The scanning ion conductance microscope for cellular physiology

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Submitted 28 June 2012; accepted in final form 5 October 2012

The scanning ion conductance microscope for cellular physiology. Am J Physiol Heart Circ Physiol 304: H1–H11, 2013. First published October 19, 2012; doi:10.1152/ajpheart.00499.2012.—The quest for nonoptical imaging methods that can surmount light diffraction limits resulted in the development of scanning probe microscopes. However, most of the existing methods are not quite suitable for studying biological samples. The scanning ion conductance microscope (SICM) bridges the gap between the resolution capabilities of atomic force microscope and scanning electron microscope and functional capabilities of conventional light microscope. A nanopipette mounted on a three-axis piezo-actuator, scans a sample of interest and ion current is measured between the pipette tip and the sample. The feedback control system always keeps a certain distance between the sample and the pipette so the pipette never touches the sample. At the same time pipette movement is recorded and this generates a three-dimensional topographical image of the sample surface. SICM represents an alternative to conventional high-resolution microscopy, especially in imaging topography of live biological samples. In addition, the nanopipette probe provides a host of added modalities, for example using the same pipette and feedback control for efficient approach and seal with the cell membrane for ion channel recording. SICM can be combined in one instrument with optical and fluorescent methods and allows drawing structure-function correlations. It can also be used for precise mechanical force measurements as well as vehicle to apply pressure with precision. This can be done on living cells and tissues for prolonged periods of time without them losing viability. The SICM is a multifunctional instrument, and it is maturing rapidly and will open even more possibilities in the near future.

cardiovascular; cell mechanics; FRET; smart patch-clamp; topography; SICM

THE RESOLUTION of conventional microscopy has light diffraction limits (Table 1). Electron microscopy surmounts this wavelength limit (15, 78), as does the nonoptical scanning probe microscope (SPM). It does this by using nanoprobes (20, 46, 52, 83, 92, 115). The probe, mounted on a three-axis actuator platform, scans a sample of interest in the X and Y dimensions and together with recording of displacement in the Z dimension generates a three-dimensional (3-D) image. Binnig and Rohrer scanned the first samples using the scanning tunneling microscope (STM) in 1982, for which they received the Nobel Prize. Electrons “tunnel” between the surface of interest and a very sharp stylus formed by a single atom enabling the mapping of surface topography. With the placement of biological samples on a conductive base, the STM went on to image their surfaces at very high resolutions (3). More recently, the self-assembly of subunits of proteins (amino acids and peptides) was studied using STM (121). The STM was the predecessor of other surface probe microscopes, including the prevalent atomic force microscope (AFM) (7) (Table 1). The AFM uses a sharp tip mounted on a flexible cantilever, and the tip-sample interactions during scanning deflect the tip that produces very high-resolution images. This technology has developed over time (1) and, with the use of an oscillating cantilever tip, has imaged large molecules (94), including DNA (9). Because of the ability of AFM to image nonconducting surfaces, it has been widely used in biology to study cellular macromolecules like protein-nucleic acid complexes, chromosomes, cellular membranes, and microbes. Recently, it has also been used for determining the mechanical properties of skinned myocardial cells (126). The technique of nanoindentation, traditionally performed via dedicated indenters, can now be reliably achieved using AFM instrumentation, enabling mechanical property determination at the nanoscale. AFM nanoindentation capabilities have provided an excellent improvement over conventional nanomechanical tools and, by integration of topographical data from imaging, enabled the rapid extraction and presentation of mechanical data for biological samples (1). For example, Jacot et al. (50) measured the elastic modulus of the epicardium using AFM indentation and found that it significantly changes at birth from an embryonic value of 12 ± 4 kPa to a neonatal value of 39 ± 7 kPa. This change is in the range shown to significantly affect the development of neonatal cardiomyocytes.

Biological cell surfaces commonly have a complex 3-D nanostructure associated with complex and dynamic functions.
The AFM’s intermittent contact imaging mode or “tapping mode” circumvents these problems somewhat and has been used for imaging of biological cells and molecules (2, 13, 14, 27, 71, 87, 95, 108).

However, the use of force in AFM can be problematic in studying biological samples. The cantilever tips can distort the image (123). Consequently this is an invasive technique, albeit at the nanometer scale. However, reducing the force reduces surface indentation and improves its resolution to reveal complex subcellular structures such as microvilli (69). The other major method of probing biological surfaces is scanning electron microscopy (SEM), but it can only indirectly study remnants of cell behavior after the cells have been fixed (frozen) (41, 76).

By contrast, optical methods, and in particular fluorescent microscopy, are more suited to study functionality by localizing fluorescently labeled molecules within the cell. But this does not directly image cell surfaces (74, 119), and neither can it reach the resolution of the above two methods (Table 1). However, the scanning ion conductance microscope (SICM) bridges the gap between the resolution capabilities of AFM/SEM and functional capabilities of conventional light microscopy (Table 1).

### Principle of SICM Operation

Scanning ion conductance microscopy is a relatively new technique first used by Paul Hansma in 1989 (40). Its development was continued by Yuri Korchev starting in 1997 (35, 55, 57–61). In this microscope, a sharp borosilicate glass nanopipette (with the inner diameter below 200 nm) is used as a probe.

The pipette can move during the scan, or as in Fig. 1, the sample moves, being similarly mounted on the XY piezo. In this case, the pipette does not move laterally, just vertically (Z). To start, the pipette approaches the sample progressively decreasing the pipette-sample distance. This gradually restricts ions flowing through the fluid-filled pipette, thus decreasing the current. The ion current through the pipette depends on the overall resistance of the tip, which is the combination of the resistance of the micropipette itself and the access resistance of the micropipette opening. Access resistance is a complex function of the distance between the sample and the probe, and the geometry and electrochemical properties of the sample surface. This current (I) through the pipette, which is measured directly, is given by

\[
I = \frac{V}{R_p + R_{ac}d}
\]

where \(V\) is voltage applied to the electrode, \(R_p\) is pipette resistance, \(R_{ac}\) is access resistance, and \(d\) is distance between sample and the probe.

A distance-modulated feedback control system keeps the ionic conductance and thus the sample/pipette distance constant. Typically, the feedback control stops the approach at a predefined distance or set point. With the feedback loop controlling ion conductance, the pipette never touches the sample: it stops at a distance equivalent to the inner radius of the pipette. This enables noncontact scanning. As the scan proceeds in the X and Y directions, the ion conductance control system detects changes in nanopipette displacement in the Z direction. These recorded signals build up a 3-D image of the sample surface (Fig. 1).

The pipette’s inner tip diameter is typically 20–100 nm. As the resolution depends on the radius of the pipette inner tip (97), the SICM can obtain a resolution of 10 nm with a tip size of 20 nm diameter. The limitation of the tip size is around 10–12 nm.

To scan living cells, we require displacements in excess of 50 μm. Therefore, the SICM uses a three-axis piezo translation stage (Jena, Germany) with 100 μm travel distance in X, Y, and Z directions. This can image an area of 100 × 100 μm in a single scan. The lateral image resolution is usually 512 × 512 pixels. A single scan can take several minutes depending on the resolution at which it is being acquired and the area scanned. For example a 10 × 10 μm scan of an isolated adult cardiomyocyte with 150-nm resolution takes between 2 and 3 min. Although the SICM’s resolution is not as high as that of the AFM, SICM is potentially an alternative to conventional high-resolution microscopy technologies, especially in the imaging of live biological samples (e.g., Fig. 2). Importantly, the pipette probe provides a remarkable set of added capabilities (55, 107, 112). As described below, this multifunctionality makes the SICM a platform for the convergence of different microscopic and investigative technologies.

### Typical Hardware of SICM

A typical SICM uses an inverted microscope (e.g., Diaphot 200 or TE200; Nikon, Tokyo, Japan). Sample positioning is achieved by a computer-controlled piezotranslation stage mounted on the microscope stage (ICnano sample scan system, Ionscope, UK) with 100 × 100 μm x-y piezo-actuator for sample movement and a 25-μm z-axis piezo-actuator for pipette movement. The piezo stage holds the glass nanopipette, which is connected to the head-stage of the feedback amplifier (Fig. 1).

The SICM can use either a resistive or capacitive feedback head-stage like Axopatch 200B or Multiclamp 700B (Molecular Devices, Sunnyvale, CA). Ag/AgCl electrodes, in the bath and pipette, provide electrical connection as in a conventional electrophysiological circuit. Additional electronic components

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**Table 1. Resolutions of microscopy techniques**

<table>
<thead>
<tr>
<th>Approximate Resolution, nm</th>
<th>Microscope System</th>
<th>Principle of Operation</th>
<th>Sample Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>~200</td>
<td>Optical—standard (116)</td>
<td>Light—wavelength limited</td>
<td>Stained or unstained in liquid or air</td>
</tr>
<tr>
<td>~5–100</td>
<td>Optical—advanced (101)</td>
<td>Below light’s wavelength</td>
<td></td>
</tr>
<tr>
<td>~5–200</td>
<td>SICM (57, 104, 106)</td>
<td>Ion conductance via nanopipette</td>
<td>In ion conducting solution</td>
</tr>
<tr>
<td>0.5–10</td>
<td>Electron—SEM (41, 76)</td>
<td>Electron beam*</td>
<td>Electron conductive sample, vacuum</td>
</tr>
<tr>
<td>10–50</td>
<td>Electron—TEM (18)</td>
<td>Impede electrons*</td>
<td>Metal staining, vacuum</td>
</tr>
<tr>
<td>~5–500</td>
<td>AFM for live cells (81)</td>
<td>Force via sharp probe</td>
<td>In liquid or air</td>
</tr>
</tbody>
</table>

SICM, scanning ion conductance microscope; SEM, scanning electron microscope; TEM, transmission electron microscope; AFM, atomic force microscope.

*Not ideal for cell surfaces.
include a digitizer and computer-controlled software. The SICM has stringent requirements to cope with vibration and electrical noise, particularly for “smart patch-clamp.” The SICM setup rests on a vibration isolation table commonly used in electrophysiology. A small Faraday cage on the microscope stage encloses the piezo axis translation stage for electrical isolation and minimizing noise.

“Hopping Probe” SICM

Tall structures on the surface of biological cells can pose problems in lateral scanning. The pipette tip may collide with and drag the structures producing image artifacts or the tip can break. To overcome this, Pavel Novak and colleagues developed the “hopping” mode of the SICM (HPSICM) as a modification of the scanning procedure (86). In a system with this modification, the pipette “hops” as it scans and it no longer needs continuous feedback to keep a fixed sample-probe distance. The pipette starts well above a surface feature, producing a maximal current at this starting distance from the surface. From this point the pipette approaches, reducing the ion current to a predefined 0.25–1%. The height of the sample at this imaging point is the recorded Z-position. With the ion current reduced by 1%, the tip is still at a one inner pipette radius from the surface and still avoids cell surface contact. The pipette withdraws and then the sample moves laterally to the next imaging point. Importantly, by the update of the maximal current continuously while the pipette is withdrawn from the surface, the system automatically compensates for slow drift in pipette current. HPSICM also minimizes image-distorting noise as the pipette spends most of its time away from the image sampling point of interest. A direct comparison between images of soft biological samples obtained with the AFM, and SICM showed that the SICM images were better than those of AFM, which distorts the image (96).

Imaging of Cell Surface Topography

The SICM has produced 3-D topographical images of cells that are unfixed, unstained, and alive, with resolutions approaching that of AFM and electron microscopes (96, 106). For SICM imaging, living cell samples need no fixing or mounting. Cells are placed in petri dishes or on the coverslips on which they were grown. In other studies, for example “smart” patch clamp, the external (bath solution) and internal (pipette solution) are determined by the requirements of the electrophysiological study [e.g., ion channel studies (35)].

Images of mouse ear hair cells were produced using SICM that have a true fidelity, as demonstrated when comparing them with images produced with a SEM after cells were fixed and shaded. Surface topographical images of cardiomyocytes show complex structures with grooves related to the Z disc, with invaginations that represent T-tubule openings (Fig. 2, A and C). One can calculate the average length of Z grooves on the surface of the myocytes to produce a measurement of the surface structure regularity. This indicator has proven to be
valuable in following surface structural changes in cardiomyocytes that occur during heart failure (72). SICM cardiovascular imaging has not been confined to myocardial cells, and for example images of aortic and heart valve endothelium have been obtained. Endothelial cells from aorta were cultured in transwell plates under static conditions or on an orbital shaker that generated shear stress. In static conditions, endothelial cells oriented randomly, but when shear stress was applied, they became aligned (93). In another study the fragments of living aortic tissues were examined. Endothelium from the outer curve of aorta showed orientation along the direction of blood flow, whereas the inner curve showed no orientation. This has been demonstrated in the past with other microscopy techniques, but SICM resolves this physical alignment in living tissue. Also SICM has been applied to study the differences in membrane compliance in these cells (93).

Structure-Function Correlation

Compartmentalization of calcium (5, 43, 89) and other second messengers (12, 47, 63) is crucial to cellular function. The plasma membrane is rich in receptors and ion channels that respond to corresponding ligands by generating second messengers in signaling microdomains. For example, G protein-coupled receptors can change their signaling properties depending on their cellular locations (11, 120). Protein kinases and phosphatases can also form structurally determined microdomains by specific localization to the cytoskeleton, thus facilitating heterogeneous compartmentation (102). The AFM has been used to localize membrane receptors in cardiac tissue by combining fluorescence imaging and topography (23), but the SICM expands this type of use, which we will describe in the following section.

Localized β-adrenergic receptor-dependent cAMP signaling. Combining SICM with Förster resonance energy transfer (FRET) technique allowed Nikolaev et al. (85) to follow nanoscale signaling changes in defined subcellular regions in living cardiomyocytes (Fig. 2) (85). The SICM resolved T-tubules openings on the membrane of cardiomyocytes (Fig. 2C), expressing a FRET sensor (EPAC2 camps) for the second messenger cAMP. When β2-adrenergic receptors (β2AR) were selectively stimulated, cAMP was only produced when applying its agonist selectively above a T tubule, and the resultant cAMP signal was spatially confined (Fig. 2D). This provides a degree of control for the β2AR signaling mechanism: a situation in contrast to the β1-adrenergic receptor signaling, which is not spatially confined and is present equally in all membrane regions. In failing cardiomyocytes (Fig. 2E), β2AR signal was no longer constrained to the T tubules (Fig. 2F), which may be
a contributory factor in the pathology of heart failure. They redistributed from the T tubules to the crests. This alters cAMP signal compartmentation with possible pathophysiological consequences.

This hybrid SICM/FRET technique may be an important avenue in studying other localized receptors. Moreover, as well as responses to pharmacological stimuli, SICM/FRET could investigate molecular mechanosensitivity. Recent studies by the Schwartz group (33) used a FRET sensor based on vinculin to resolve piconewton stresses on vinculin in focal adhesions. We could envisage a possible future development of SICM/FRET in this direction. By the application of pressure via the SICM pipette, a combination of SICM and a FRET sensor could investigate these dynamic processes. It could also investigate cellular networks and contacts to monitor the effects of stimulating single cells in a network of adherent cells.

Mapping ion channels with an added patch system. The SICM has been combined with a conventional whole cell patch-clamp setup. In this combination, the SICM noncontact scan can provide a map of the location of ATP-sensitive K⁺ (KₐTP) channels (60). In this setup, current images were recorded over some 40 min and showed low lateral mobility of KₐTP channels, but they concentrate in sarclemmal Z grooves. The SICM pipette with K⁺ inside applied K⁺ while scanning the cell surface. A second patch-clamp pipette in the whole cell mode records patch currents as the SICM probe scans over and activates a KₐTP channel. This information can be related to the position of the SICM probe to create a distribution map of KₐTP channels or with appropriate activation and markers, other particular ion channels.

Mapping ion channels with SICM as the patch-clamp system: the “smart” patch-clamp technique. The mapping of ion channels has been facilitated by using the same pipette both to image the surface of a cardiomyocyte and to function as the electrophysiological probe. In this technique, a high-resistance glass nanopipette first scans the cell surface to produce a high-resolution topographic image of the cell surface. The topographic image is used to precisely position the pipette onto a cellular nanodomain of choice (we have the XY coordinates). The same glass nanopipette is then used to obtain single ion channel currents using the patch-clamp technique. In the conventional patch-clamp technique, the pipette approaches the target manually and often obliquely to visualize the pipette tip placement through the microscope. By contrast, pipette approach with the SICM is vertical, almost fully automatic, and precisely controlled by the distance modulated feedback system. At a predetermined distance from the precisely selected target location, the feedback control is switched off to allow a controlled contact with the membrane. The frequency of successful patches is significantly higher with this approach than with the conventional method (31). SICM distance-modulated feedback control is useful as the glass pipette will not touch the cell until required, and damage to the cell or pipette is minimized. As a result, “smart” patch-clamp technique records ion channel activity from precise nanodomains on the surface of live cells and thus can generate a spatial functional map of surface ion channels. This is in contrast to conventional patch clamping where the position of the pipette with respect to the cell topography cannot be controlled with a nanoscale precision. The method is also superior to the current conventional microscopic techniques (immunofluorescence) that target all the channels including the dormant ones, whereas the “smart” patch-clamp technique locates only the functional ion channels.

Yuri Korchev and coworkers (31, 35) developed and successfully applied the “smart” patch clamp to record ion channels from several different cell types. Ion currents were recorded from very small cells (e.g., sea urchin sperm cells) and fine focal swellings (boutons) in neuronal processes of 0.5–1.0 μm in diameter, which otherwise would have been extremely difficult to patch. That is, it can successfully record from membrane structures that are too small to be resolved conventionally or that cannot be detected by light microscopy e.g., from T tubules of cardiomyocytes. The “smart” patch-clamp system produced a spatial map of L-type calcium channels on a cardiomyocyte surface (setup shown in Fig. 3B). Figure 3A shows the optical image of an isolated cardiomyocyte and position of the nanopipette. Figure 3C shows a 10 μm × 10 μm topography scan of the same cardiomyocyte. “Smart” patch clamp at different locations (e.g., T tubules, crest or groove, Fig. 3D) provided the first direct evidence of functional ion channel location. A representative L-type calcium channel activity from a T tubule is shown in Fig. 3E. The study showed a preferential location of L-type calcium channels to the T tubules (Fig. 3F) where they are in close proximity to other proteins involved in excitation-contraction coupling (6, 88).

The “smart” patch clamp also recorded ion channel currents from opaque samples (e.g., aorta, brain slices, and cells grown on filters). Optically guided patch clamp prevents the collection of any information on the position or type of cellular structure. In another study, Gu and coworkers (35) mapped not only calcium channels but also chloride channels on the surface of cardiomyocytes and found that these are not randomly distributed but have a specific location on cardiomyocyte membrane (3 Cl⁻ channels are located only in Z grooves and T-tubule openings) where they may have synergistic roles in excitation-contraction coupling.

Dutta and coworkers (24) used “smart” patch clamp to record maxi-anion channels from cardiomyocytes that could not be recorded using conventional patch clamp (24). The advantage of “smart” patch clamp here would be to visualize structures where the ion channels could be clustered that could easily be missed in conventional patch clamp.

Tracking Dynamic Biological Processes

Dynamics of membrane structures. Unlike the other high-resolution microscopes, the fact that the SICM can scan live cells in real time means it can follow biological processes taking place at cell membrane surfaces (125) including large membrane proteins (104, 106). In the high-resolution mode, the SICM can scan an area of 1 μm² in 9 s per frame. This can follow the appearance and disappearance of microvilli and the opening and closing of endocytotic pits. With the improved topographical resolution, Shevchuk et al. (105) found that the SICM can visualize the opening and closing of clathrin pits, which are ~200-nm indentations in the membrane. It appears that most pits close with the help of a membrane protrusion that forms beside the pit. After simultaneous confocal detection of fluorescence in cells expressing green fluorescent protein-labeled proteins, they found that clathrin, dynamin, and several other actin-binding proteins, for example flotillin 1 and 2,
colocalize with pits when these undergo morphological changes.

The SICM has revealed movements and reorganization of microvilli in real time on the surface of different types of epithelial cells (32). Microvilli appear and grow at a rate of about 5 nm/s over 2.5 min and after reaching a plateau lasting some 5 min and retract at about 1.2 nm/s. Moreover, they tend to form migrating clusters.

**Cell volume measurements.** Cell volumes can dynamically change. Osmotic changes following early myocardial ischemia and cell changes in myocardial hypertrophy require complex cell shape and volume reorganization (38, 45). Several indirect techniques (16, 53) and some more direct methods (84) have been used to measure and monitor cell volume. The latter method is only applicable to cells of nearly spherical shape and in suspension. Light microscopy methods have also been used (26, 75). An advanced method for cell volume measurement uses scanning laser confocal microscopy (25, 36, 127). An advanced method for cell volume measurement uses scanning laser confocal microscopy (25, 36, 127). However, generally, these methods have limited spatial resolution, and the cell plasma membrane cannot always be clearly visualized. The SICM can measure cell volumes between 10^{-19} and 10^{-20} liter resolution.

**SICM as a Noninvasive Mechanical (Distance and Force) Probe**

**Contraction.** Hovering over a contracting myocyte, the SICM distance feedback control can follow and record Z-displacement of the nanopipette and therefore record the event of contraction as an upward membrane movement. Figure 4A diagrams the system for recording contraction and monitoring calcium transients. The system records and digitizes the Z piezo position which follows the physical contraction of the membrane (Fig. 4B, black trace).

**Cell membrane compliance.** Cellular mechanical properties are determined by its intracellular mechanical characteristics such as cell structures, molecules, and downstream signals associated with the cytoskeleton (51). On the other hand, mechanical properties of the cell membrane contribute to cellular function (90). Being heterogeneously supported by the cytoskeleton, membrane

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**Fig. 3. L-type calcium channel (LTCC) distribution in the cardiomyocyte membrane: mapping of ion channels by the “smart” patch-clamp technique. A: adult rat cardiomyocyte and micropipette as seen by optical microscope. B: smart patch circuitry with vertical pipette. 3-D, three-dimensional. C: cardiomyocyte topography showing the regular Z grooves and crests (scalloped surface) with T tubules. D: myocyte cross-section with probe positioning in sarcomere units (Z groove, T-tubule opening and scallop crest). Probabilities of forming a G9 seal as a function of surface position are shown in parenthesis. E: cell-attached Ba^{2+} currents at 0 mV showing several current traces and ensemble average of 12 traces showing typical LTCC current kinetics. F: statistical distribution of LTCCs with the highest density near the T-tubule opening. Modified from (35) with permission.**
corresponding change in pipette position. Control experiments on the cantilever bends the lever, and we can measure the cantilever with a spring constant of 0.01 N/m. Pipette pressure is converted to force (100), and the pressure applied at the tip ensures the pipette follows the bending lever. Pipette pressure bending the cantilever. The SICM distance feedback control ensures that the pipette follows the indentation, which is a function of the applied pressure. This quantitatively probes the cell surface without direct contact. Knowing the effective force (right block arrow) not only produces a larger indentation but elicits a contraction (sharp upward deflection).

The pipette radius, \( R_p \), can be calculated from the pipette resistance \( (R_p) \) using the following:

\[
R_p = 1 / (\pi \xi R_0 \tan \theta)
\]

where \( \theta \) is the half cone angle of the inner wall of the pipette (1.5 degrees in our case) and \( \xi \) is the conductance of the solution. This formula was verified experimentally using scanning electron microscopy or optical microscopy of pipettes of measured resistance to directly determine \( R_0 \). We then obtained a plot of the force exerted on the cantilever per kilopascal for pipettes of different resistances.

Specifically, SICM has been used to explore the mechanical properties of cardiomyocytes with their characteristic repetitive scalloped topographic features. The distance feedback control ensures that the pipette follows the indentation, which is a function of the applied pressure. This quantitatively probes the cell surface without direct contact. Knowing the effective force and distance, one can determine a Young’s modulus of elasticity. A hydraulic jet produces an initial small 200-nm indentation (Young’s modulus, 2.8 kPa) (100). This component of the SICM not only probes mechanical properties of cells but can also map any nanoheterogeneity in mechanical properties. A study in over a glass coverslip showed no detectable change in pipette position with applied hydrostatic pressure, indicating that this does not alter the ion current. The relationship between the cantilever deformation and applied pressure was linear, with no hysteresis. This was also observed using pipettes of different resistance and hence inner radius. Since we know the spring constant of the cantilever, the distance moved by the cantilever can be directly converted into force using \( F = k \) (spring constant) \( \times \) (distance), allowing a direct conversion of applied pressure into applied force. The force exerted on the cantilever should also depend on the applied pressure and pipette radius. The pipette radius, \( R_0 \), should also depend on the applied pressure and pipette radius. The pipette radius, \( R_0 \), can be calculated from the pipette resistance \( R_p \) using the following:

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cardiomyocytes from heart failure shows changes in surface topography, Z grooves and T tubules (72), and the precise factors leading to this change are unclear.

Nanomechanotransduction and mechanosensitivity. Mecha-notransduction and mechanosensitivity is highly conserved and exists throughout biology (79). It involves mechanosensitive ion channels (29, 80), membrane and intracellular molecules, downstream intracellular signals, and intracellular structures, including the cytoskeleton. Cellular mechanosensitivity is a burgeoning study area of rapidly increasing importance (39), particularly in heart (4, 10, 49, 56, 66, 118) and the vasculature (34, 48, 54, 82). The SICM was first used to study the mechanics of neurons (100) where a hydraulic jet could clearly indent the membrane. However, in cardiac myocytes a hydraulic jet (system diagrammed Fig. 4C) cannot only indent the membrane (Fig. 4D, subthreshold pressure, left-hand block arrow), but this indentation reached a threshold magnitude to activate contraction of the cardiomyocyte (65) (Fig. 4D, threshold pressure right-hand block arrow), that is, a mechanically induced potentially arrhythmogenic beat.

In an extended study (77), myocardial cells were loaded with fluo-4 AM, a calcium-sensitive dye. The SICM pipette initially scanned the cell membrane with no applied pipette pressure, producing a 3-D image of membrane topology. The pipette pressure is then applied to selected coordinates, targeting crests, Z grooves, or T tubules. The jets indent the membrane activating a mechanosensitive calcium response, and these are determined by the cell’s regional mechanical properties. The calcium response remains highly localized when pressure is applied over a groove and spreads through the whole cell when the pressure is applied over a crest. The SICM has revealed the normal regular repetitive surface topography (see Fig. 2A) of a myocardial cell, but cardiomyocytes from failing myocardium show surface disruption with no regular striations (see Fig. 2E), and strikingly, hydraulic jets can generate an abnormal activating calcium signal regardless of the position of applied pressure.

General Perspectives and Conclusion

The nanopipette platform lends itself to label-free biosensing, using specific recognition compounds for analysis (19). For use in microinjection, it confers advantages over conventional techniques with superior control over delivery and the possible use of voltage instead of pressure to drive delivery. Thus more cells survive injection (the force produced by applying voltage across the liquid/liquid interface, which changes the surface tension, is enough to produce pipette flow). A few studies have provided results with this technique (8, 67, 91, 110). The SICM is not just a microscope as indicated in the left-hand side of Fig. 5. Because it uses a nanopipette as its scanning probe, it is a multifunctional convergent instrument (Fig. 5, right). It is a rapidly maturing technology. The potential of the “smart” patch-clamp method is yet to be fully explored. It can potentially be used to identify electrophysiological changes associated with morphological changes that occur in different circumstances, e.g., with cell differentiation from precursor cells (30). Moreover, combining “smart” patch-clamp and FRET-based signaling may open new avenues of SICM application in cell physiology and pathology. An electron microscopic estimate of populations of membrane channels showed that stereo imaging is superior to non-stereo imaging for quantifying surface channels and receptors (62). Electron microscopy can provide a means of counting receptors and ion channels on freeze-fractured membranes. But because it uses freeze fracture, the number of functional channels cannot be estimated. Improved SICM scanning and electrophysiological procedures may provide accurate estimates of functional channels with nanometer precision.

Also, delivery through the pipette using pressure and/or voltage opens a host of possibilities (122). A multi-barreled pipette can deliver multiple ligands such as agonists/inhibitors (98, 112). The SICM nanopipette may be turned into highly sensitive electrochemical sensor for spatiotemporal distribu-

Fig. 5. Summary diagram of overall functionality of the SICM. The pipette can function as various probes (input to central SICM sphere from below). The spokes radiating out from the SICM sphere reach toward other spheres, which indicate the range of functionality. The spheres on the left subserve its original and basic function as a microscope. It can image large molecules through to a whole cell. The spheres on the right motion the SICM’s diverse hybrid function: top two spheres, signal and electrophysiological surface location (ion channels, receptors); and the bottom two, mechanical.
tion of electrochemical mediators. The amount of substance released at different pressures and voltages can be calibrated, and the release of chemicals from the pipette can be precisely controlled. By this, the nanopipette can concentrate and control chemicals at the tip to trigger localized receptor-mediated response, opening the possibility of functional mapping of receptor-mediated responses in cardiomyocytes and other biological cells. Local delivery enables replication in delivery of chemical agents to the same cellular structures at multiple points in the same dish without exposing neighboring cells, or indeed other parts of the cell, to the drug. This could enable rapid drug testing experiments effectively at the nanolevel that has not been previously possible.

The fact that the SICM uses a glass pipette for its probe not only confines many purely biological applications (55), it is also useful in mechanobiology. In the latter case, force-based studies similar to that in the AFM (70), but noninvasively, will show whether the stiffness (Young’s modulus of elasticity) changes during development, repair and regeneration, neoplasia, and heart pathology. Finally, the pipette lends itself to sense probing and nanofabrication.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.J.L., A.B., P.T.W., and J.G. edited and revised manuscript; M.J.L., A.B., P.T.W., and J.G. interpreted results of experiments; M.J.L., A.B., P.T.W., and J.G. performed experiments; M.J.L. and J.G. analyzed data; M.J.L., A.B., P.T.W., and J.G. conceived and design of research; M.J.L., A.B., P.T.W., and J.G. conception and design of research; M.J.L., A.B., P.T.W., and J.G. conception and design of research; M.J.L., A.B., P.T.W., and J.G. contributed equally to this work.

REFERENCES


