SUPPORTING INFORMATION

Fabrication of Two-Layered Channel System with Embedded Electrodes to Measure Resistance Across Epithelial and Endothelial Barriers

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Table of Contents:

Supporting Information:

1. circuit_fun.m:

```
function F = circuit_fun (rc_component)

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Import data from autolab output .dfr file (experimental)
autolab_data1 = importdata ('Experimental File Name.dfr');
% Extract numerical data part from the data structure
autolab_data1 = autolab_data1.data;

% Import data from autolab output .dfr file (baseline data)
autolab_data2 = importdata ('Baseline File Name.dfr');
% Extract numerical data part from the data structure
autolab_data2 = autolab_data2.data;

% Number of measurement points in each impedance spectrum
point_num = 64;
% Number of impedance measurement sets
data_set = autolab_data1 (1)/point_num;
data_set = autolab_data2 (1)/point_num;
```

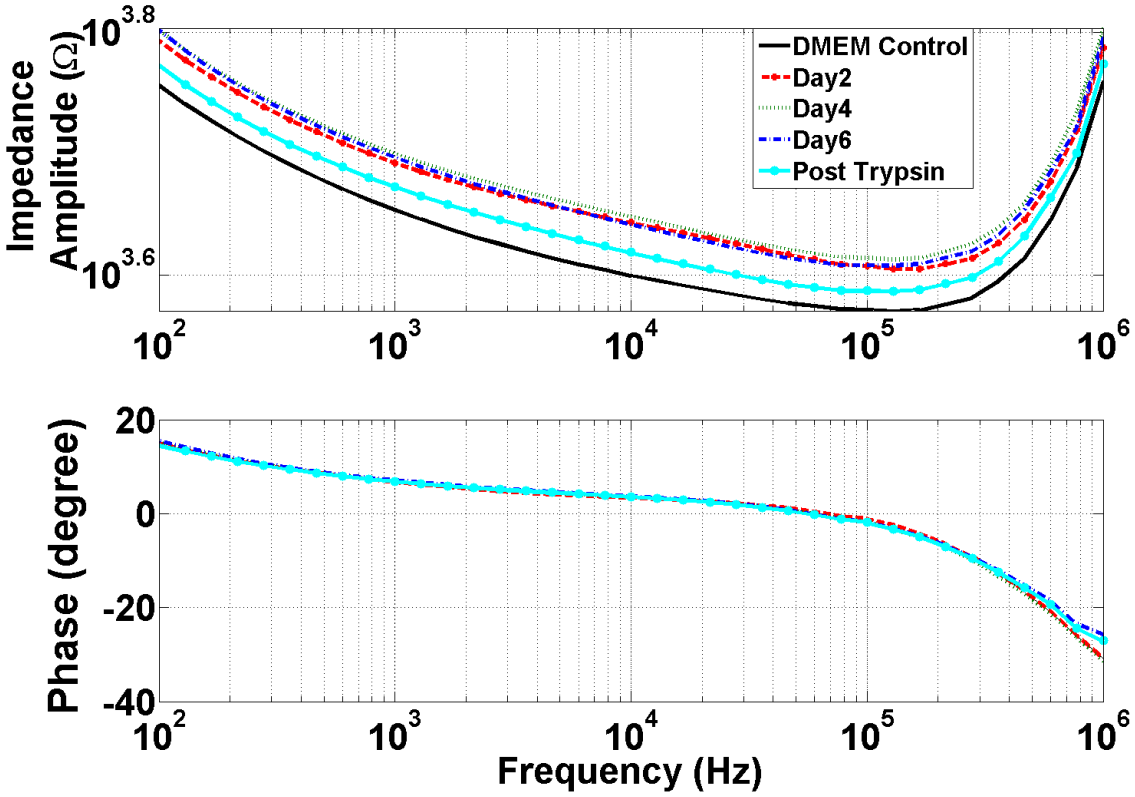
```
% Number of lines containing numerical non-impedance data
head_line = 2;

for a = 1:1:point_num
    for b = 1:1:data_set
        frequency (a,b) = autolab_data1 (head_line + (a-1)*data_set*9 + (b-
1)*9 + 1);
        impedance_rel1 (a,b) = autolab_data1 (head_line + (a-1)*data_set*9 +
(b-1)*9 + 2);
        impedance_img1 (a,b) = autolab_data1 (head_line + (a-1)*data_set*9 +
(b-1)*9 + 3);

        frequency (a,b) = autolab_data2 (head_line + (a-1)*data_set*9 + (b-
1)*9 + 1);
        impedance_rel2 (a,b) = autolab_data2 (head_line + (a-1)*data_set*9 +
(b-1)*9 + 2);
        impedance_img2 (a,b) = autolab_data2 (head_line + (a-1)*data_set*9 +
(b-1)*9 + 3);
    end
end

impedance1 = impedance_rel1 + j*impedance_img1;
impedance_amp1 = abs (impedance1);
impedance_phase1 = angle (impedance1) * (180/pi);

impedance2 = impedance_rel2 + j*impedance_img2;
impedance_amp2 = abs (impedance2);
impedance_phase2 = angle (impedance2) * (180/pi);

%%%%%%%%%%%% Use data set 2 as background
Z_exp_amp = impedance_amp1 - impedance_amp2;
Z_exp_phase = impedance_phase1- impedance_phase2;

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

frequency = frequency(:,1);
omega = 2*pi*frequency;

R1 = rc_component (1)*1000;
R2 = rc_component (2)*1000;
C1 = rc_component (3)*1.E-9;

Z_R1 = R1;
Z_R2 = R2;
Z_C1 = 1./(j*omega*C1);

Z = Z_R1 + (Z_C1.*R2)./(Z_C1+R2);
```

```
Z_amp = abs(Z);
Z_phase = angle(Z)*180/pi*-1;

if or(or((R1 < 0),(R2 < 0)),(C1<0))
      F = 1.e12;
else
      F = sum(((log10(Z_amp) - log10(Z_exp_amp))).^2);
End
```

2. circuit_fun.m:

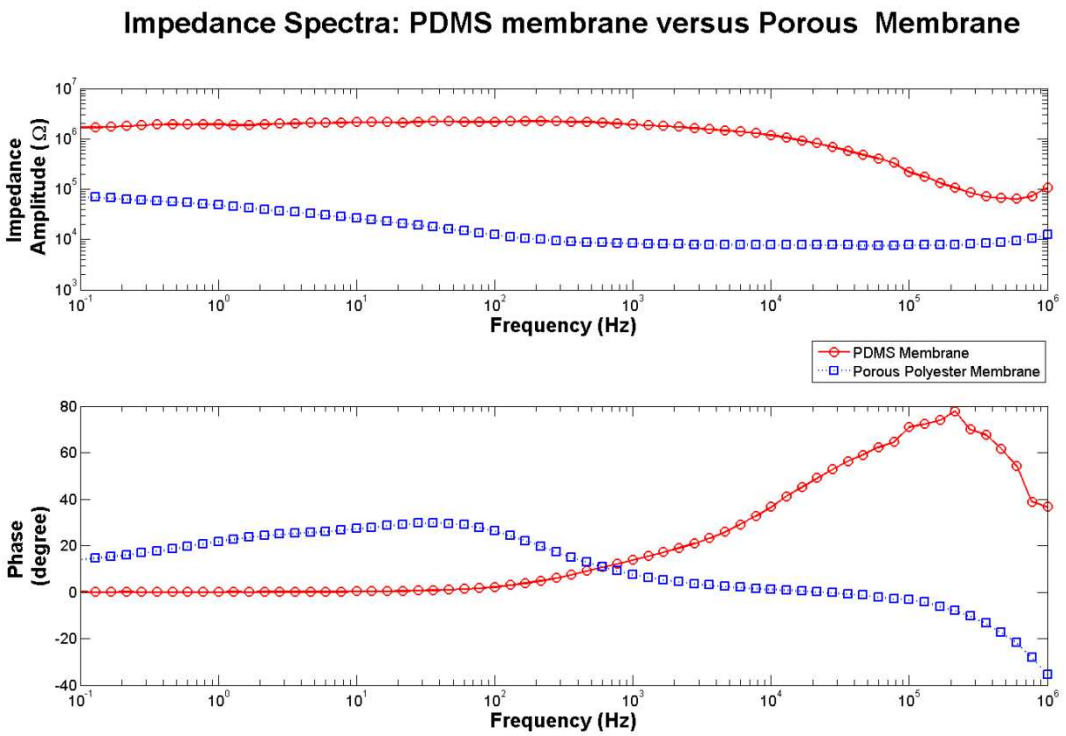
```
clear all;

R1 = 1;      % KOhm
R2 = 3;      % KOhm
C1 = 1000;   % nF

rc_component0 = [ R1 R2 C1 ];

options = optimset('MaxIter',1.E4,'MaxFunEvals',1.E4);

[rc_component,F] = fminsearch(@circuit_fun,rc_component0,options)

r1 = rc_component(1)*1000
r2 = rc_component(2)*1000
c1 = rc_component(3)*1.e-9
```

3. **Representative Impedance Spectra for bEND.3 cells over One Week Growth**:



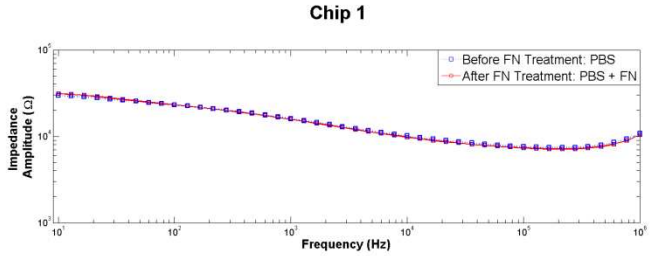
Representative Impedance Spectra for bEND.3 Cells over One Week Growth. The impedance increases from baseline chip (DMEM control) to Day 2 and again from Day 2 to Day 4. The impedance plateaus near Day 4 before decreasing slightly on Day 6. Trypsinization decreases the impedance below Day 2 levels but remains higher than the baseline chip (DMEM control). Note, only every other day graphed for clarity.

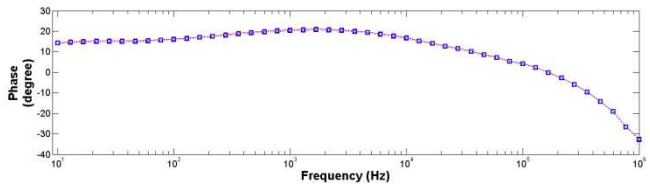
4. **<u>Impact of Membrane Material on Impedance Spectra</u>**:

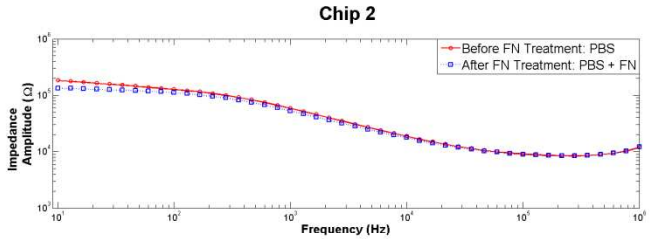


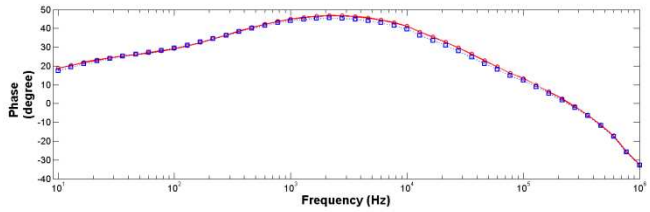
Impact of Membrane Material on Impedance Spectra. To verify that our system was capable of detecting large changes in impedance, we tested the impact of membrane material on impedance. First we characterized the standard system (polyester membrane with 400 nm pore size) sandwiched between the upper and lower PDMS channels. We compared this impedance to impedance measured in a system fabricated with a PDMS membrane in the place of the polyester membrane. Because PDMS is an insulator, we expected the resistance at low to intermediate frequencies to be much greater than the baseline (porous system). Our experimental results agreed with the expected results: the impedance amplitude was an order of magnitude greater in the system containing the PDMS membrane compared to the standard, porous membrane. Furthermore, at high frequencies, the impedance gap decreased (as the phase of the chip containing the PDMS membrane rose). This indicates that the PDMS membrane has a capacitive effect on the net impedance of the system.

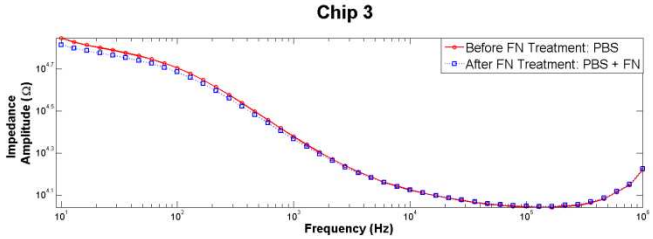
5. **Impact of FN Treatment on Impedance Spectra**:

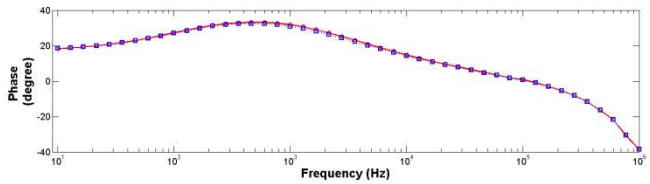






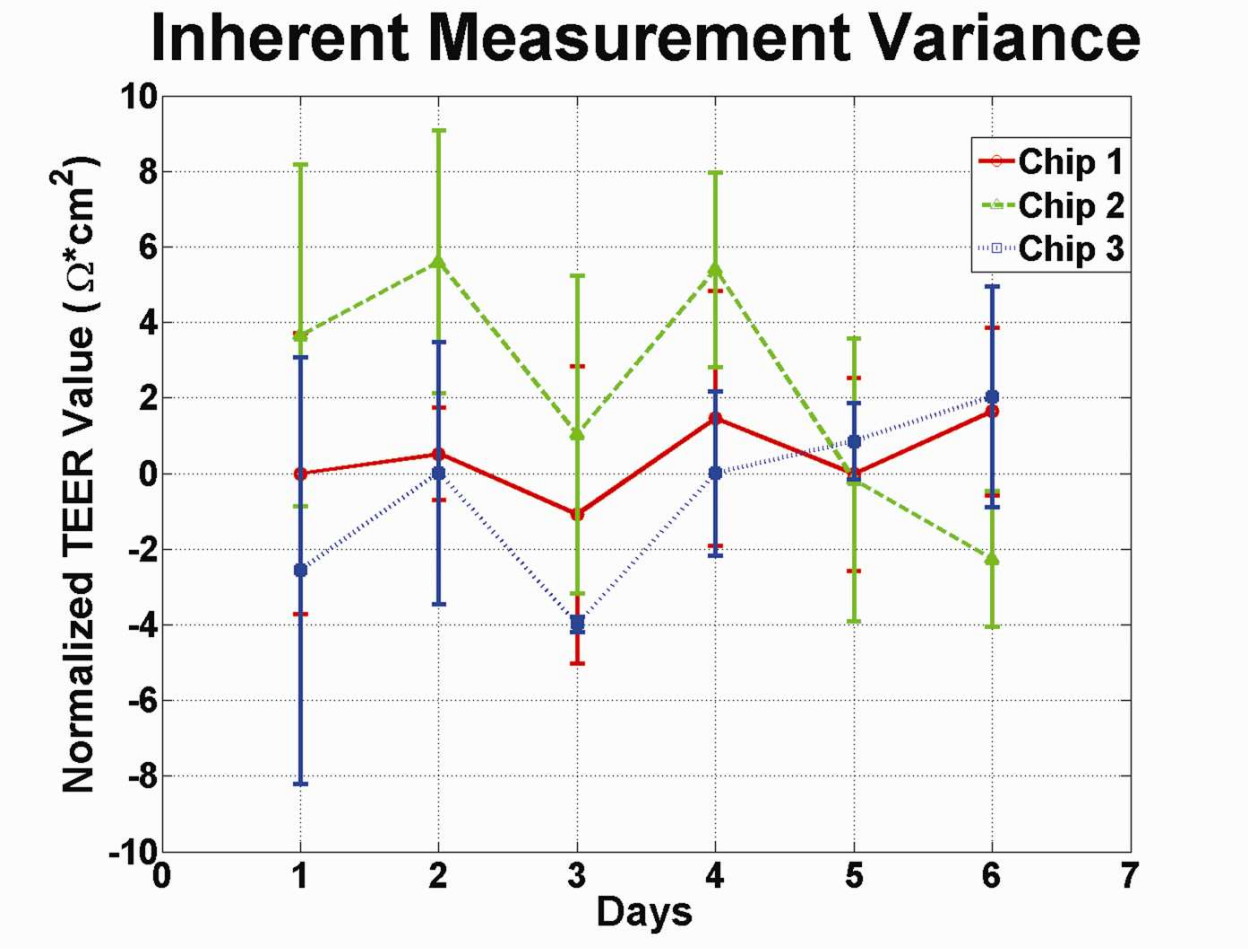






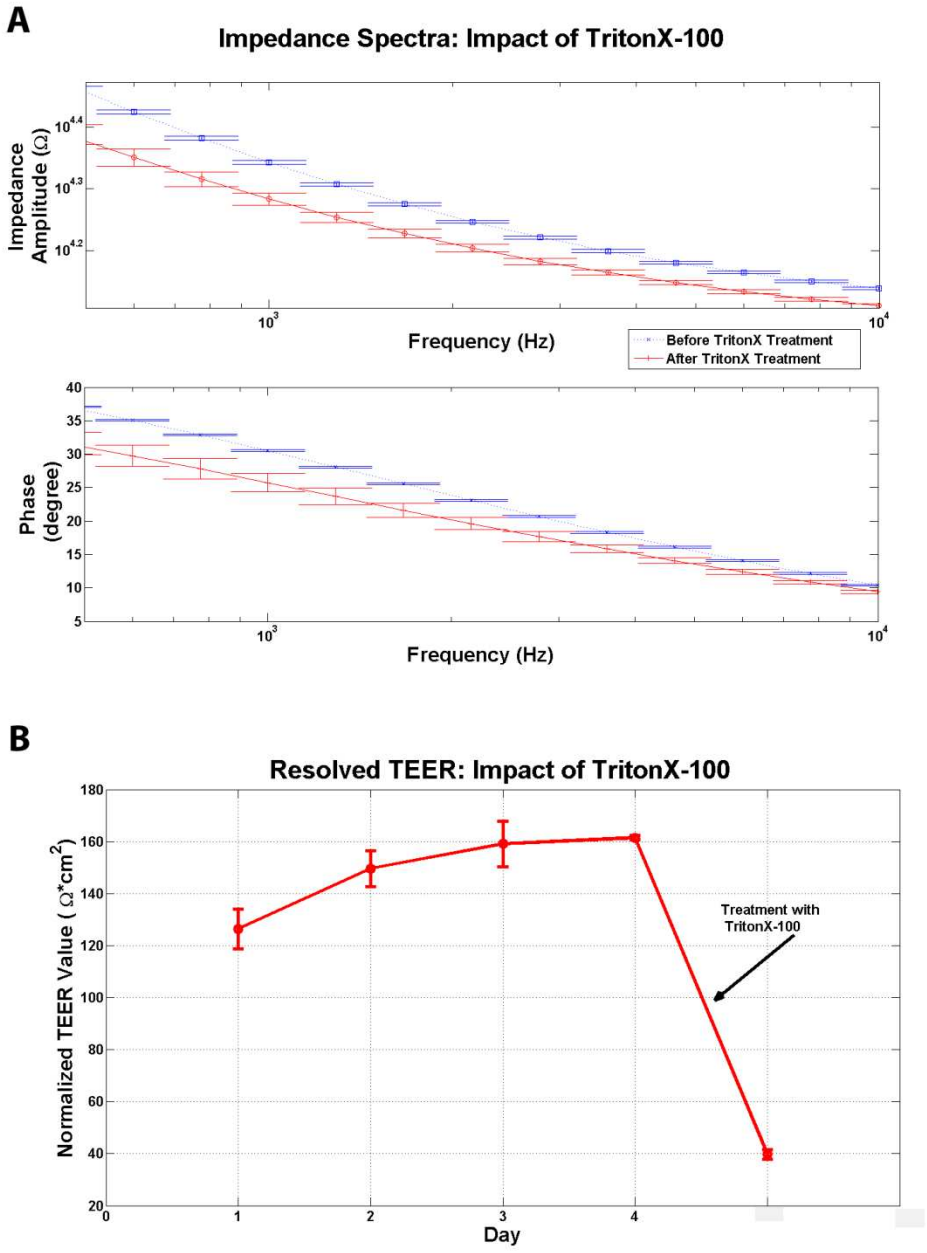
Impact of FN Treatment on Impedance Spectra. To test the impact of FN treatment on the baseline impedance of our system, we measured the impedance of 3 different Chips filled only with PBS. We then treated the system with fibronectin using an identical protocol to the one used in preparation for cell culture (30 minutes of 100 µg/mL FN in PBS under UV light). After 30 minutes of treatment, fresh PBS (without FN) was flowed into the system and the impedance was re-measured. We found that FN treatment had a minimal impact on the impedance spectra, actually decreasing amplitude slightly at low frequencies.

6. **Inherent Measurement Variance**:



Inherent Measurement Variance. As a control experiment, an identical protocol to the cell culture experiment was maintained for the acellular condition. Growth media was loaded into the system, changed twice per day, and stored in the incubator at 37 ° C. Impedance measurements were resolved daily for 6 days. The data show that trans-membrane resistance values fluctuate between +6 $\Omega*cm^2$ and -4 $\Omega*cm^2$. The resistance measurements do not appear to trend either upward or downward as a function of time. The small measurement variance provides a window where TEER values as opposed to system drift can be resolved.

7. Impact of Treatment with TritonX-100:



Impact of Treatment with TritonX-100. Treatment with the non-ionic surfactant, TritonX-100 is expected to increases the permeability of an endothelial cell monolayer. To demonstrate that this microfluidic system can detect dynamic changes in impedance, a confluent bEND.3 cell monolayer was treated for 30 minutes with 0.1% TritonX-100

then completely flushed with fresh growth media. The impedance amplitude decreased significantly at each frequency point (partial spectrum shown in the Supporting Information, Figure 7a) and the TEER decreased from a maximum value of 161.51 ±0.755 $\Omega*cm^2$ to 39.73 ± 1.925 $\Omega*cm^2$ (shown in Supporting Information, Figure 7b) demonstrating that treatment with TritonX-100 increases the permeability of the cell monolayer.