SUPPORTING INFORMATION FOR

ACTIVE MODULATION OF DRUG RELEASE BY IONIC FIELD EFFECT TRANSISTOR FOR AN ULTRA-LOW POWER IMPLANTABLE NANOFLUIDIC SYSTEM

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Nanofluidic device fabrication process.
The nanofluidic devices were composed of a silicon substrate and a pyrex cap with integrated electrodes. The processing of the silicon substrates started with double side polished p-type 4 inch wafers. To fabricate the nanochannels, a pad oxide layer was deposited followed by a layer of nitride on wafers. Then, standard lithography was applied to generate nanochannel patterns. CF4 reactive ion etching (RIE) was then used to remove nitrides on these channel patterns. Etching time was controlled so that there was a thin layer of silicon dioxide remaining to prevent reactive ions from attacking the silicon underneath. A diluted HF solution was used to clean the oxide in channel patterns. The silicon wafers were put into thermal oxide furnace and a desired amount of oxide was grown. The thickness of oxide defined the height of nanochannels and was measured by using ellipsometry. HF solution was then used to remove all dielectrics on the substrates. A new layer of silicon nitride was deposited for the second major step to define the inlet and outlet microchannel areas. After patterning these on top of nanochannels, RIE was performed. The third major step was creating the back side openings. A new layer of silicon nitride was deposited on the silicon substrates. The exit ports were patterned on back of the wafers by double side alignment. CF4 RIE was used to open the exit port pattern. Then KOH etching was performed (approximately eight hours) to etch through silicon wafers. After removing dielectric materials using HF solution, a 15 nm thick silicon oxide layer was deposited as an insulating layer for easy wire bonding. The real nanochannel height was measured using AFM. 5 selected points were measured for each substrate. The completed microfabricated silicon substrate is shown in Figure S1.

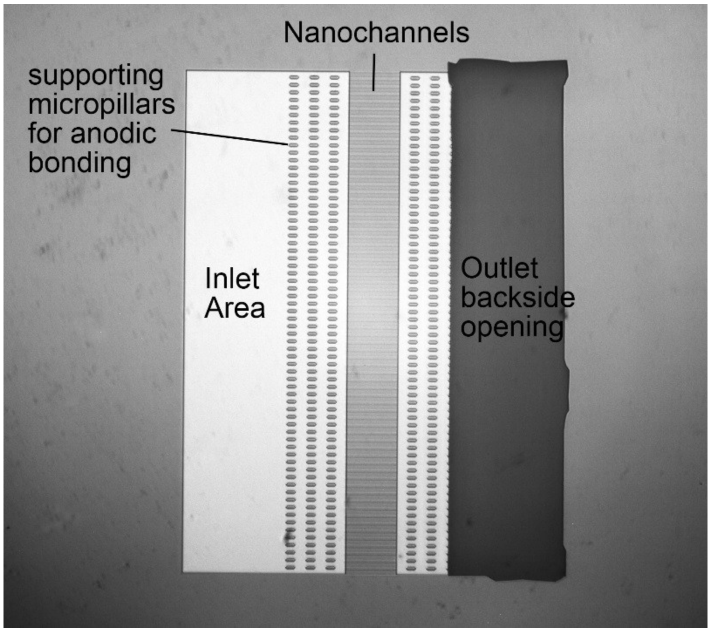


Figure S1. Microfabricated nanofluidic silicon substrate with parallel set of slit-nanochannels Pyrex glass fabrication.

A sandwich of titanium, gold and titanium was evaporated on four inch pyrex wafers. Then, standard lithography was used to pattern the electrodes. Multi-step wet etching was applied to etch the Ti-Au-Ti sandwich, and the remaining photoresist was then cleaned up. Here, the height of electrodes was 150 nm above the pyrex surface. Then, a lift-off process was performed to match the thickness of electrode with evaporated pyrex glass film. To do so, a photolithograph was performed to cover the electrodes followed by a 150 nm glass film evaporation using CHA. Next, the wafer was sonicated in acetone to remove the photoresist and clean the deposited pyrex on the top of electrodes. After that, a 100 nm thick pyrex film was evaporated to cover electrodes. Then, a CMP step was applied to polish the surface to reach desired roughness. Much effort was used to optimize the CMP process. During CMP process, many parameters such as pressure, speed, time, and pads, needed to be controlled. The surface roughness was finally reduced to 1 to 2 nm. The CMP processing was monitored by AFM.

Anodic bonding.

Inlet openings were drilled into the pyrex wafers by Bullen Ultrasonics. The anodic bonding of pyrex and silicon wafers were done using Ohio State University facilities. The bonding conditions were 350 °C, 450 V, compressive force 500 N and were maintained for 20 minutes. Finally, the wafers were cleaned and diced.

AFM measurements.

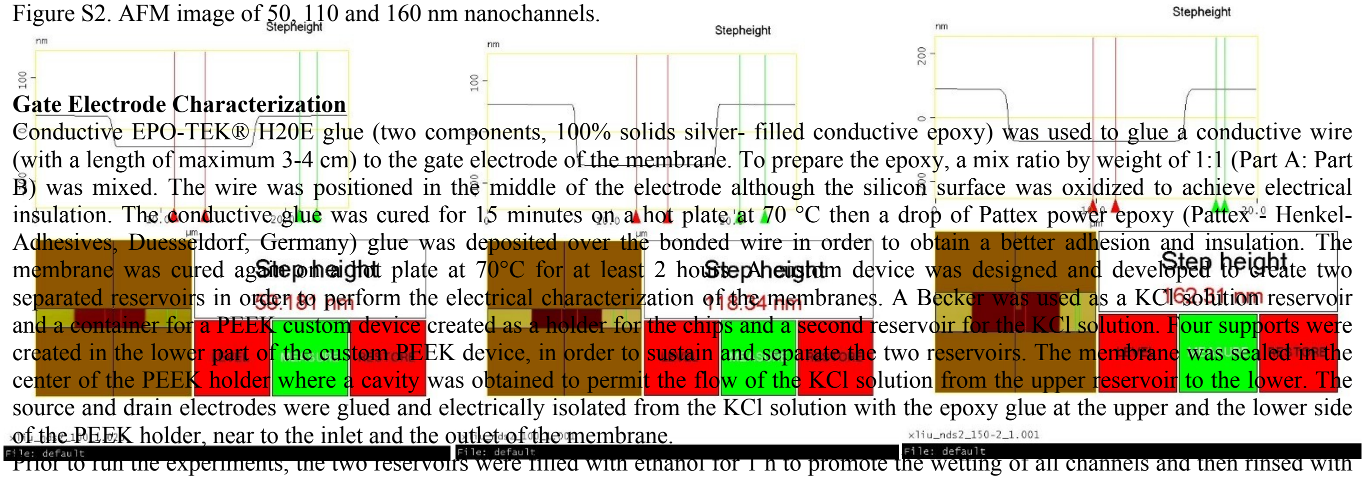
The measurement of the nanochannel depth was performed through Atomic Force Microscopy (AFM) (Digital Instrument Dimension 3000). Table 1 lists the measured values of the nanochannel depth. The measurements were performed on 4-5 different points of the wafer, located in different area of the wafer. A percentage standard deviation of nanochannel size smaller that 2.48% was observed for each fabricated substrate. Figures S2-S4 show representative example for the AFM measurements.

Table 1. AFM measurement results. The standard deviation (STDEV) and the % standard deviation (%STDEV) are also listed.

Substrate#	top	center	left	right	bottom	STDEV	%STDEV
50-1	56.685	55.868	54.85	56.36	56.395	0.72	1.29
50-2	56.275	55.54	57.418	56.376	59.181	1.41	2.48
50-3	56.286	57.063	55.667	55.272	56.434	0.70	1.24
110-1	118.45	113.79	118.59	117.89	117.77	1.99	1.70
110-2	121.77	118.34	121.4	120.59	122.26	1.54	1.28
160-0	159.06	159.45	159.7	159.72	154.34	2.32	1.46
160-1	157.39	159.92	159.58	154.28		2.60	1.65
160-2	155.87	162.31	160.65	161.43	160.5	2.50	1.56

Nominal Size (nm)	Measured Average (nm)	STDEV	%STDEV
50	56	1.0	1.8
110	119	2.5	2.1
160	159	2.5	1.6

Figure S2. AFM image of 50, 110 and 160 nm nanochannels.



Gate Electrode Characterization

Conductive EPO-TEK® H20E glue (two components, 100% solids silver- filled conductive epoxy) was used to glue a conductive wire (with a length of maximum 3-4 cm) to the gate electrode of the membrane. To prepare the epoxy, a mix ratio by weight of 1:1 (Part A: Part B) was mixed. The wire was positioned in the middle of the electrode although the silicon surface was oxidized to achieve electrical insulation. The conductive glue was cured for 15 minutes on a hot plate at 70 °C then a drop of Pattex power epoxy (Pattex - Henkel-Adhesives, Duesseldorf, Germany) glue was deposited over the bonded wire in order to obtain a better adhesion and insulation. The membrane was cured again on a hot plate at 70°C for at least 2 hours. A custom device was designed and developed to create two separated reservoirs in order to perform the electrical characterization of the membranes. A Becker was used as a KCl solution reservoir and a container for a PEEK custom device created as a holder for the chips and a second reservoir for the KCl solution. Four supports were created in the lower part of the custom PEEK device, in order to sustain and separate the two reservoirs. The membrane was sealed in the center of the PEEK holder where a cavity was obtained to permit the flow of the KCl solution from the upper reservoir to the lower. The source and drain electrodes were glued and electrically isolated from the KCl solution with the epoxy glue at the upper and the lower side of the PEEK holder, near to the inlet and the outlet of the membrane.

Prior to run the experiments, the two reservoirs were filled with ethanol for 1 h to promote the wetting of all channels and then rinsed with Millipore water. Finally, the chips were filled with KCl solution. Measurements were collected at different ionic concentrations of KCl prepared by serial dilution, ranging from 0.01 to 1000 mM. Each set of experiment started with the KCl solution with the lower ionic concentration. Between experimental sequences, membranes were rinsed in Millipore water for ~5 min. Platinum wires (Sigma Aldrich, St. Louis, MO) were employed for the drain-source voltage, while a Ag/AgCl electrode (Harvard Apparatus, Holliston, MA) was utilized as reference directly connected to the system's ground.

The drain source voltage was generated by a 33522A function/arbitrary wave generator (Agilent Technologies, Santa Clara, CA), while the gate voltage was applied by a 33250A arbitrary waveform generator (Agilent Technologies). Both instruments were controlled by a custom Matlab (The MathWorks Inc., Natick, MA) though COM ports, maximizing both accuracy and repeatability. The currents flowing tough all three cables (source wire, drain wire, and gate wire) were monitored using a34410A multimeter (Agilent Technologies) multiplexed by a series of high-speed relays (American Relays, Inc., CA, USA). An Arduino UNO (Arduino, Italy), which was controlled was a Matlab script, was employed to modulated the switching terminals of the latter relays. The data were collected and post-processed on Matlab.

In Vitro Drug Release Experiment

To quantify the amount of drug released through the nanochannels an identical setup to the one previously described was adopted, with the only difference of the solution in which the membranes were immersed. Atenolol (Sigma Aldrich) was dissolved in a solution of 1 mM KCl achieving a concentration of 0.316 mg/ml. Similarly, 500 µg of perindopril (Sigma Aldrich) were dissolved in 1 mM KCl for a final concentration of 0.5 mg/ml. 500 µl of the high concentrated drug solution were placed in the drug reservoir chamber of the membrane holder, while 2 mL of 1 mM KCl were disposed in the sink reservoir. The sink solution was periodically sampled and replaced with fresh solution to analyze the drug concentration with a spectrophotometer (PerkinElmer Lambda 35, Waltham, MA). Data collection and post-processing were performed using Excel (Microsoft Office, WA).

Figure S3 represents the cumulative release of perindopril over time with different gate voltages. These data were acquired by sampling 1 ml of the sink solution every two hours, which was then analyzed with the spectrophotometer. The sampled volume was then replaced with 1 ml of fresh 1 mM KCl solution. Drain-source potential was alternated between 0 V and 2 V every 8 hours.

The schematic shown in Fig. S4 illustrates how the gate electric field redistributes the charged therapeutics within the nanochannels. When a positive gate voltage is present an accumulation of perindopril occurs in proximity of the gate electrode, increasing the diffusion gradient between the area near the gate electrode and the sink solution. The same phenomenon occurs when a negative gate potential is applied for positively charged molecules, such as atenolol.

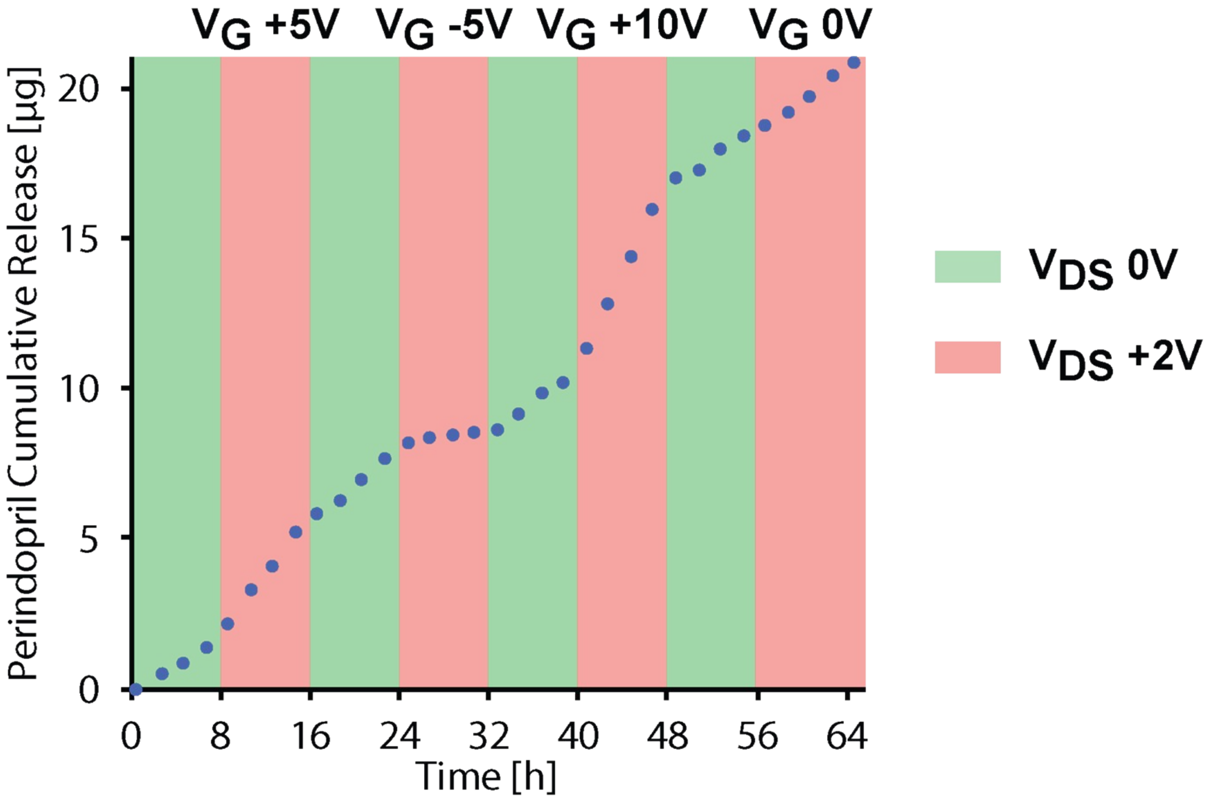


Figure S3. Cumulative release of perindopril over time with different applied voltages.

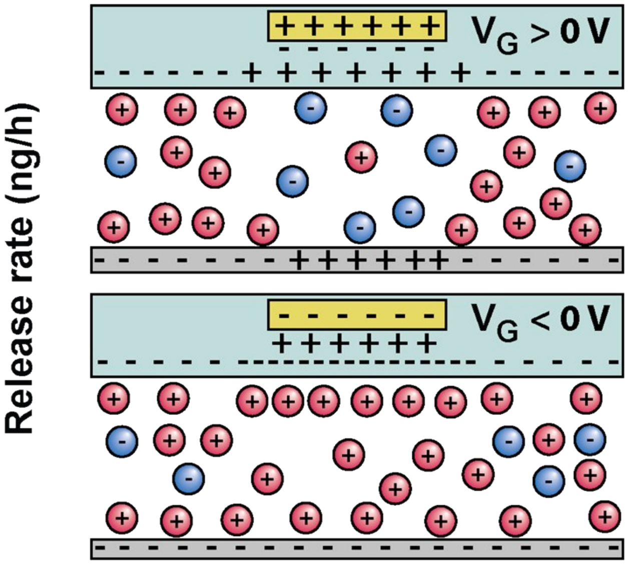


Figure S4. Schematic representation of charge flow in the nanochannel at positive and negative gate voltages.