and was followed by a sustained nicotine level for 4h. During patch-OFF (6-12h), nicotine level decreased and was below detection limit after 2-3h. This suggests nicotine depot formed in skin and released nicotine for 2-3 h after patch removal (skin lag for nicotine removal). Figure 6B shows dialysate nicotine collected in microdialysis probe when CNT membrane device was turned-ON and OFF for 6h each. During ON state (active diffusion, -1.5V bias), dialysate nicotine levels reached to maximum at 2h, and followed by a steady state level for 4h. When battery was switch OFF (passive diffusion, 0 mV), nicotine levels decreased and reached another steady state level, corresponding to diffusion through the membrane in the OFF state. A lag time of ~2-3h was observed to reach a new steady state concentration, indicating the switching limit of CNT device with nicotine emission from skin depot (Figure 6B). The average ON/OFF dialysate (in microdialysis membrane probe) nicotine ratio was 5-7, demonstrating the useful switching operation of our device *in-vivo*. Importantly the nicotine level of CNT device (ON state) was 4 times of commercial nicotine patch indicating the therapeutic potential of our device to switch between patch background levels and nicotine gum used for relapse events.

Treatment	Plasma cotinine level (ng/ml)	
	ON	OFF
Nicotine patch	29 ± 2.8	2.5 ± 1.7
CNT Device	39.4 ± 27.3	6.6 ± 4.0

Table 1 Average plasma cotinine levels obtained in hairless guinea pig after 6hr application of nicotine patch and device

To show a correlation between dialysate nicotine levels with respect to plasma cotinine levels, blood samples were collected after 6h of patch/device-ON and 6h of patch/device-OFF. Table 1 shows average plasma cotinine levels of nicotine patches and devices. Plasma cotinine ON/OFF nicotine ratio of device was ~ 6 times, which is equivalent to dialysate nicotine ON/OFF flux ratio of 5-7 times (Figure 6B). This confirms that microdialysis is an appropriate method to show switching fluxes of CNT membrane device.

6 CONCLUSION

CNT membranes achieved target fluxes of 0.1-0.3 µmoles/cm^2/h and 0.9-1.1 µmoles/cm^2/h, corresponding to commercial patch and nicotine gum dosing. *Ex-vivo*, dialysate ON/OFF nicotine flux ratio as high as 8 showed the switching potential of CNT membrane for transdermal nicotine delivery and microdialysis as an appropriate method to detect switching fluxes across a skin barrier. The ON/OFF dialysate nicotine flux ratio of ~ 5-7 times and plasma cotinine ON/OFF ratio of ~6 times shows the *in-vivo* switching potential of membrane device. A 4 times higher dialysate nicotine flux of device during ON state than commercial nicotine patch meet the requirement of nicotine gum dose (3.6 times). These findings allow the development of a Blue-tooth enabled CNT membrane device that is programmed by a smartphone based counseling program. This will enable the delivery of variable nicotine doses match to patient needs using remote psychological counseling and dramatically improve smoking cessation therapy.

ACKNOWLEDGEMENTS

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