The Effect of Electrovascular Streaming Potential on Endothelium

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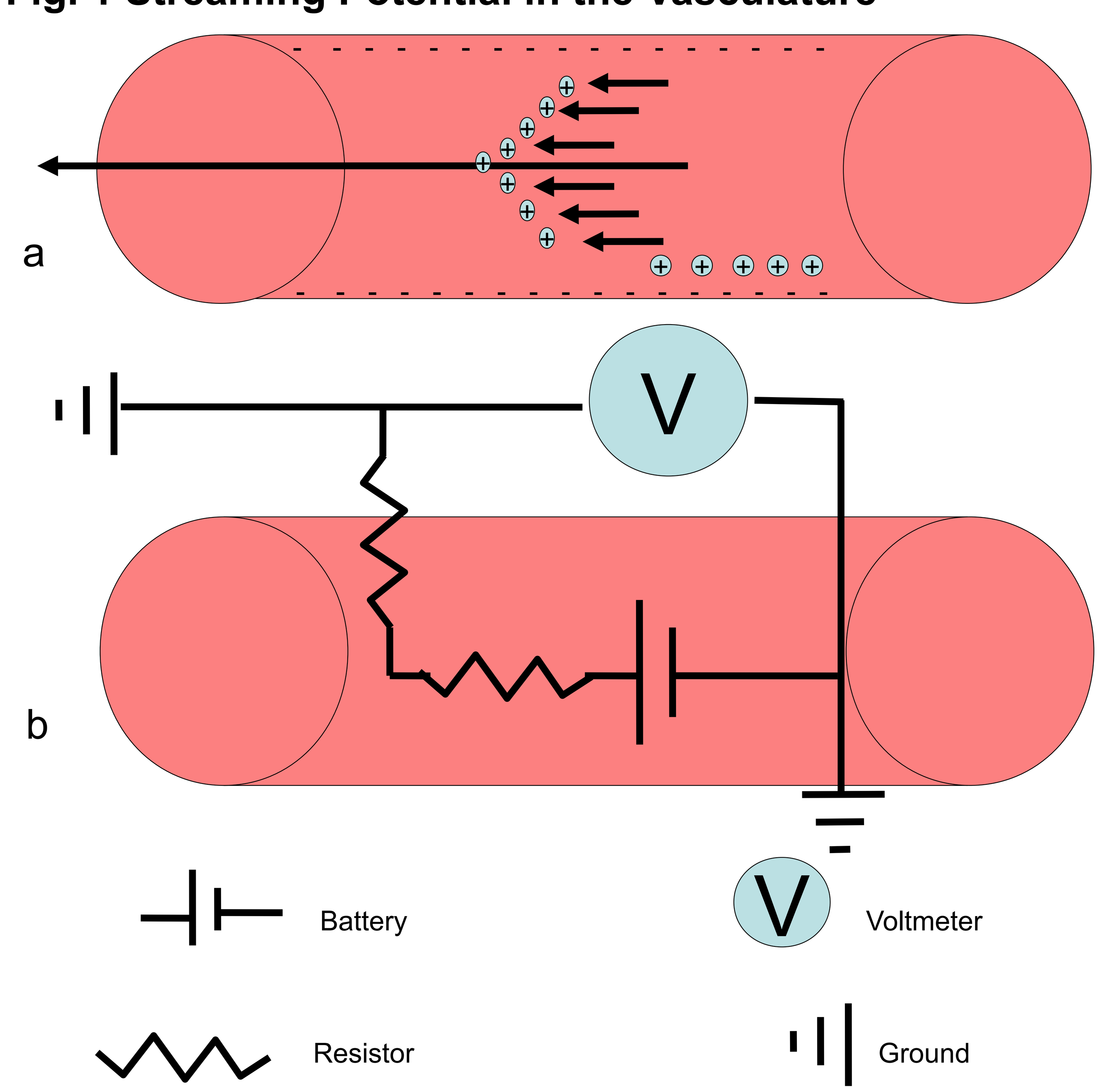
ABSTRACT

Objective - This work investigates how a biophysical vascular phenomenon, the electrovascular streaming potential (EVSP), affects the membrane potential of endothelial cells (ECs).

Background - Stroke is caused by atherosclerosis. It is the leading causes of disability in the Western world. Though atherogenesis has been extensively studied, it may be further explained, in part, by the physical context in which this dysfunction occurs. The EVSP is a dynamic electric field generated by charge separation caused by blood flow along the vascular wall. Previous studies have shown the existence of the EVSP, in vivo, and have demonstrated its effect in vascular smooth muscle cells (SMCs), in vitro. In SMCs, the EVSP has been shown to induce a steady-state depolarization of the plasma membrane, potentiate the response to membrane depolarizing agonists, and alter extracellular matrix accumulation. ECs comprise the vascular luminal surface and, therefore, are exposed to the EVSP, as well.

Fig. 1 Streaming Potential in the Vasculature



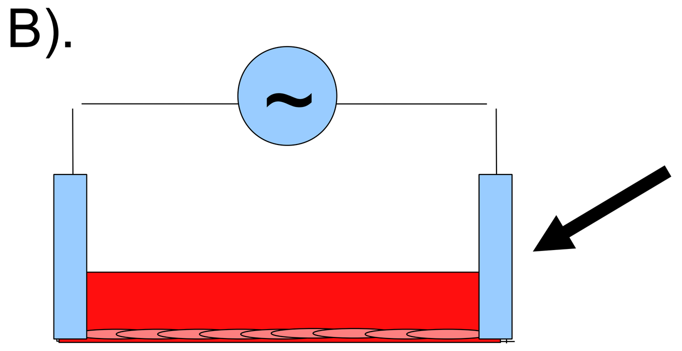
The luminal surface of a typical blood vessel is negatively charged due to the rich protein content of the endothelial plasma membranes. Balancing this charge are positively-charged ions in the blood. **A).** Without blood flow, electrostatic forces would dominate and the ions would migrate and coat the luminal surface. However, due to the pressure differential in vessels, blood flow occurs, generating a shearing force that strips the ions off the luminal surface and forces them further down the vessel, thereby separating the two opposite charges. Assuming laminar flow, the lamina furthest from the luminal surface will experience the least friction and therefore have the highest velocity, causing greater charge separation. This generates a potential field called the electrokinetic vascular streaming potential (EVSP). **B).** Circuit diagram representing the EVSP.

Fig. 2 Application of Electric Field and Determination of Membrane Potential

A). $E_s = [Ze_0P/nk][f(Y_a)]$

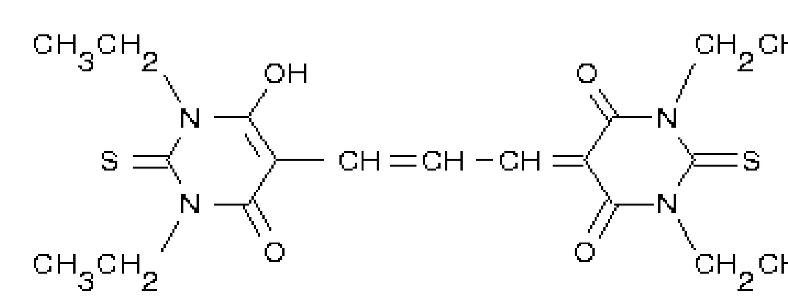
E_s = streaming potential (volts)
e = electrolyte dielectric constant
P = systolic pressure (N/m2)
k = conductivity (S/m)
Z = zeta potential (volts)
e_0 = permittivity of free space
n = viscosity (kg/m*s)
$f(Y_a)$ = correction for pulsatile flow

B).



Hydroxyethylmethacrylate (HEMA) hydrogel bridges, preferred over traditional Agar salt bridges, due to greater power dissipation and virtually non-existent failure rate.

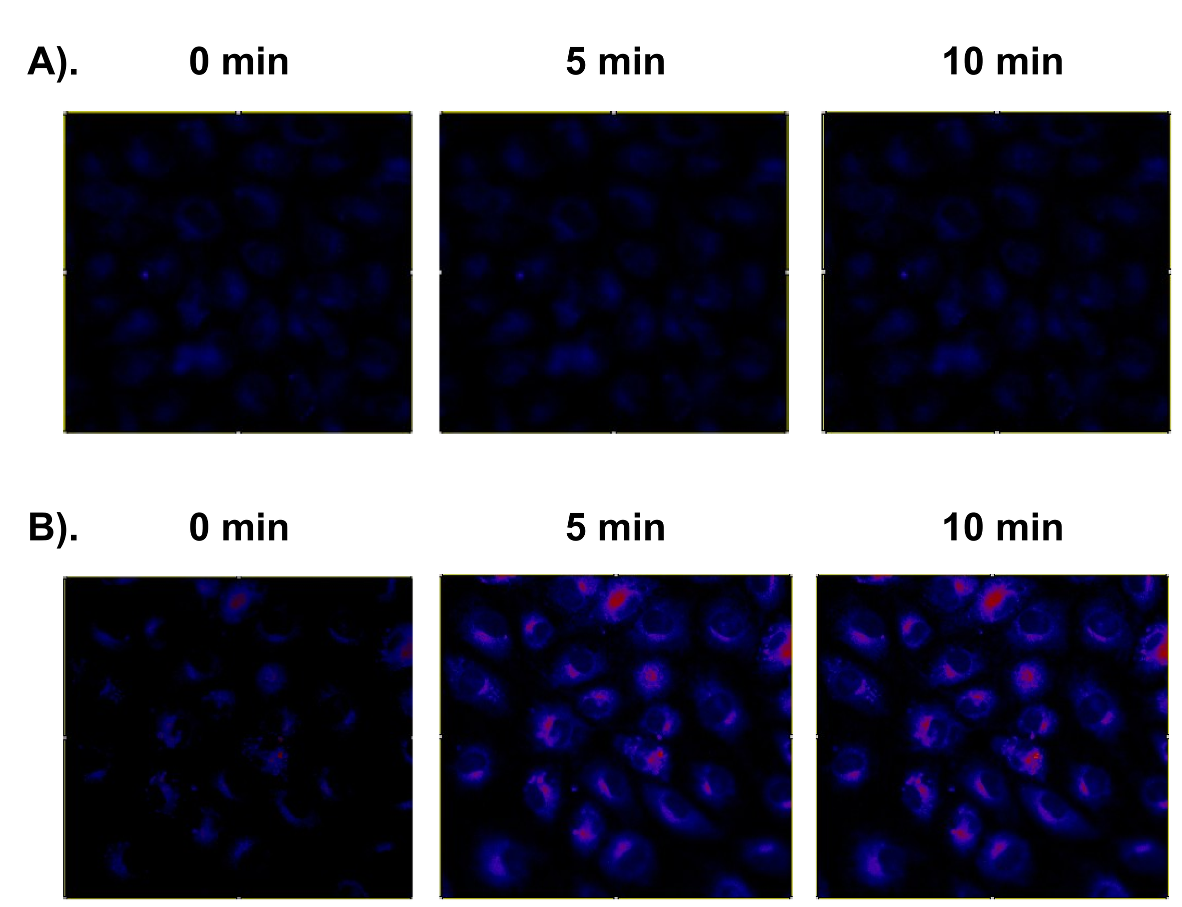
C).



BIS-OXONOL – Membrane Potential Sensitive Dye
Excitation – 490 nm, Emission – 530 nm

A). The streaming potential, E_s, as described in the equation assuming i). no surface conduction, ii). no influence of thermal forces on ionic diffusion, iii). unvarying laminar flow. **B).** View of single well from plate. The EVSP will be calculated using Eq. A. and then applied to cells grown to confluence in the wells. Electrodes will be fed into the growth plate and the EVSP will be applied to the cells. **C).** Cells will be dosed with 1uM Bis-Oxonol in 5 ml Puck's Saline.

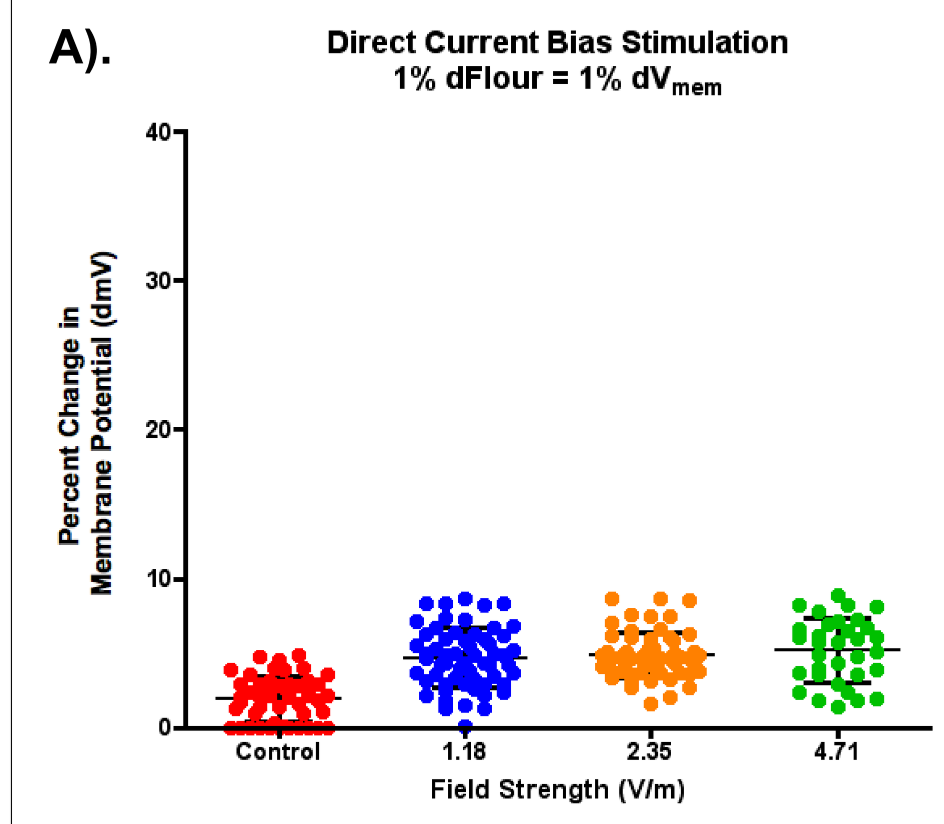
Fig. 3 Observed Steady State Depolarization



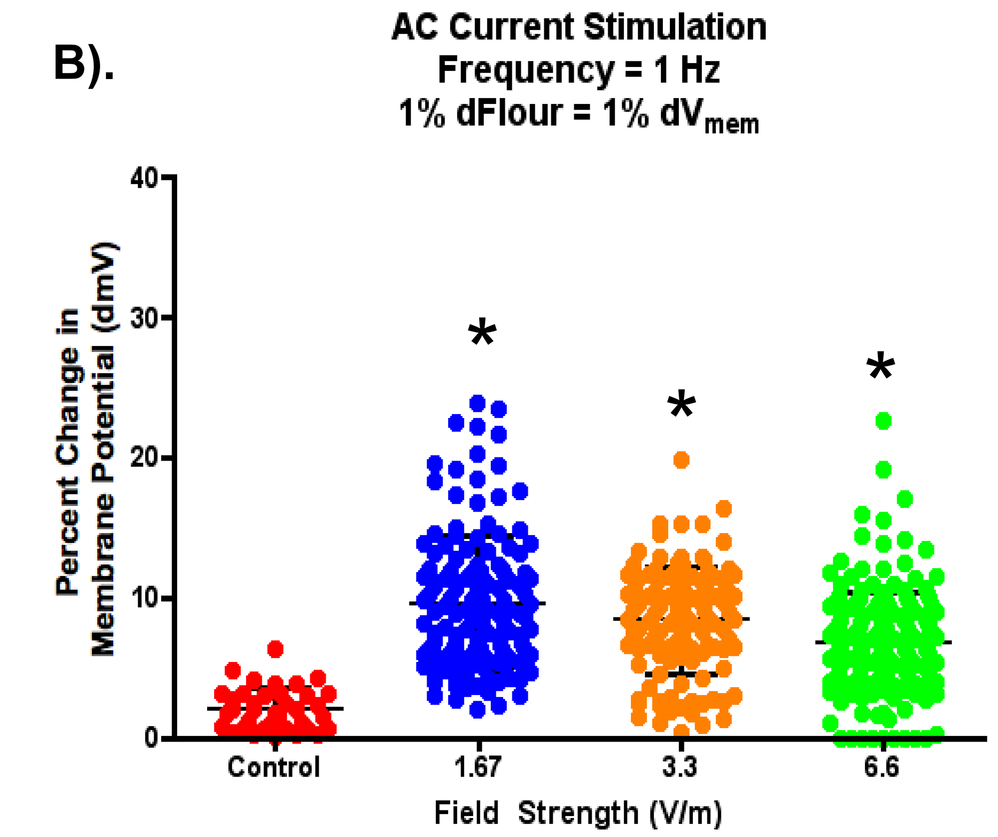
3.3 V/m, 1 Hz AC current stimulation experiment. Images were captured every 20 seconds over 10 minutes. **A).** Control studies showed little change over the course of the study. **B).** Stimulated cells showed a 10.5% change in fluorescence and based on the results of the null point calibration, this correlates to a 10.5% change in membrane potential in the presence of the modeled EVSP.

Fig. 4 Frequency Dependence

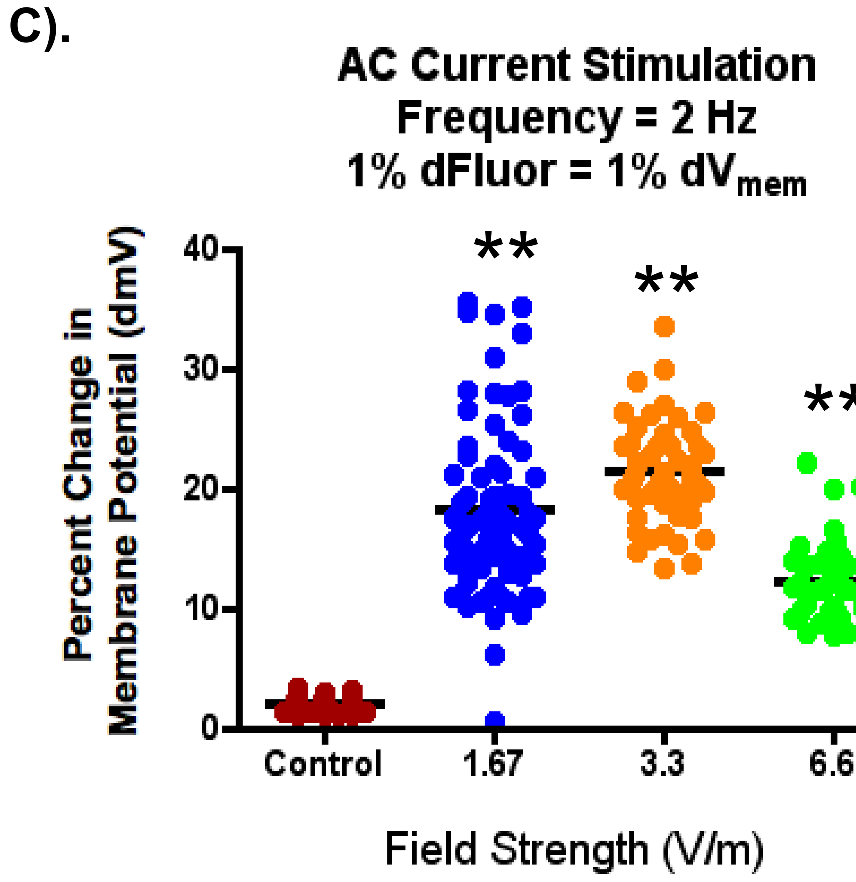
A).



B).



C).



A). DC Bias experiments served as a zero-frequency state for determining frequency dependence of the observed depolarization. They did not show significant difference from controls. Alternating Current (AC) stimulation was performed at B). 1 Hz and C). 2 Hz. This is equivalent to an average pulse of 60 beats per minute and 120 beats per minute, respectively. Compared to controls, the 1 Hz stimulated cells showed a significant average depolarization of ~10-12 mV (*$p < 0.05$), in a non-linear manner with increasing field strength. The 2 Hz stimulated cells also showed a significant depolarization of ~15-20 mV (**$p < 0.01$) compared to controls, and also behaved in a non-linear manner with increasing field strength.

Results - We have demonstrated that the parameterized AC electric field induces a steady-state depolarization of bovine aortic ECs. Average depolarization in the range of 10-20 mV has been shown above 1.67 V/m, with a non-linear response afterward. This observed depolarization was shown to increase with an increase in AC stimulation frequency.

Conclusion - These results show that the EVSP induces a steady-state depolarization of the EC plasma membrane in a non-linear fashion. This indicates that the EVSP is a depolarizing physical force acting on the ECs in the vessel wall. Its maintenance and possible derangement should therefore be further studied as a potential component of atherogenesis.

Acknowledgements

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