Use of translucent indium tin oxide to measure stimulatory effects of a passive conductor during field stimulation of rabbit hearts

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1Department of Biomedical Engineering, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill; 2College of Engineering, North Carolina State University, Raleigh, North Carolina; and 3Cardiac Rhythm Management Laboratory, Department of Biomedical Engineering, School of Engineering, University of Alabama at Birmingham, Birmingham, Alabama

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Knisley, Stephen B., and Andrew E. Pollard. Use of translucent indium tin oxide to measure stimulatory effects of a passive conductor during field stimulation of rabbit hearts. Am J Physiol Heart Circ Physiol 289: H1137–H1146, 2005. First published May 13, 2005; doi:10.1152/ajpheart.00064.2005.—Biomathematical models and experiments have indicated that passive extracellular conductors influence field stimulation. Because metallic conductors prevent optical mapping under the conductor, we have evaluated a passive translucent indium tin oxide (ITO) thin-film conductor to allow mapping of transmembrane potential ($V_m$) and stimulatory current under the conductor. A 1-cm ITO disk was patterned photolithographically and positioned between 0.3-cm$^2$ mesh shock electrodes on the ventricular epicardium of isolated perfused rabbit hearts stained with 4-[2-(6-dibutylamino)-2-naphthylenal]ethenyl]-1-(3-sulfopropyl)-hydroxide, inner salt (di-4-ANEPPS). For a 1-A, 10-ms shock during the action potential plateau, optical maps from fluorescence collected using emission ratioometry (excitation at 488 nm and emissions at 510–570 and >590 nm) indicated that the disk altered $V_m$ by as much as the height of an action potential. $\Delta V_m$ became more positive near the edge of the disk, where the ITO conductance gradient was parallel to applied current, and more negative near the opposite edge, where the gradient was not parallel to current. For diastolic shocks, the disk expedited membrane excitation at the sites of positive $\Delta V_m$ in the heart and in a cardiac model with realistic ITO disk surface and interfacial conductances. Optical maps of ITO transmittance and the model indicated that the disk introduced anodal and cathodal stimulatory current at opposite edges of the disk. Thus ITO allows study of the stimulatory effects of a passive conductor in an electric field.

The theoretical basis for understanding responses of heart tissue to defibrillation shocks primarily involves predictions of how changes in transmembrane potential ($\Delta V_m$) at sites distant from stimulating electrodes relate to local variations in the tissue’s volume conductor characteristics or the applied electric field. Considerable attention has been directed toward mechanisms resulting from intracellular resistance variations at gap junctions, anisotropic fiber structure, changes in fiber geometry, such as the curvature and rotation of fibers that occur in moving from the endocardium to the epicardium, gradients of the electric field, and surface boundaries (7, 8, 14, 20, 27, 30, 38, 41, 43, 45). Current redistribution resulting from unequal ratios of intracellular to interstitial resistances along and across fibers is also recognized as a mechanism for $\Delta V_m$ (42). Limited attention has been focused on the contribution of local resistance variations in the interstitium, vascular space, or extracellular conductors outside the endocardium and epicardium (9, 31, 37). Girouard and Ideker (15) demonstrated depolarization wavefront initiation on the left ventricle with an inactive wire contacting the left and right ventricles during right ventricular stimulation. The results indicate that conductive coupling by the passive wire during stimulation induced sufficient current in the wire to depolarize the tissue. Entcheva et al. (7) showed that epicardial $\Delta V_m$ induced by shocks from an intraventricular electrode was modulated by alteration of the epicardial interface and was more dependent on tissue anisotropy when the epicardium contacted an insulating face of the chamber than an extracellular bath.

Although these findings demonstrate that the combined effects of cardiac tissue characteristics and interface conditions influence shock-induced $\Delta V_m$, details of the tissue response in arrangements analogous to that considered by Girouard and Ideker (15) are complicated by the use of standard electrode materials. Optical mapping with $V_m$-sensitive fluorescent dyes during shocks allows $\Delta V_m$ measurement. The mapping requires excitation light to travel onto the tissue from an external source and fluorescence to travel from the tissue to a light detector. Examination of interactions between passive electrodes and tissue by optical mapping, therefore, requires use of an electrode material that does not block light.

We undertook the present study to assess the suitability of indium tin oxide (ITO) as such a material. Optical mapping was ratiometric, involving simultaneous fluorescence collection in two emission wavelength bands to allow correction for effects of changes in ITO transmittance in $\Delta V_m$ measurement as opposed to single-wavelength fluorescence collection. Consistent with our previous work with ITO, which includes characterization of the interface between ITO and physiological saline, we found reversible transmittance changes on the boundary of the ITO material associated with charge movement across the boundary with the heart (20, 23, 24, 27, 33). Transmittance can change when oxidation or reduction produces materials with different optical properties, e.g., tin results from reduction of tin oxide when ITO is a cathode (29). Therefore, to estimate charge entering and leaving the heart from the passive ITO electrode in the experiments, we quantified transmittance changes with nonratiometric mapping using either of the emission wavelength bands. Companion bidomain computer simulations in which ITO material resis-
tance and ITO-saline interfacial resistances were incorporated into grid generation were used to interpret $\Delta V_m$ and transmittance measurements.

**MATERIALS AND METHODS**

Fiberation of the conductive disk. ITO (Thin Film Devices, Anaheim, CA) was sputtered to a thickness of 850 nm onto one side of borosilicate glass plates. Average ITO sheet resistivity was 1.5 $\Omega$ per unit square area (a standard unit of material resistance), and transmittance was 0.75–0.85 for light wavelengths in the range 450–800 nm (2). After the ITO was cleaned with acetone and dehydrated, it was lithographically patterned with photoreist and etched for 45 min in a solution consisting of 75 parts H2O, 20 parts HCl concentrate (36.5%), and 5 parts HNO3 concentrate (69.5%) at 50–55°C (33). The pattern of remaining ITO was a 1-cm-diameter disk on the glass.

Experiments with rabbit heart. Hearts were removed from six New Zealand White rabbits with approval of the Institutional Animal Care and Use Committee and arterially perfused with physiological saline and stained with 4-[2-(6-(dibutylamino)-2-naphthalenyl)ethenyl]-1-(3-sulfopropyl)-, hydroxide, inner salt (di-4-ANEPPS; Molecular Probes, Eugene, OR) (22). Experiments were performed at a single ventricular epicardial region in one heart and at anterior and posterior regions in five hearts. Five regions that produced acceptable ratiometric emission bands were used for analysis. The ventricular epicardium was held in contact with an ITO disk (Fig. 1) by gentle pressure from a flexible sponge on the opposite side of the heart. Stainless steel 3 cm × 10 mm mesh electrodes for shock current application were positioned outside the left and right edges of the disk and 20 mm apart. A 6 × 12 mm grid of 128 laser spots was positioned between the mesh electrodes. Distance from the grid to each mesh electrode was ~4 mm to avoid measurement of $\Delta V_m$ within a few space constants of each electrode (18). The plate was mounted on a manipulator to laterally slide the ITO disk into the grid area or away from it, leaving glass in contact with the heart.

In experiments to measure $\Delta V_m$, hearts were paced from a bipolar Ag-AgCl electrode located to the right of the grid at a cycle length of 300 ms with 3-ms S1 pulses, alternating polarity, and a strength of two to three times threshold. A 10-ms, 1-A S2 field stimulus was applied during the plateau phase of an action potential from the mesh electrodes that were in contact with the heart. The S2 was not applied from the ITO disk. The S2 polarity was reversed in different trials. In experiments to measure excitation times, the S1 was removed, and field stimulation was applied from the mesh electrodes during diastole with a cycle length of 300 ms, duration of 5 ms, alternating polarity, and strength of 30–1,000 mA in 11 steps.

Optical mapping was performed with a laser scanner system controlled with a microprocessor (33, 34). Fluorescence excited at each spot by a 488-nm laser beam was collected in two wavelength bands for ratiometry with a dichroic mirror (model 590dcplxr, Chroma Technology, Rockingham, VT) positioned 6 cm from the heart and on the same side as the laser. Red light passed through the mirror and a filter (model OG590, Melles Griot, Rochester, NY) into a photomultiplier tube while green light reflected from the mirror and passed through a filter (510–570 nm; model D540/60M, Chroma Technology) into another photomultiplier tube. Direct current-coupled signals proportional to laser intensity, fluorescence, and applied current were recorded at a sampling rate of 1 kHz.

Analysis of fluorescence recordings. To examine ITO transmittance changes, we measured averages of fluorescence for each of the red and green emission bands during individual cardiac cycles before and after the cycle that received S2 and used the square root of the recorded fluorescence intensity to account for attenuation of the excitation and fluorescence light by ITO (Fig. 2) (33). No baseline adjustments were performed for measurements of the transmittance changes.

$\Delta V_m$ and excitation times were measured using a ratio of green fluorescence to red fluorescence. Each of the $\Delta V_m$ measurements was defined as the average ratio during S2 minus the average ratio at the same time relative to phase-zero depolarization in a preceding action potential with no S2 and was expressed as a percentage of the height of the phase-zero depolarization. The effect of the disk on $\Delta V_m$ was determined by subtracting $\Delta V_m$ produced by S2 with no disk on the heart from $\Delta V_m$ produced by an identical S2 with the disk on the heart (difference in $\Delta V_m$). Times of excitation produced by diastolic field stimulation were determined at the maximum slope of phase-zero depolarization.

Wavelength dependence of the change in transmittance or slight nonlinearity of light detection systems may cause unequal changes in red and green fluorescence signals during a shock. To check whether this affected our results, we also analyzed $\Delta V_m$ in all experiments after adding constants to green fluorescence signals so that percent changes in the red and green signals due to transmittance were the same (33).

Recordings were analyzed with programs written in Matlab (Mathworks, Natick, MA). Optical recordings were digitally smoothed with a 3-ms boxcar filter. Significance at $P < 0.05$ was evaluated with two-tailed large-sample test or t-test using Matlab or Graphpad Prism. Numerical summaries are given as mean (SD).

Active bidomain simulations. Simulations represented a layer of ventricular tissue with intracellular (i) and interstitial (o) components coupled to an extracellular (e) layer with or without an ITO disk. Electrical activity in the tissue layer was modeled by

$$\nabla \cdot \left[ \sigma \nabla (V_m + \varphi_s) \right] = \beta \left( \frac{\partial V_m}{\partial t} + I_{ion} \right) $$

and

$$\nabla \cdot \left[ (\sigma_i + \sigma_o) \nabla \varphi_s \right] = -\nabla \cdot (\sigma_i \nabla V_m)$$

where $\sigma$ is specific conductivity tensor, $V_m$ is transmembrane potential, $\varphi$ is potential, $\beta$ is the ratio of membrane surface to element...
Equations 1 and 2 were discretized using a finite difference approach (25, 40). Values along fibers (for $\sigma_l$ and $\sigma_i$) were based on the report by Kléber and Riegger (18), who measured specific intracellular ($R_l$) and extracellular ($R_i$) resistivities of 166 $\Omega \cdot$cm ($=6.02$ mS/cm) and 63 $\Omega \cdot$cm ($=15.85$ mS/cm), respectively, in perfused rabbit papillary muscles. We prescribed longitudinal intra- and interstitial conductivities of 4.82 and 3.17 mS/cm, consistent with an intracellular volume fraction of 0.8, on the basis of morphometric measurements by Polimeni et al. (39). Values across fibers (for $\sigma_l$ and $\sigma_i$) were based on longitudinal-to-transverse conductivity ratios reported by Clerc (4). Use of intracellular and interstitial ratios of 9.4 and 2.7 led to transverse conductivities of 0.51 and 1.18 mS/cm, respectively. The value for $\beta$ was set to 6,350/cm to reflect rabbit left ventricular epicardial myocytes (13). $C_{in}$ was set to 1 $\mu$F/cm$^2$ to reflect nominal values for biological tissue. $I_{ion}$ was determined from solution of the Luo-Rudy dynamic membrane equations (36). The overall dimensions for the tissue layer were 2 cm (longitudinal) $\times$ 1.25 cm (transverse). The layer was discretized using space steps of 100 $\mu$m for the nodes placed along fibers and 25 $\mu$m for the nodes placed across fibers, which produced 100,000 nodes. No-flow boundary conditions were assigned on all edges.

Electrical activity in the extracellular layer was modeled by

$$\sigma_e \nabla^2 \varphi_e = f_{es},$$

(3)

where $f_{es}$ is extracellular injected stimulus current density. In discretizing Eq. 3, we used space steps identical to those in the tissue layer to place 100,000 extracellular nodes directly above the tissue nodes. Here, we separated extracellular and tissue nodes by 20 $\mu$m to represent a layer of perfusate on the tissue surface (1, 47). To represent the case with no disk, we prescribed isotropic conductivity of 15.85 mS/cm (18). To represent the arrangement with the ITO disk, we identified all extracellular nodes located inside a 1-cm-diameter circle centered in the 20-$\mu$m extracellular layer. For all internal connections between those nodes, we prescribed isotropic conductivity of 318,000 mS/cm. With this conductivity, resistance between opposite sides of a 20-$\mu$m cube is 1.5 $\Omega$, in agreement with the...
nominal resistivity value of the ITO. To represent the interfacial resistance between extracellular and adjacent interstitial nodes, we prescribed conductivity of 0.2 mS/cm in the direction perpendicular to the extracellular layer. This value produced conductance across a 20-μm layer of 1,000 S/m², which agrees with our measurements of interfacial conductance between ITO and adjacent solution (33).

Difference equations resulting from discretization of Eqs. 1–3 were solved iteratively using the conjugate gradient method to produce 100,000 \( V_m \) values, 100,000 \( \phi_e \) values, and 100,000 \( \phi_i \) values at 2-μs time steps for a 35-ms simulation. During the first 1 ms of each simulation, no stimuli were applied, so model variables achieved stable initial conditions. Then depolarization was initiated with S1 transmembrane current injection \( (I_m = 19 \mu A/cm², 2\text{-ms duration}) \) at every tissue node. At 25 ms, S2 (0.079 mA, 10-ms duration) was injected into nodes located on one model edge and removed from nodes located on the opposite model edge 2 cm away.

For each simulation, action potentials along fibers on the model’s centerline were recorded, and values for \( V_m, \phi_e, \) and \( \phi_i \) were archived for all nodes. To determine \( \Delta V_m, V_m \) during S2 was offset by \( V_m \) at the corresponding time in a different simulation with no S2 (36.2 mV). For comparison with the change in transmittance in experiments, interfacial charge was determined as the time integral of current between interstitial nodes in the tissue layer and adjacent extracellular nodes in the layer containing ITO.

**Passive bidomain simulations.** To examine the influence of the additional tissue layers associated with the three-dimensional ventricular wall in experiments with hearts, we measured \( \Delta V_m \) and interfacial current in simulations with passive bidomain models. Because of the computational complexity of three-dimensional models, we viewed the completion of passive simulations as practical to demonstrate effects of deeper tissue layers on \( \Delta V_m \). For the passive simulations, we used a computational procedure analogous to that in the active simulations and solved the following equations

\[
\nabla \cdot [\sigma \nabla (V_m + \phi_i)] - \frac{V_m}{R_m} = 0 \quad (4)
\]

and

\[
\nabla \cdot (\sigma \nabla \phi_e) + \nabla \cdot [(\sigma + \varepsilon) \nabla \phi_i] = 0 \quad (5)
\]

simultaneously with Eq. 3. In Eq. 4, \( R_m \) was set to 5 kΩ·cm² to represent ventricular myocytes. The number of tissue layers was adjusted from 4 (60-μm tissue thickness) to 25 (480-μm tissue thickness). Changes in \( \Delta V_m \) and interfacial current were quantified relative to changes measured in simulations with only four tissue layers.

**RESULTS**

**Fluorescence recordings under ITO during stimulation.** Recordings of the green and red fluorescence at a laser spot under the ITO disk, their ratio, the modified green signal, the modified ratio, and the simultaneously recorded S2 shock current are shown in Fig. 2. For polarity 1, the mesh electrode on the left of the grid was the anode and the mesh electrode on the right was the cathode. Leads were then reversed and S2 was repeated (polarity 2).

Green and red fluorescence signals were morphologically complex and included deflections that did not correspond to phases of an action potential. Deflections consistent with the \( V_m \)-sensitive response of di-4-ANEPPS during phase-zero depolarization occurred in opposite directions in green and red signals. [These deflections occur simultaneously with the action potential upstrokes in the ratios (11, 35).] Deflections produced by cardiac motion had a common direction in green and red signals, e.g., motion artifacts encircled in the green and red signals for polarity 1 (16, 22, 28). In addition, common changes in ITO transmittance during S2 consisted of a small increase for polarity 1 and a decrease for polarity 2. The changes in transmittance persisted after S2, producing slightly elevated levels of green and red signals after S2 for polarity 1 and depressed levels for polarity 2. These effects of motion and transmittance obscured plateau and repolarization phases of the action potential and the \( \Delta V_m \) in green and red signals. Computation of the ratio of these two signals (green signal divided by red signal), which cancels any changes that are equivalent to multiplication of red and green signals by a function, revealed \( V_m \)-dependent deflections (22, 28, 33).

The transmittance-induced changes in the averages of the fluorescence during cycles before and after S2 were not always the same for green and red fluorescence. For polarity 1 in Fig. 2A, the percent changes in green and red fluorescence, 100 * (b – a)/a and 100 * (d – c)/c, were 1.7%, and there was negligible change in the ratio. For polarity 2, the change in green fluorescence was −5.3%, while the change in red fluorescence was −4.5% and the ratio decreased 1%. To determine whether this affected measurements of \( \Delta V_m \), we measured \( \Delta V_m \) with the ratio computed as the green waveform divided by the red waveform (e.g., 3rd waveform in Fig. 2A) and with a modified ratio (5th waveform). The modified ratio was computed with a modified green waveform (4th waveform) produced by adding a constant, \( k = (ad – cb)/(c – d) \), which satisfies \( (a + k)/c = (b + k)/d \). This modification ensured that the percent changes in the modified green and red fluorescence due to transmittance were identical. The constant was added only to the segment indicated by the horizontal bar beneath the modified green signal, which contained the action potentials used to measure \( \Delta V_m \) (Fig. 2B).

**Heterogeneous extracellular conductance and \( \Delta V_m \).** To test whether the ITO disk affected \( \Delta V_m \), we measured S2 polarities with and without the disk on the epicardium. Figure 3 shows ratiometric action potentials at spots on the left and right halves of the laser grid in a single heart. Plots show the enlarged segment of the recordings containing the phase-zero depolarization and the S2. With an ITO disk on the heart, the recording nearer the anodal mesh electrode underwent positive \( \Delta V_m \) (first and last recordings in the top row), whereas the recording nearer the cathodal mesh electrode underwent negative \( \Delta V_m \). At each of these two spots, reversal of the polarity of leads produced reversal of the signs of \( \Delta V_m \), although signs of \( \Delta V_m \) at some of the other spots did not reverse. When the two S2 shocks were repeated without the ITO disk on the heart, the recording nearer the anodal mesh electrode underwent positive \( \Delta V_m \) while the recording nearer the cathodal mesh electrode underwent negative \( \Delta V_m \). Figure 4 shows \( \Delta V_m \) for each S2 polarity, with the disk on the heart and after the disk was removed. With the disk, areas in the half of the grid closer to the anodal mesh electrode underwent positive or negligible \( \Delta V_m \) (e.g., green and yellow areas). Without disk, \( \Delta V_m \) in these areas was negative (e.g., blue areas). To distinguish effects of the disk on \( \Delta V_m \), the difference between \( \Delta V_m \) without the disk and \( \Delta V_m \) at the same spots with the disk, i.e., the difference in \( \Delta V_m \), is shown. Plots of the difference in \( \Delta V_m \) contained red areas on the half closer to the anodal mesh electrode and blue areas on the half closer to the cathodal mesh electrode.
In three mapped regions, the difference in $\Delta V_m$ analyzed using the ratio and expressed as percentage of action potential amplitude for S2 with polarity 1 was 10 (SD40) at all laser spots in the half of the grid closer to the anodal mesh electrode ($P < 0.001$ vs. zero, $n = 192$ spots) and $-22$ (SD30) at the spots in the half closer to the cathodal mesh electrode ($P < 0.001$). For polarity 2, the difference in $\Delta V_m$ was 4.3 (SD32) at the spots in the half closer to the anodal mesh electrode ($P = 0.031$) and $-20$ (SD40) at the spots in the half closer to the cathodal mesh electrode ($P < 0.001$). The same recordings were analyzed using the modified ratio for all cases in which the percent change in red fluorescence due to transmittance was $\geq 1\%$. With the use of the modified ratio, the difference in $\Delta V_m$ with polarity 1 was 11 (SD41) in the half closer to the anodal mesh electrode and $-27$ (SD34) in the half closer to the cathodal mesh electrode; with polarity 2, it was 8.8 (SD33) in the half closer to the anodal mesh electrode and $-23$ (SD42) in the half closer to the cathodal mesh electrode ($P < 0.001$ in each test).

Consistent with these effects of the disk, we found that the field stimulation during diastole produced early excitation at the edge of the disk closer to the anodal mesh electrode (i.e., the edge where positive $\Delta V_m$ was found). For an applied current of 62 mA (SD26), the excitation was as much as 5.1 ms (SD2.5) earlier than the excitation with no disk ($P = 0.027$, $n = 4$, regions $\times$ polarity). Results were qualitatively similar when diastolic stimulation was applied in the active bidomain model.

**Local heterogeneity-induced stimulatory current.** We believe that $\Delta V_m$ observed near the disk during shocks resulted from the abrupt change in resistance between the heart’s extracellular space and the disk, with that change establishing stimulatory current that was maximal at the ITO edge. Evidence in support of this interpretation is presented in Fig. 4 as $\Delta$transmittance during trials with and without the disk on the heart. As we showed previously, $\Delta$transmittance corresponds to applied current during experiments with a current source connected directly to the ITO disk (33).
With the disk, Δtransmittance decreased in the half closer to the anodal mesh electrode and increased in the half closer to the cathodal mesh electrode. This finding is consistent with cathodal stimulatory current (from the tissue to the disk) at the side of the disk nearer the anodal mesh electrode and anodal stimulatory current (from the disk to the tissue) at the side nearer the cathodal mesh electrode. As a check of our method, the absence of Δtransmittance when there was no ITO disk on the heart is shown in Fig. 4. In combined results using the red fluorescence from three mapped regions, Δtransmittance for polarity 1, expressed as percentage of preshock transmittance, was −1.1 (SD1.4) at the laser spots in the half closer to the anodal mesh electrode (P < 0.001 vs. zero, n = 192 spots in this half of the grid for all 3 regions combined) and 0.9 (SD1.6) at the spots in the half closer to the cathodal mesh electrode (P < 0.001). For polarity 2, Δtransmittance was −1.7 (SD1.7) at the spots in the half closer to the anodal mesh electrode (P < 0.001) and 1.1 (SD1.3) at the spots in the half closer to the cathodal mesh electrode (P < 0.001). With the use of the green fluorescence, Δtransmittance for polarity 1 was −1.2 (SD1.5) in the half closer to the anodal mesh electrode and 1.1 (SD1.9) in the half closer to the cathodal mesh electrode. For polarity 2, it was −2.0 (SD2.0) in the half closer to the anodal mesh electrode and 1.3 (SD1.6) in the half closer to the cathodal mesh electrode (P < 0.001 in each test).

As expected, differences in ΔVm and Δtransmittance were largely uncorrelated. To test whether there was a relation between the difference in ΔVm and Δtransmittance, for three mapped regions we plotted measurement pairs for the difference in ΔVm and Δtransmittance at all 128 laser spots and performed minimum mean-square-error linear fits. Figure 5 shows the relation between these two variables measured at the laser spots during shocks of each polarity in one experiment. Negative signs of correlation coefficient indicate a trend for a decrease in Δtransmittance as difference in ΔVm increased. Overall correlation coefficients (R) from linear regressions of difference in ΔVm vs. Δtransmittance were −0.65 for polarity 1 and −0.58 for polarity 2. When the data points with magnitudes of Δtransmittance >3% were omitted in Fig. 5, we found R = −0.75 for polarity 1 and R = −0.66 for polarity 2. Across all experiments and data points, we found R = −0.32 (SD0.30) (n = 6, regions × polarities).

Active bidomain simulations. Responses in the active simulations were qualitatively similar to those in the experiments. Figure 6A shows action potentials recorded on the central axis during simulations with and without the central region representing the ITO disk. Because models were symmetrical about the vertical axis, results are shown for only one S2 polarity (the other polarity reverses left and right recordings). Action potentials were recorded 1–9 mm from the anodal edge and 1–9 mm from the cathodal edge of the model at 1-mm intervals. Near the anode and cathode (1-mm locations), comparable ΔVm were recorded with and without the disk. Near the disk, ΔVm differences were observed with ΔVm of comparable magnitude to the action potential upstroke recorded at the edge of the disk (5 mm) and smaller-magnitude ΔVm with increasing distance from the disk edge (4 and 6 mm). Without the disk there was negligible ΔVm in the central area. Figure 6B shows contour maps of ΔVm with the disk, ΔVm without the disk, the difference in ΔVm and the charge during shock application from the active bidomain simulations. As in the experiments, ΔVm differences were observed over a larger spatial extent than the interfacial charge during shock application.

Although qualitatively similar, one main quantitative difference between the experiments and simulations was related to interfacial charge. In the active bidomain simulations, total charge measured in a disk half was 25 nC, which corresponded to 317 nC/mA of applied current. By comparison, the result from experimental measurements was 163 nC/mA of applied current, which was calculated using the average magnitude of Δtransmittance with red fluorescence (mean 1.2%), the area mapped within each half of the disk (0.28 cm²), and the reported calibration factor of 20% change in ITO transmittance per 1 A/cm² for a 10-ms shock (33).

Passive bidomain simulations. To quantify the extent to which modeling a single tissue layer affected ΔVm and inter-
facial charge, we completed passive bidomain simulations in which tissue layers were systematically added from 60 μm (4 layers) to 480 μm (25 layers). Figure 7A shows ∆V_m and interfacial current during shock application in simulations with 4 and 25 layers. Here, ∆V_m without the disk and the difference in ∆V_m were not determined, because Fig. 6B indicated that such measurements provide no additional information in the regions away from the left and right edges of the model. Also interfacial current, rather than charge, is reported, because these simulations had no time dependence. The main consequences of adding tissue layers were a reduction in peak values of ∆V_m and interfacial current at the disk edge and increased spatial extents over which ∆V_m and interfacial current were induced near that edge (Fig. 7B). Figure 7C shows the peak and total interfacial current on half of the disk, expressed as percent change from the four-layer model for all simulations. The peak interfacial current reduced to 57%, while the total current increased to 228%, when 25 layers were included, indicating that tissue thickness affects the magnitude and distribution of the current.

Fig. 6. Results from active bidomain model containing a single tissue layer. A: effect of S2 field stimulation for V_m along the fiber axis on the model's centerline between mesh electrodes. Solid traces, simulations with no disk; dashed traces, simulations with the ITO disk. Waveforms on left were recorded 1–9 mm from the anode, which was located on the left edge of the model; those on right were recorded 1–9 mm from the cathode, which was located on the right edge. Horizontal dashed lines on each upstroke denote 0 mV. B: maps of ∆V_m and charge associated with local heterogeneity-induced current flow between the extracellular layer and the interstitium in the tissue layer during plateau phase S2 field stimulation. Top map shows ∆V_m at the end of S2 with the disk in the extracellular layer. Tick marks indicate locations from which recordings are shown in A. Second map shows ∆V_m with no disk. Third map shows the difference between top map and the second map, calculated as for hearts. Fourth map shows distribution of charge at interface of extracellular and tissue layers during S2. A positive value of charge (white) indicates that local current flowed from the tissue to the ITO layer.

Fig. 7. Results from passive bidomain models showing effects of inclusion of additional tissue layers on ∆V_m and interfacial current during field stimulation with the ITO disk. A: ∆V_m with 4 and 25 tissue layers (top) and interfacial current with 4 and 25 tissue layers (bottom). A positive value of current (white) indicates flow from the tissue to the ITO layer. B: V_m and interfacial current vs. distance along the centerline of the models with 4 (solid line) and 25 (dotted line) tissue layers. C: magnitudes of peak and total interfacial current for half of the disk vs. tissue thickness.
Figure 8 shows plots of $\Delta V_m$ vs. interfacial current in the region of the passive bidomain models containing the ITO disk. As in experiments with hearts, there was a trend for decreased interfacial current as $\Delta V_m$ increased (Fig. 8, A and B). Also, similar to results from hearts, the plots tended to flatten at large values of interfacial current. The correlation coefficient between $\Delta V_m$ and interfacial current was larger than that in hearts and ranged from $-0.86$ for the 60-µm tissue thickness passive model to $-0.98$ for the 480-µm tissue thickness passive model (Fig. 8C).

**DISCUSSION**

**ITO for optical mapping of cardiac stimulation.** The ITO conductor contained oxides of indium and tin sputtered onto a substrate to produce a thin conductive film suitable for lithographic patterning. In contrast to many other solid conductive materials, ITO was translucent, which allowed us to perform the present study, because excitation light could be passed to the heart and fluorescence could be collected from the heart for optical mapping of $V_m$-sensitive fluorescence and ITO transmittance. The film was thin and optically flat, which prevented optical distortion of the excitation and fluorescence light and allowed the glass substrate and disk to be moved laterally on the moist epicardium without noticeable friction. Although we used a simple disk pattern, ITO can be patterned to produce an arbitrary planar geometry. Also, sheet resistance of ITO can be varied by altering the thickness deposited during sputtering or by thinning the ITO film with timed exposure to acid (20). It is conceivable that ITO may be produced with electrical conductance and size approximating cardiac extracellular features such as coronary vessels and interstitial spaces, as well as artificial conductors (9, 10, 15, 19, 44).

In a previous study, we found that ITO transmittance can change when current is delivered directly across the ITO-heart interface, decreasing during cathodal stimulation from ITO or increasing during later anodal stimulation due to electrochemical changes that occur at the surface of the ITO, where current flows between the ITO and the saline (33). Although we found that transmittance changes can indicate interfacial current distribution with nonratiometric optical mapping, the alteration of the amount of fluorescence that reaches the light collector during a shock interferes with measurements of $\Delta V_m$, when the fluorescence in only a single-emission wavelength band is collected. To overcome this for $\Delta V_m$ measurement, we used a ratiometric optical mapping system containing two photomultiplier tubes coupled by direct current to digitizers to produce signals proportional to the intensities of red and green light reaching the respective photocathodes.

In contrast to motion and transmittance changes, the $V_m$-dependent shift of di-4-ANEPPS fluorescence toward shorter wavelengths during membrane depolarization produces a decrease in the intensity of the longer-wavelength red fluorescence and an increase in the intensity of the shorter-wavelength green fluorescence (11, 22). These opposite effects in red and green signals are evident during phase-zero depolarization (Fig. 2). We found that the ratio of the signals canceled the large deflections due to ITO transmittance changes or cardiac motion common to green and red signals (22, 28). However, because the ratio of the signals retained $V_m$-dependent deflections, we were able to measure $\Delta V_m$ optically under the electrode and without addition of motion-inhibiting drugs to the perfusion solution.

It is possible to describe the theoretical effects of ratiometry in terms of multiplicative vs. additive changes in the measured fluorescence. In the case of the same percent change in transmittance for the red and green emission, the effect is equivalent to multiplication of the numerator and denominator by the same value, which will be cancelled in the ratio. A motion artifact that alters the red and green signals proportionally will cancel, similar to our results showing reduced motion artifacts using the ratiometric signal compared with the red and green signals (22). On the other hand, the opposite changes in the...
green and red signals due to alterations in $V_m$ can be described as addition of a value to the green signal (numerator) and subtraction of a value from the red signal (denominator), which will not cancel in the ratio.

Because full cancellation of the effects of transmittance is expected only for proportional changes in the green and red signals, a difference in $\Delta$transmittance for the two signals may influence ratiometric measurements of $\Delta V_m$ under the disk. To test this, we analyzed the data using the original ratio and again using the modified ratio, for which transmittance-induced percent changes in red and modified green signals were the same (Fig. 2A) (33). Magnitudes of the difference in $\Delta V_m$ under the passive conductor using the modified ratio were larger by an average of 1–5% of the action potential amplitude. Both analyses indicated the same conclusions regarding the signs of the difference in $\Delta V_m$ under the left and right halves of the passive conductor.

$\Delta V_m$ produced by conductance heterogeneity. In experiments, $\Delta V_m$ with the ITO disk differed from $\Delta V_m$ with no disk. With no disk, $\Delta V_m$ were negative in the half of the grid closer to the anodal mesh electrode and positive in the half closer to the cathodal mesh electrode (Fig. 4). These $\Delta V_m$ may be due to factors such as heart structure or electric field (9, 27, 46). The disk produced additional effects on $\Delta V_m$ indicated by comparing maps with the disk with maps with no disk (Figs. 4 and 6B). The difference in $\Delta V_m$ was positive in the half closer to the anodal mesh electrode and negative in the half closer to the cathodal mesh electrode.

Local heterogeneity-induced stimulatory current and $\Delta V_m$. We found that ITO transmittance decreased in the half of the disk closer to the anodal mesh electrode, which indicates cathodal current across the interface (33). Also, ITO transmittance increased on the half closer to the cathodal mesh electrode, indicating anodal current across the interface. The finding that $\Delta$transmittance decreased as difference in $\Delta V_m$ increased (measurements in left and right halves of the disk and negative correlation coefficients in Figs. 4 and 5) supports the conclusion that local interfacial current altered $V_m$; however, these variables are not well correlated in hearts. A full explanation may need to incorporate possible nonlinearities of the transmittance change with interfacial current and the membrane response to shocks during the action potential (26). However, even in our passive models, in which the membrane was linear and current was determined without using transmittance, plots of $\Delta V_m$ vs. interfacial current for all spots did not fit a straight line (Fig. 8). The contributions of nodes undergoing a change in $V_m$ where there was little interfacial current produce marked variations from a straight line in Fig. 8, A and B (e.g., points describing a steeper slope near the centers of the plots). Variations may occur, because some of the extracellularly injected current flows away from its point of injection before it crosses the membrane (e.g., current injected at a point produces $\Delta V_m$ distributed over several millimeters) (21, 27, 42, 48). The large correlation coefficients compared with those from experiments suggest that contributions of many nodes away from the centers may increase the coefficient.

Our bidomain models indicated that interfacial charge became more widely distributed when additional tissue layers were added (Fig. 7). This is consistent with the finding that $\Delta$transmittance in hearts was distributed as far as several millimeters from the disk edge (Fig. 4). A wider distribution of $\Delta$transmittance and interfacial current in experiments and in the multilayer models than in the single-layer model may occur when current in deeper tissue layers flows farther toward the center of the disk before emerging at the ITO-tissue interface.

Implications for far-field stimulation. With a 1-A shock during the action potential plateau, we found that magnitudes of the difference in $\Delta V_m$ under the disk were frequently as large as the action potential amplitude (Fig. 4). A smaller positive $\Delta V_m$ in diastole is sufficient to activate membrane sodium channels (5, 12, 49). Thus far-field stimulation with the disk should occur with a weaker applied shock, which is consistent with our measurements of early excitation under the disk produced with diastolic stimulation having a mean strength of 62 mA. Also, $\Delta V_m$ and early excitation with the disk support theoretical predictions that extracellular heterogeneity can play a role in the mechanism of field stimulation and defibrillation (3, 10, 32).

Limitations. The magnitude of $\Delta V_m$ per unit S2 current near the edge of the disk in the models was larger than the $\Delta V_m$ in hearts [e.g., peak difference in $\Delta V_m$ was 140 mV for 79-$\mu$A S2 current (Fig. 6A) and ~100 mV for 1-A S2 current (Fig. 4)]. This is partly explained by small underestimation of $\Delta V_m$ using the ratio and effects of additional tissue layers. For example, with 25 layers, $\Delta V_m$ at the edge of the disk was approximately one-third of that obtained with 4 layers (Fig. 7B). A previous three-dimensional model gave a similar discrepancy, even though in that case the ITO was the active electrode and spatial averaging was performed to mimic the optical averaging in experiments (33). (That model used a 0.010-ms time step, rather than 0.002 ms in the present active model, similar space steps and membrane capacitance, membrane resistance similar to the present passive models, membrane surface-to-volume ratio half of the present value, and specific tissue conductivities 0.27–0.63 of present values, but similar anisotropy ratios for the intracellular and extracellular spaces. Also that model omitted the ITO resistance and polarization impedance.) We also noted that $\Delta V_m$ was distributed over a wider area in hearts than in the models. A wider distribution in hearts may depend on complexities of tissue structure, such as fiber rotation and heart curvature, not included in our models. $\Delta V_m$ measurements in hearts may be influenced by lateral and depth averaging in optical measurements due to light scattering in the tissue (6, 17). Finally, the magnitude of interfacial charge depends on tissue thickness (Fig. 7C) and may differ in experimental measurements compared with models, because the experimental charge was determined indirectly using estimated changes in transmittance. However, there is still an order of magnitude agreement between the values for interfacial charge in the experiments and in the models.

GRANTS

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REFERENCES


