Measuring surface potential components necessary for transmembrane current computation using microfabricated arrays

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Wiley, J. James, Raymond E. Ideker, William M. Smith, and Andrew E. Pollard. Measuring surface potential components necessary for transmembrane current computation using microfabricated arrays. Am J Physiol Heart Circ Physiol 289:H2468–H2477, 2005. First published August 5, 2005; doi:10.1152/ajpheart.00570.2005. —This study was designed to test the feasibility of using microfabricated electrodes to record surface potentials with sufficiently fine spatial resolution to measure the potential gradients necessary for improved computation of transmembrane current density. To assess that feasibility, we recorded unipolar electrograms from perfused rabbit right ventricular free wall epicardium (n = 6) using electrode arrays that included 25-μm sensors fabricated onto a flexible substrate with 75-μm interelectrode spacing. Electrode spacing was therefore on the size scale of an individual myocyte. Signal conditioning adjacent to the sensors to control lead noise was achieved by routing traces from the electrodes to the back side of the substrate where buffer amplifiers were located. For comparison, recordings were also made using arrays built from chloridized silver wire electrodes of either 50-μm (fine wire) or 250-μm (coarse wire) diameters. Electrode separations were necessarily wider than with microfabricated arrays. Comparable signal-to-noise ratios (SNRs) of 21.2 ± 2.2, 32.5 ± 4.1, and 22.9 ± 0.7 for electrograms recorded using microfabricated sensors (n = 78), fine wires (n = 78), and coarse wires (n = 78), respectively, were found. High SNRs were maintained in bipolar electrograms assembled using spatial combinations of the unipolar electrograms necessary for the potential gradient measurements and in second-difference electrograms assembled using spatial combinations of the bipolar electrograms necessary for surface Laplacian (SL) measurements. Simulations incorporating a bidomain representation of tissue structure and a two-dimensional network of guinea pig myocytes prescribed following the Luo and Rudy dynamic membrane equations were completed and 33%, 76%, and 96% reductions in peak-to-peak SLs. Maintenance of comparable SNRs for source electrograms was therefore important because microfabrication provides a highly attractive methods to achieve spatial resolutions necessary for improved computation of transmembrane current density.

microimpedance; action potential propagation; Laplacian electrograms; signal conditioning; bidomain modeling

THE TRANSMEMBRANE CURRENT density (Im) in heart tissue depends on the balance between active sarcolemmal ionic currents and the passive current flowing in the intracellular, interstitial, and extracellular volume conductors. Because Im provides a direct measure of electrical source and load influences on adjacent myocytes, its in vivo computation remains an attractive goal in cardiac electrophysiological studies. Significant emphasis has been placed recently on the balance between the active ionic currents and the passive intracellular current flowing through the myoplasm and gap junctions in considering Im (14, 15, 28, 29). However, it is important to recognize that intracellular access is not required for Im computation (26, 32). In fact, Im computation from the epicardium is possible on theoretical grounds provided that J�a system of electrodes with sufficiently fine spatial resolution is available for surface potential gradient measurements in orthogonal directions, 2) a method to either measure or eliminate the potential gradient between the interstitium and the extracellular volume conductor is available, 3) a method to either measure or eliminate the transmural interstitial potential gradient is available, and 4) three-dimensional interstitial and extracellular impedance data are available to measure directional currents from the potential gradients.

The goal of the present study was to assess the feasibility of using microfabricated sensor arrays for the necessary surface potential recordings to measure surface potential gradients in orthogonal directions. Here we hypothesized advantages of using microfabrication compared with traditional hand-assembled approaches because microfabrication allows finer spatial resolution and more precise localization of individual electrodes. Although mature techniques for neural recordings using microfabricated sensors are available (8, 35), application in cardiac electrophysiological studies has been more limited (7, 12). After considering design steps associated with careful signal conditioning to achieve high-quality unipolar electrograms, we measured surface potential gradients and surface Laplacians (SLs) with microfabricated arrays at comparable signal-to-noise ratios (SNRs) to surface potential gradients and SLs measured using hand-assembled arrays in experiments involving perfused rabbit hearts. Influences of electrode separation on potential gradient and SL measurements were additionally considered in simulations incorporating a bidomain representation of tissue structure and a two-dimensional network of guinea pig myocytes prescribed following the Luo and Rudy dynamic (LRd) membrane equations (9, 10, 17, 23). Collectively, our findings support microfabrication-based

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strategies for accurate determination of surface potential contributions for improved in vivo computation of $I_m$.

**GENERAL THEORY**

The importance of the surface potential gradient and the SL for improved in vivo epicardial $I_m$ computation is apparent from inspection of the governing equation expressed in terms of the interstitial volume conductor (20, 27)

$$I_m = - \frac{\partial}{\partial x} \left( g_{o,x} \frac{\partial \phi_o}{\partial x} \right) - \frac{\partial}{\partial y} \left( g_{o,y} \frac{\partial \phi_o}{\partial y} \right) - \frac{\partial}{\partial z} \left( g_{o,z} \frac{\partial \phi_o}{\partial z} \right)$$

In Eq. 1, $g_o$ are specific interstitial bidomain conductivities in the x, y, and z directions and $\phi_o$ is the interstitial potential. Under experimental conditions in which recording electrodes are embedded in an insulating medium that limits current flowing from the interstitium to the extracellular volume conductor and activation sequences are initiated epicardially in close proximity to the electrodes to limit current flowing intramurally within the interstitium, $z$ components to Eq. 1 can be neglected. Furthermore, conditions under which directional differences in $g_{o,x}$ and $g_{o,y}$ are assumed place the main emphasis on accurate determination of the surface potential gradient terms.

Such spatial differences in surface potentials were used by Witkowski et al. (32), who assumed uniform planar depolarization wavefront expansion within the interrogation region and derived an expression for $I_m$ with the temporal derivative of the magnitude for the surface potential gradient as the sole time- and space-dependent term. Combinations of surface potentials were therefore recorded with 5-electrode arrays. Using electrodes separated from one another on a size scale of 2–3 myocytes (210 $\mu$m), those investigators estimated $I_m$ in canine ventricular epicardium and characterized shape changes in these estimates to index sodium channel activation intervals and the ratio between inward and outward sarcloemmal current during depolarization wavefront expansion. Because their approach assumed constant conduction velocity and homogeneous surface potential gradients, they were able to demonstrate consistency in the shapes for estimated $I_m$ between recordings made with 210-$\mu$m electrode separation and recordings made over a range from 65- to 420-$\mu$m electrode separation. With separations wider than 1 mm, however, estimated $I_m$ duration prolonged markedly and the timing for peak inward and outward estimated $I_m$ shifted dramatically. More recently, Coronel et al. (3) estimated $I_m$ with surface Laplacians (SLs) measured from porcine hearts using 300-$\mu$m interelectrode separation. Those investigators found analogous SL shape changes to those described by Witkowski et al. (32) with increases in electrode spacing in their $I_m$ estimates.

Although Witkowski et al. (32) suggested cellular level spatial resolution was not required to estimate $I_m$ under their assumptions, there are compelling reasons to support finer resolution if all necessary component terms for improved $I_m$ computation using Eq. 1 are to be made available. Inhomogeneities in the surface potential gradients diminish with refinement, leading to improved gradient measurements. Action potential propagation at a microscopic size scale is discontinuous (5, 30), suggesting an approach following Coronel et al. (3) is more readily applicable to cardiac tissue than the approach of Witkowski et al. (32), which assumes uniform conduction. Four-electrode impedance measurements with widely spaced electrodes provide effective tissue impedances that are complex combinations of the intracellular, interstitial, and extracellular impedances (21), whereas microimpedances measured with stimulating and recording electrodes separated on a cellular size scale include no intracellular contributions (19). Systems including small, closely spaced recording electrodes for precise potential gradient measurements in orthogonal directions that are bounded by stimulating electrodes to allow interstitial microimpedance measurements in these directions (24) will therefore be required.

**METHODS**

**Heart preparation.** Experiments were approved by the Institutional Animal Care and Use Committee. Six New Zealand White rabbits (3–5 kg) were anesthetized with intramuscular ketamine (44 mg/kg) and xylazine (20 mg/kg), followed by intravenous pentobarbital sodium (2.5 ml) and heparin (2.0 ml). Hearts were rapidly excised after a medial sternotomy and retrogradely perfused through the aorta with 3–4 liters of recirculating, oxygenated (95% $O_2$-5% $CO_2$) normal solution containing (in g/l) 7.36 NaCl, 3.96 glucose, 0.2 MgCl$_2$, 2.5 taurine, 0.65 creatine, 0.55 sodium pyruvate, 0.14 NaH$_2$PO$_4$, 3 NaHCO$_3$, and 0.12 CaCl$_2$; pH was 7.3–7.4. Temperature was maintained at 36 ± 1°C. Flow rate was fixed at 45–50 ml/min. Hearts were positioned with the cannula oriented horizontally and the right ventricular (RV) free wall facing up. Before electrophysiological study, excess lung and fat tissue was trimmed from the preparation, although a portion was left attached to the heart so that the indifferent electrode could be placed in this inactive tissue.

**Electrode arrays.** Hand-assembled and microfabricated arrays were built using a consistent configuration. Schematic diagrams of the arrangements are summarized in Fig. 1. Two line arrays containing seven electrodes with constant interelectrode spacing were oriented perpendicular to one another such that the central point was shared on each line. This allowed surface potentials recorded with a range of interelectrode spacing during individual acquisition intervals to be completed rapidly during experiments. Hand-assembled arrays were built following the method of Witkowski et al. (32). Briefly, that method involved threading silver wires of different diameters through alternate openings in nylon mesh cloth (Small Parts). This controlled wire arrangements, with the alternation minimizing the adjacent electrodes’ influence on the potential measurements, which was identified as an important issue in SL measurements by Witkowski et al. (32). Tensioned wires were epoxied in place, then cut and sanded flush with the epoxy surface to create 13-electrode plaques. Here, the epoxy served as an insulating layer to restrict extracellular current flow away from the epicardium. One such array was built using relatively coarse wires (California Fine Wire, Grover Beach, CA) of 250-$\mu$m diameter threaded through mesh with 300-$\mu$m openings and thread diameters of 140 $\mu$m (Fig. 1A). Center-to-center interelectrode spacing therefore measured 880 $\mu$m. Here, electrodes were widely separated compared with myocytes. Another array was built using fine wires of 50-$\mu$m diameter threaded through mesh with 74-$\mu$m openings and thread diameters of 50 $\mu$m to achieve center-to-center interelectrode spacing of 240 $\mu$m (Fig. 1B). Individual electrodes in this array were therefore separated more widely than individual myocytes, with separation being on a size scale comparable to that of Witkowski et al. (32) and Coronel et al. (3). Microfabricated arrays were built by Compunetics (Monroeville, PA) who exposed 25-$\mu$m silver pads using a laser drill at 50 $\mu$m edge-to-edge separation on one side of circuit boards whose traces were routed to interface and amplification circuitry located on the opposite side of the board. Center-to-center interelectrode spacing was 75 $\mu$m (Fig. 1C), resulting in electrodes separated on the size scale of individual myocytes. By design, packages were made flexible by using DuPont AP7156 as
arrays based on findings of Hofer et al. (7), considerable emphasis was placed on signal conditioning near the electrodes themselves. Each of the 13 trace leads from the electrodes in the microfabricated arrays was routed to the input of a low-noise instrumentation amplifier (AD623, Analog Devices, Norwood, MA) that was bonded to the circuit side of the flexible board. Each instrumentation amplifier served as a buffer amplifier stage in advance of filtering and acquisition. A comparable arrangement was achieved with the hand-assembled arrays by soldering the lead wires from each array to amplifier inputs on flexible “dummy” boards in which no microfabricated electrodes were included. Lead wire lengths to dummy boards were shorter than 5 cm.

Electrogram measurements. Heats were stimulated with ventricular pacing from bipolar electrodes applied to RV epicardium located near the recording array in each experiment. Basic drive cycle lengths ranged from 300 to 500 ms, with the cycle length selected to ensure activation sequence initiation from the RV epicardium. Unipolar electrograms were acquired simultaneously and bandpass filtered at 0.05 Hz and 4 kHz cutoff frequencies before sampling at 16,000 samples/s with 14-bit resolution. For acquisition, we used 13 channels from a custom built 528-channel mapping system (34) that allows simultaneous archival of data from 528 channels at a 2,000 samples/s rate or up to 66 channels of data at the 16,000 samples/s rate. Here, the high sampling rate was required to resolve rapid signal characteristics for SLs. In each of the six experiments, 13 unipolar electrograms were recorded with coarse wire, fine wire, and microfabricated arrays under conditions with signal conditioning in close proximity to the electrodes and with signal conditioning distant (~1 m) from the electrodes. This allowed the influence of signal conditioning proximity to be quantified in terms of signal-to-noise ratios (SNRs) measured as peak-to-peak electrogram amplitude divided by root mean square (RMS) noise during diastole. All data from these 6 hearts were used for our analyses. In practice, an array was placed on the epicardium using a micromanipulator, and electrograms were collected with adjacent and distant signal conditioning. Recordings with each array were made over the course of a few minutes. In making the switch from one array to the next, we attempted to maintain the general location, although it was not practical to preserve orientation of the central recording site or of the electrode axes with each switch. A total of 78 SNR values were tabulated from electrograms recorded with and without adjacent signal conditioning for each array type.

Surface potential differences. Surface potential differences were assembled from the unipolar electrograms recorded with adjacent signal conditioning using the electrogram recorded from the central site (a) as the reference using

$$\Delta \phi_{o, b} = \phi_{o, b} - \phi_{o, a}$$

with subscript j denoting electrodes in north (N), south (S), east (E), and west (W) directions and subscript k denoting electrodes separated from a at b, c, or d as shown in Fig. 1. With coarse wires, bipolar electrograms were therefore assembled using unipolar electrograms recorded at electrodes spaced 830, 1,710, and 2,590 µm from the central reference, while separations with fine wires were 224, 348, and 720 µm and separations with microfabricated sensors were 75, 150, and 225 µm. A total of 312 bipolar electrograms were then analyzed for each spacing and each electrode type, recognizing the orthogonal orientation of the microfabricated arrays. Second-difference electrograms were then assembled from component bipolar electrograms using

$$\Delta^2 \phi_{o, a} = \Delta \phi_{o, N a} + \Delta \phi_{o, S a} + \Delta \phi_{o, E a} + \Delta \phi_{o, W a}$$

such that 78 second-difference electrograms were analyzed for each spacing and each electrode type. SNRs were calculated for all bipolar and second-difference electrograms.

Potential gradients and surface Laplacians. Potential gradients were assembled by scaling each bipolar electrogram by its associated electrode spacing. SLs were assembled by scaling each

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Fig. 1. Schematic diagrams of the electrode arrangements for the coarse wire (A), fine wire (B), and microfabricated arrays (C). The central electrode (a) in each array was bounded by additional electrodes (b, c, d) emanating in north (N), south (S), east (E), and west (W) directions. All schematics were drawn to scale with a 100-µm calibration bar included in C.
second-difference electrogram by the square of its associated electrode spacing.

**Bidomain simulations.** LRd simulations were completed using models of cardiac tissue represented as a bidomain with intracellular and interstitial portions coupled to one another via cell membrane (20, 27). In addition to Eq. 1, electrical activity was therefore described by

$$I_m = \frac{\partial}{\partial x} \left[ g_{o,x} \left( \frac{\partial V_m}{\partial x} + \frac{\partial \phi_m}{\partial x} \right) \right] + \frac{\partial}{\partial y} \left[ g_{o,y} \left( \frac{\partial V_m}{\partial y} + \frac{\partial \phi_m}{\partial y} \right) \right] \tag{5}$$

with $g_i$ specific intracellular bidomain conductivities in the $x$ and $y$ directions and $V_m$ the transmembrane potential. Domain coupling was then described by

$$I_m = \beta \left( C_m \frac{\partial V_m}{\partial t} + I_{im} \right) \tag{6}$$

with $\beta$ the ratio of membrane surface to element volume [6,350/cm following Giles and Imaizumi (6)], $C_m$ the specific membrane capacitance (1 $\mu$F/cm$^2$ as nominal for biological tissue), and $I_{im}$ the total transmembrane current density resulting from ion channels, pumps, and exchangers as described by the LRd membrane equations. To discretize Eq. 5, we used space steps of $\Delta x = \Delta y = 12.5$ $\mu$m. We prescribed values of $g_{o,x} = 3.17$ $\mathrm{mS/cm}$ and $g_{o,y} = 4.82$ $\mathrm{mS/cm}$ assuming fibers oriented in the $x$ direction, following the Kleber and Rieger (13) measurements in perfused rabbit papillary muscles after accounting for intracellular ($f = 0.8$) and interstitial ($1 - f = 0.2$) volume fractions (22). Values of $g_{o,y} = 1.17$ $\mathrm{mS/cm}$ and $g_{i,x} = 0.51$ $\mathrm{mS/cm}$ were then derived from directional conductivity ratios of 2.7 and 9.4, respectively, as reported by Clerc (2). Models measured 5 mm $\times$ 5 mm (400 nodes $\times$ 400 nodes). Full discretization resulted in sparse linear systems that we solved using the method of conjugate gradients as described in our earlier report (24). Time steps were fixed at $\Delta t = 2$ $\mu$s to ensure stable and accurate solutions during action potential depolarization.

We assumed the center point of the model was located in the center of a recording array that included 399 total electrodes oriented along fibers and 399 total electrodes oriented across fibers. Bipolar electrograms, second-difference electrograms, potential gradients, and SLs were calculated using the same approach as in the experiments, with the difference that 198 electrograms were available for analysis in each direction moving from the model center point. Action potential propagation was initiated by transmembrane current injection (300 $\mu$A/cm$^2$, 2-ms duration) in a 4 $\times$ 4 group of nodes at the model edge where electrodes oriented along fibers were located.

**RESULTS**

**Unipolar electrogram recordings.** Positioning the buffer amplification stage on the flexible substrate with microfabrication was a significant step in using these arrays for high-quality SL measurements. Adjacent signal conditioning was necessary for the microfabricated sensors because the electrode impedance was considerably larger than the impedances associated with standard wire electrodes. Figure 2 shows electrode impedance as a function of frequency with coarse wires ($n = 13$; Fig. 2A), fine wires ($n = 13$; Fig. 2B), and microfabricated sensors ($n = 13$; Fig. 2C). While impedance increased inversely with electrode diameter as expected and the impedance changes with frequency were consistent between electrode types, suggesting the major factor influencing the overall electrode impedance was the electrode size, it is important to note that the microfabricated sensors had direct-current (DC) impedances 250 times those of the coarse wires. Adjacent signal conditioning compensated for this discrepancy in our measurements as overall SNRs measured with adjacent signal conditioning were 21.2 $\pm$ 2.2, 32.5 $\pm$ 4.1, and 22.9 $\pm$ 0.7 using coarse wires, fine wires, and microfabricated sensors, respectively. Overall SNRs measured with distant signal conditioning were 23.3 $\pm$ 3.4, 18.8 $\pm$ 4.2, and 13.4 $\pm$ 2.2 using coarse wires, fine wires, and microfabricated sensors, respectively. Figure 3 shows the unipolar electrograms with the highest SNRs for each group. Electrograms shown on the left side were recorded with adjacent signal conditioning while those on the right side were recorded with distant signal conditioning. SNRs are marked to the left of each waveform. As a separate index of lead noise, each electrogram was differentiated in time since that process is known to amplify lead noise in practice. With the use of coarse wires (Fig. 3A), the largest measured SNRs were comparable at 41.6 and 49.7, with adjacent and distant signal conditioning, respectively. Temporal derivatives associated with these electrograms suggested increased lead noise with distant signal conditioning, however, as baseline oscillations became pronounced. With the use of fine wires (Fig. 3B), the largest measured SNR with adjacent signal conditioning measured 87.6 and with distant signal conditioning measured 64.1, while using microfabricated sensors (Fig. 3C), the largest measured SNR with adjacent signal conditioning measured 38.9 and with distant signal conditioning measured 28.9. Increases in baseline temporal derivative magnitudes with movement of the buffer amplifiers from the fine wire and microfabricated electrodes were comparable to the increases with movement using coarse wire electrodes.

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**Fig. 2.** Electrode impedance (M$\Omega$) as a function of frequency (Hz) in arrays built with coarse wire (A), fine wire (B), and microfabricated sensors (C).
Bipolar electrograms and potential gradients. The main reason that high SNRs for unipolar electrograms with microfabricated sensors are needed for improved in vivo computation is related to the use of these recordings for surface potential gradient measurements. The importance of fine spatial resolution is highlighted in waveforms assembled from surface potentials recorded in our bidomain simulations and shown in Fig. 4. Figure 4A, left, shows bipolar electrograms recorded with electrode separations ranging from 75 to 750 μm. This range corresponded, approximately, to the range between the finest spatial resolution achieved with our microfabrication strategy and the finest spatial resolution achieved with the coarse wire electrode arrays. As expected, both the widths and peaks of these electrograms increased as the electrodes were separated more widely. With 750-μm separation, an additional feature of the bipolar electrogram was a prolongation of the time over which the electrogram remained at peak potential. This characteristic is consistent with the positioning of recording electrodes outside the width of the electromotive surface of the depolarization wavefront (31), and its presence was associated with marked deviations in estimated $I_m$ identified by Witkowski et al. (32). Figure 4A, right, shows bipolar electrograms recorded with electrode separations of 12.5, 25.0, and 37.5 μm, which were finer separations than we achieved with microfabrication. Bipolar electrogram magnitudes fell with finer separation, suggesting high susceptibility of SNRs in experimental recordings at such separation with lead noise. Figure 4B, left, shows the potential gradients associated with the bipolar electrograms recorded using 75- to 750-μm electrode separation. Inaccuracies in both the peaks and widths of the gradient accompanied wider separation. For comparison, Fig. 4B, right, shows the potential gradients associated with the bipolar electrograms recorded with finer spacing. Although three separate waveforms were drawn, the potential gradients are indistinguishable from one another, suggesting sufficiently fine resolution for accurate gradient measurement. Figure 4C shows the maximum potential for bipolar electrograms (left) and the maximum potential gradient (right) recorded at all electrode spacings in the simulations. The range of separations used experimentally with microfabricated sensors, fine wires, and coarse wires are divided by dashed vertical lines in the right panel. Furthermore, the maximum potential gradient measured with 75-μm separation, which was the finest separation achieved with microfabrication, is marked with the filled arrow. Here, at that resolution, we did observe a reduction in the peak gradient by 10% relative to the finest separation available in the simulations, but measurement was clearly superior to that with wider separations. For example, peak gradient reduced by 42% with an electrode separation of 237.5 μm, which was a comparable spacing to the finest available with the fine wire array; and by 81% with an electrode...
ricated sensors were brought to 225 \( \mu m \) of one another, as shown in Fig. 5, indicating that separation of 875.0 \( \mu m \), which was a comparable spacing to the finest available with the coarse wire array.

Because signal conditioning was adjacent to the microfabricated sensors in our experimental arrangement, we were able to record high-quality bipolar electrograms at 75-\( \mu m \) electrode separation. Figure 5A shows SNRs from all experimentally recorded bipolar electrograms, plotted as a function of inter-electrode separation. Although mean SNRs did increase with that separation, as expected, we note that the mean SNR achieved using microfabricated sensors at 75-\( \mu m \) electrode separation exceeded that for the unipolar electrograms recorded using microfabricated sensors. Figure 5B shows coarse wire bipolar electrograms with electrodes separated by 2,590 \( \mu m \) (top), 1,710 \( \mu m \) (middle), and 830 \( \mu m \) (bottom) from one experiment. Consistent with expectations for widely spaced electrodes, peaks and widths for these electrograms shortened as the recording electrodes approached one another. These changes were even more pronounced as the fine wire electrodes were brought to 720 \( \mu m \) (top), 348 \( \mu m \) (middle), and 224 \( \mu m \) (bottom) of one another, as shown in Fig. 5C, and microfabricated sensors were brought to 225 \( \mu m \) (top), 150 \( \mu m \) (middle), and 75 \( \mu m \) (bottom) of one another as shown in Fig. 5D.

Second-difference electrograms and SLs. Advantages associated with fine spatial resolution to measure potential gradients are highly pertinent to improved in vivo \( I_m \) computation because that computation involves determination of spatial differences in directional currents. Because interstitial microimpedances were not measured here, we considered SLs as a surrogate and identified influences of electrode separation on SL that will necessarily impact \( I_m \). Figure 6A, left, shows second-difference electrograms recorded with electrode separations ranging from 75 to 750 \( \mu m \) in the simulation. Consistent with our analysis of the bipolar electrograms, second-difference electrogram magnitudes and widths increased as recording electrodes were more widely separated. Figure 6A, right, shows second-difference electrograms recorded with electrode separations of 12.5, 25.0, and 37.5 \( \mu m \). With such fine spatial resolution, electrogram magnitudes were considerably lower than magnitudes measured with wider electrode spacing. Individual traces are not marked in the panel because of the low magnitudes. Figure 6B shows SLs measured at these same electrode spacings. As with the potential gradients, marked improvements in SL measurements accompanied refinements in spatial resolution. Here, the SL measured with 75-\( \mu m \) electrode separation (left) grossly underestimated the SLs measured with the finest available spatial resolution. Peak-to-peak SL measured with 75-\( \mu m \) electrode separation more closely represented SLs measured with finer spatial resolution, although peak-to-peak magnitude was reduced by 33%. Figure 6C shows peak-to-peak second-difference electrogram (left) and peak-to-peak SL (right) magnitudes for all electrode separations considered in the simulation. Here, it is important to recognize that with electrode separations comparable to the finest available with the fine and coarse wire arrays, peak-to-peak SLs were reduced by 76% and 96%, respectively.

Figure 7A shows SNRs for all experimentally recorded second-difference electrograms. Although we observed wide variability in SNR with the most closely spaced microfabricated sensors, mean SNR was comparable to mean SNRs recorded using widely spaced coarse wires. Characteristics of the second-difference electrograms were consistent with those from previous reports and our simulations. Figure 7, B–D, shows second-difference electrograms recorded using coarse...
wires, fine wires, and microfabricated sensors, respectively. All electrograms were acquired from one experiment. With wide separation and coarse wires (Fig. 7B), the widths and peak-to-peak second differences were much more pronounced than the widths and peak-to-peak second differences with fine wire (Fig. 7C) and microfabricated (Fig. 7D) arrays.

To further appreciate the differences between second-difference electrograms and the SLs associated with improved $I_m$ computation, we scaled second-difference electrograms recorded with the microfabricated array in three different experiments by the square of the associated electrode separation. Figure 8 shows these SLs, with the records in Fig. 8A being the scaled versions of the second-difference electrograms from Fig. 7D. For reference, the SL measured using coarse wires with 830-µm electrode separation is included below the SLs measured using microfabricated sensors. As in the simulations, peak-to-peak SL diminished with electrode separations from 75 to 150 and 225 µm, suggesting the spacing available with microfabrication likely improves in vivo $I_m$ computation. SL available with the coarse wire dramatically underestimates peak-to-peak SL. SLs from the two additional experiments shown in Fig. 8, B and C, share common features with the SLs shown in Fig. 8A, as increases in peak-to-peak SL accompanied refinements in electrode spacing.

**DISCUSSION**

The goal of the present study was to assess the feasibility of using microfabricated electrode arrays to measure surface potential components necessary for improved in vivo $I_m$ computation. Arrays were assembled in such a way that small electrodes separated on the size scale of individual myocytes were patterned onto one side of an insulated flexible substrate, with trace routings to adjacent signal conditioning on the other side of the substrate profoundly impacting SNRs for unipolar electrogram recordings. By carefully controlling SNRs for unipolar electrogram recordings, we were able to combine surface potentials in bipolar and second-difference electrograms and achieve comparable SNRs to those in electrograms recorded with chloridized silver wires of the type used routinely in cardiac electrophysiological studies. SNR control was critical because surface potential gradient measurements involve scaling of bipolar electrograms by the inverse of electrode separation and SL measurements involve scaling of second-difference electrograms by the inverse of the square of that separation. We believe that the importance of the work presented here is that it is possible to acquire Laplacians on the size scale of cardiac myocytes. Because of the size of the structures involved, it was of great concern that the high impedances and resulting noise would render the measurements unusable. The fact that the SNR of the microfabricated arrays is similar to or, in some cases, better than those of the larger arrays leads us to believe that we will be able to characterize depolarization wavefront expansion at the cellular level with this approach. These findings are significant because they will facilitate device development for improved $I_m$ computation in a much wider range of preparations and situations than those considered here.

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**Fig. 6.** A: simulated second-difference electrograms recorded using different electrode separations (left) showed alongside second-difference electrograms recorded using the finest available separations (right). B: SLs measured using the second-difference electrograms from A. C: peak-to-peak potential in second-difference electrograms (left) and surface Laplacians (SLs; right) as a function of electrode separation (in µm).

**Fig. 7.** A: SNR as a function of electrode separation (in µm) for all experimentally recorded second-difference electrograms. Second-difference electrograms recorded with coarse wire (B), fine wire (C), and microfabricated sensors (D). Electrode separation for each second-difference electrogram is indicated on the left side of the trace.
Microfabrication. Our work is therefore unique compared with previous investigations that focused on the acquisition of unipolar electrograms using microfabricated sensors because we emphasized the potential gradient and SL measurements. Although the precision and consistency of silicon-based arrays used for neural electrophysiological studies (8, 35) is well documented, there have been relatively few attempts to use microfabricated arrays in cardiac electrophysiological studies. In a landmark report, Hofer et al. (7) demonstrated high-quality electrograms recorded from superfused guinea pig papillary muscles using 15 × 15 μm chloridized silver electrodes patterned onto glass with a thin-film technique. Their recordings revealed discontinuous conduction and depolarization wavefront fractionation occurring at a microscopic size scale. More recently, Kim et al. (12) evaluated the suitability of micromachined silicon probes with single or multiple shanks and iridium sensors for intramural unipolar electrogram recordings in perfused rabbit papillary muscles and isolated mouse hearts. Consistent with the present investigation, adjacent signal conditioning was identified as an essential design step for recording with sufficiently high SNR.

Cardiac mapping. Although the use of microfabrication distinguished our work because of the available spatial resolution, it is important to recognize that previous investigations involving surface potential combinations for \( I_m \) estimation have focused, primarily, on waveform morphology of the resultant electrograms for improved cardiac mapping. Many of those investigations support the use of SL for unambiguous activation time detection. During uniform action potential propagation, \( I_m \) in one cell includes an initial positive (outward current) phase whose rise is a consequence of capacitive charging of the cell membrane as a depolarization wavefront approaches the cell and whose fall is a consequence of sodium channel activation as the cell reaches threshold. That positive phase is followed by a negative (inward current) phase over which sodium channels inactivate. Assuming the time of \( I_m \) zero crossing to be correlated with peak sodium current therefore provides a measure of cellular activation that is presumably superior to standard measures based on analyses of unipolar electrogram waveform characteristics. The unipolar electrogram includes contributions from local and distant sources that complicate activation time detection in fractionated signals, leading to ambiguities in the representation of depolarization wavefront expansion with isochrone maps (1, 11). The second-difference electrogram is suitable for this purpose because the time of its zero crossing is largely independent of directional differences in the interstitial microimpedances. Superiority for SL-based isochrone map construction was therefore suggested by Witkowski et al. (32) and Coronel et al. (3), with a detailed comparison of isochrone maps assembled using unipolar electrograms recorded at 1- to 2-mm electrode spacing from canine epicardium being completed by Punske et al. (25), who found maps assembled using the time of SL zero crossing correlated well with maps assembled using the time of peak surface potential gradient magnitude under conditions where errors were observed in maps assembled using the time of the minimum derivative of unipolar electrogram. Although the focus here was not on activation time detection, we do note likely advantages to using microfabricated sensors for this purpose because peak-to-peak SL magnitude increases so dramatically with fine spatial resolution.

Although both Witkowski et al. (33) and Coronel et al. (3) recognized the importance of improved activation time detection available from zero crossing times, each group found additional features of waveform morphology illustrative in understanding the spatial and temporal evolution of these arrhythmias. For example, Coronel et al. (3) identified positive SLs at sites of depolarization wavefront collision, negative SLs at sites of depolarization wavefront initiation, and sustained positive or negative baseline SLs in regions of conduction block during VF mapping. Witkowski et al. (33) systematically compared the ratio of the area under the estimated inward \( I_m \) curve to the area under the estimated outward \( I_m \) curve during VF and found that ratio was balanced when surface unipolar potential deflections resulted from local cellular activation identified using \( V_{m} \)-dependent fluorescence collected in an adjacent optical fiber. Surface unipolar deflections associated with electrotonic interactions showed marked reduction in the inward current as indexed by its area. Routine segmentation of active from passive deflections was therefore available. Our findings suggest finer spatial resolution would provide addi-
tional detail, with improved activation sequence reconstruction as a likely consequence.

**Electrical source-load interactions.** While improvements in cardiac mapping represent one way in which data of the type we analyzed can benefit from the use of microfabrication-based strategies, investigations in which $I_m$ estimates have been used as indexes for electrical source-load relationships suggest $I_m$ measurement will improve analyses of those relationships because they will be quantified. For example, de Groot et al. (4) recently used SL measurements from Langendorff-perfused rabbit hearts in combination with voltage-clamp experiments in isolated myocytes and current-clamp recordings from cell pairs to support the specificity of gap junctional uncoupling with carbeneoxide. Those investigators assumed maintenance of $V_m$ distributions after carbeneoxide perfusion and interpreted a 50% reduction in SL amplitudes assembled from second-order measurements as a consequence of gap junctional uncoupling alone. Support for the assumption regarding $V_m$ included voltage-clamp experiments that demonstrated minimal influence of carbeneoxide on L-type calcium current, delayed rectifier potassium current, or inward rectifier potassium current and action potential recordings that showed maintenance of upstroke velocity and consistent action potential durations at 20%, 50%, and 90% repolarization. Support for gap junctional uncoupling as opposed to increased interstitial microimpedance included reduced coupling conductance measured during current clamps in cell pairs. They argued that the decrease in SL amplitude reflected gap junction uncoupling alone because $V_m$ and tissue impedance remained constant in their experiments. Interstitial microimpedance data, however, were not provided.

**Integration of microimpedance data.** We view the integration of interstitial microimpedance data as a valuable and straightforward next step because feasibility of surface potential gradient and SL measurements that contribute to improved in vivo $I_m$ computation was successfully implemented here. Microfabrication will be advantageous for this purpose because it will allow us to integrate electrodes just outside the interrogation region for use in stimulation. In a recent report (24), we showed that both the intracellular and interstitial cardiac microimpedances can be measured by analyzing a set of interstitial potential differences recorded centrally during multisite stimulation with a set of interstitial electrodes bounding the recording pair. Constant microimpedances were prescribed to one-dimensional models that included LRd membrane equations, and simulations involving 29 different electrode combinations allowed interstitial and intracellular microimpedance measurement at errors below 1%. A noteworthy aspect of that approach was the stable and independent measurement of $g_{o,x}$ and of $g_{l,x}$. Because no intracellular access was required, $V_m$ recording was unnecessary. Highly accurate $g_{o,x}$ measurements were obtained because fine spacing allowed precise four-electrode impedance measurements, consistent with theoretical descriptions by Plonsey and Barr (19). Those investigators showed that interstitial potential distributions resulting from current injection and removal in an idealized anisotropic passive bidomain model depended critically on stimulating electrode separation. With separation at 20% of the resting space constant, no intracellular current was established as the spatial distribution of transmembrane potential was equal and opposite to the distribution of interstitial potential. Using microfabrication, we anticipate bounding the set of recording electrodes necessary for the surface potential gradient measurements with stimulating electrodes separated from one another on a size scale consistent with the Plonsey and Barr (19) requirements. Such an arrangement would allow straightforward measurements of $g_{o,x}$ and of $g_{l,x}$ to be used in combination with the surface potential gradient terms in Eq. 1 to further facilitate in vivo $I_m$ computation.

**Limitations.** In assessing our findings, it is important to recognize certain limitations. 1) We compared SNRs between unipolar electrograms recorded with adjacent and distant signal conditioning for the different types of electrodes but made no attempt to shield lead wires for the distant signal conditioning recordings. Therefore, associated noise contributions in the distant signal conditioning recordings were likely higher than contributions to be expected with shielding. 2) Although all electrograms were recorded during epicardial stimulation adjacent to the electrode arrays, the electrodes themselves were not necessarily oriented along or across epicardial fibers. As a consequence, depolarization wavefronts expanding through the interrogation regions had varying amounts of curvature. Using detailed theoretical modeling, Leon and Witkowski (16) compared the shapes, magnitudes and durations of their estimated $I_m$ to the $I_m$ calculated in simulations. With decremental conduction, conduction block, depolarization wavefront initiation, and depolarization wavefront collision, those correlations decreased systematically, consistent with deviation from the underlying assumptions formulated in the initial Witkowski et al. (32) analyses. By comparison, they found high correlations during uniform depolarization wavefront expansion through the interrogation region. While their correlations suggest the moderate variability in depolarization wavefront curvature that was likely associated with our approach minimally influenced our overall findings, that influence was not demonstrated here. 3) We did not reduce spacing in the microfabricated arrays to the extent suggested by our simulations. While our findings strongly support the feasibility of using microfabricated sensors for the necessary component potential gradients, further refinements in electrode separation will likely be necessary for improved in vivo $I_m$ computation.

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