Micromachined Nanocalorimetric Sensor for Ultra-Low-Volume Cell-Based Assays

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Current strategies for cell-based screening generally focus on the development of highly specific assays, which require an understanding of the nature of the signaling molecules and cellular pathways involved. In contrast, changes in temperature of cells provide a measure of altered cellular metabolism that is not stimulus specific and hence could have widespread applications in cell-based screening of receptor agonists and antagonists, as well as in the assessment of toxicity of new lead compounds. Consequently, we have developed a micromachined nanocalorimetric biological sensor using a small number of isolated living cells integrated within a subnanoliter format, which is capable of detecting 13 nW of generated power from the cells, upon exposure to a chemical or pharmaceutical stimulus. The sensor comprises a 10-junction gold and nickel thermopile, integrated on a silicon chip which was back-etched to span a 800-nm-thick membrane of silicon nitride. The thin-film micromachining to miniaturize a thermoelectric sensor and to permit highly sensitive and rapid measurements. In contrast to alternative thermal sensing strategies, the device has the advantage of library compounds and low numbers of cells. The use of single cells or a small cluster of cells represents the fundamental minimum in the design of cell-based analytical microsystems, with the potential for producing high-throughput formats.

The development of this microarray technology requires the use of thin-film nanofabrication methods, together with silicon micromachining to miniaturize a thermoelectric sensor and to permit highly sensitive and rapid measurements. In contrast to alternative thermal sensing strategies, the device has the advantage that it is both self-referring and, importantly, that it does not dissipate energy into the biological sample. The thermoelectric devices have previously been used to study energy balances in living cells, including heat generation by muscle, nerve, and large populations of isolated mammalian cells. Calorimetry has also been applied as the analytical tool in the screening of receptor agonists and antagonists using large populations of animal or microbial cells. Although miniaturized calorimetric systems have been developed, to date, it has not been feasible to make quantitative temperature measurements from isolated cells. We have therefore sought to apply silicon microfabrication technology to develop an ultrasensitive nanocalorimetric biological sensor to make real-time measurements of changes in heat generation in order to assess the metabolic activity of individual cells in a noninvasive manner. The approach allows the use of individual cells from primary cultures or tissue biopsies and provides a route toward the development of a flexible generic assay. For example, it enables the testing of a broad range of cell agonists and antagonists, without an a priori knowledge of their activity. The developed sensor is compatible with a low-volume microarray format, which lends itself to the use of (small amounts of) library compounds and low numbers of cells. The use of single cells or a small cluster of cells represents the fundamental minimum in the design of cell-based analytical microsystems, with the potential for producing high-throughput formats.

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transducer comprises a 10-junction gold and nickel thermopile (Figure 1) with a Seebeck coefficient of 220 $\mu$V K$^{-1}$. The device was fabricated by direct-write electron beam lithography on a thin silicon nitride membrane (800 nm), which was back-etched to span an air pocket, designed to minimize heat loss (Figure 2). This provides a format in which the thermal mass of the system is greatly reduced, with an improved insulation from the ambient environment. The sensing junctions located in the center of the membrane (Figure 1a,b) measured $5 \times 3$ $\mu$m$^2$ each and formed a circle whose inner circumference had a radius of 10 $\mu$m (the outer circumference thus being 15 $\mu$m from the center). The diameter of the thermopile, including the $40 \times 40$ $\mu$m$^2$ large reference junctions located on top of the bulk silicon, was 500 $\mu$m. In the center of the thermocouple ring was a heater element (Figure 1b) which provided a method for calibration of the sensor. To

Figure 1. Thermoelectric sensor. (a) Photomicrograph (scale bar, 100 $\mu$m) showing the $300 \times 300$ $\mu$m membrane of Si$_3$N$_4$ (1) supporting 10 radially arrayed sensing junctions of the gold and nickel thermopile (220 $\mu$V K$^{-1}$) and a central calibration heater. The reference junctions, positioned 250 $\mu$m away on the bulk silicon frame, which provided a uniform reference temperature, are not visible. A cell chamber defined in a 200-$\mu$m-diameter, 23-$\mu$m-thick layer of polyimide is centered on top of the membrane (2) and accommodates the culture solution (~700 pL). (b) Scanning electron micrograph (SEM) (scale bar, 10 $\mu$m) of the sensing junctions covering a circular area of 30 $\mu$m in diameter (3). The thin-film calibration heater of nickel (4) is positioned in close proximity to the sensing junctions. The internal resistance of the thermoelectric sensor was 450 $\Omega$. (c) SEM (scale bar, 50 $\mu$m) of the sensor and cell chamber (5). The walls bend slightly upward due to the internal stress of the polymer layer.

Figure 2. Physical geometry. (a) Microscopic photograph of the thermopile showing the calibration heater (1), the thermoelectric elements of gold (2) and nickel (3), respectively, the Si$_3$N$_4$ membrane (4), reference junctions (5), sensing junctions (6), and cell chamber (7). The reference junctions were positioned at the outer edge of the sensor array on the bulk silicon, encapsulated within the polyimide layer. (b) Cross section of the sensor illustrating the material composition including polyimide (8), Si$_3$N$_4$ (9), air pocket (10), silicon (11), and glass (12). Dimensions have been exaggerated for clarity. Note the position of the insulating air pocket below the 800-nm-thick Si$_3$N$_4$ membrane supporting the center of the thermopile. (c) CAD drawing of the complete sensor chip illustrating the multilayer structure of the device. The bonding pads for the DEP (dielectrophoresis) electrodes (13), calibration heater (14), and thermopile (15) are shown. The patterning of electrodes used for experiments on field trapping of cells by negative DEP was (optionally) performed with the aid of electron beam lithography and UV-3 photoresist. All dimensions are given in micrometers.
theoretically relate the temperature from the calibration heater to the heat generated from a group of cells situated above the thermopile, the microsystem was modeled with a partial differential equation solver (Pdease 2.53; SPDE Inc.) using finite element analysis. The cell chamber (Figure 1c), which was 200 μm in diameter and 23 μm deep, was formed in a biocompatible photopatternable polyimide on the sensor array. The titer chamber provided a constrained volume in which the diffusion distances (e.g., for ligands or drugs) were short and response times fast.11

MATERIALS AND METHODS

Reagents. Bovine serum albumin (BSA), Dulbecco modification of Eagles medium (DMEM), glucose, triiodothyronin (T3), sodiumascorbate, glutamine, N-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), penicillin, and streptomycin were used as supplied from ICN Biomedicals Inc. Collagenase (type II), foetal calf serum, noradrenaline (NE), carbonyl cyanide m-chlorophenyl hydrazone (CCCP), ethyleneglycol-bis(2-aminomethyl)tetraacetic acid (EGTA), 3-(N-morpholino)-propanesulfonic acid (MOPS), and phosphate-buffered saline (PBS) were from Sigma-Aldrich. Insulin (human Acrapid) was supplied by Novonordisk A/S. Reagent grade ethanol and acetonitrile in addition to KOH and spectroscopic grade paraffin oil were supplied from Merck BDH. Microcapillaries were supplied from Intrael. Single crystalline 3-in. silicon wafers were supplied by DuPont. The electron beam patterneable poly(methyl methacrylate) (PMMA) resists were delivered in low molecular weight forms from Aldrich and in high molecular weight forms from Elvesite, respectively. Durimide 7020 photopatternable polyimide was from RACH Semiconductor Merials, whereas UV-3, S1818, and S1828 e-beam and photoresists came from Shipley. S25 Chrome Etchant was from Laborte Electronics Ultra Pure Chemicals. Metals, including gold, nickel, and NiCr were from Goodfellers. Printed circuit board (PCB) edge connectors were obtained from Radio Spares. Currus Logic Corp. donated the CS3001 amplifiers used in the amplifier design.

Brown Adipocytes. Brown adipose tissue (BAT) was dissected from the interscapular, subscapular, and cervical regions of 4-week-old male Naval Medical Research Institute (NMRI) mice. Preadipocytes were isolated12 and seeded in three 30-mm culture dishes and incubated in medium containing sodium bicarbonate (3.7 g L−1) under 8%CO2 atmosphere for 6 days, after which time they were harvested. The culture medium based on DMEM at pH 7.4, was modified with 10%foetal calf serum, 20 mM insulin, 1 mM T3, 25 μg ml−1 sodium ascorbate, 4 mM glutamine, 10 mM HEPES, 50 IU ml−1 penicillin, and 50 μg ml−1 streptomycin. Insulin was required for the expression13 of mitochondrial uncoupling protein (UCP) whereas the presence of T3 increased the thermogenic response14 due to more β-adrenergic receptors on the cells.15 The cells were stimulated with 1.4 μM NE.

Cardiomyocytes. Isolated cells were obtained from adult rabbit left ventricle by collagenase digestion and kept in base Krebs solution, containing the following: 120 mM NaCl, 20 mM HEPES, 5.4 mM KCl, 0.52 mM NaH2PO4, 3.5 mM MgCl2·6H2O, 20 mM taurine, 10 mM creatine, 11.1 mM glucose, and 0.1%BSA, pH 7.4. Cells were transferred to the sensor chips using a glass capillary and placed onto the thermocouple junctions. Cellular heat production were stimulated with 11.3 μM CCCP and 33.9 μM oligomycin.

Mitochondria. Purified mitochondria were obtained from adult rat liver by tissue homogenization prior to centrifugation and resuspension in isolation buffer containing the following: 225 mM mannitol, 70 mM sucrose, 0.4 mM EGTA, and 2 mM MOPS, pH 7.2. Liver extracts and mitochondrial preparation were kept at 4 °C prior to experiment. The assay buffer used in the experiment was based on a phosphate buffer of 10 mM NaH2PO4, 10 mM Na2HPO4, 220 mM mannitol, 50 mM sucrose, 20 mM MOPS, and 5 mg ml−1 BSA. The pH was adjusted to 7.2 with 0.1M NaOH. Respiration was stimulated by the addition of 6.25 mM each of malate and glutamate and 12.5 mM each of succinate, ascorbate and β-hydroxybutyrate, 12.6 μM CCCP, and 37.8 μM oligomycin.

Thermoelectric Sensor. Eleven consecutive levels of microfabrication were required to develop the thermoelectric sensor. The sensor geometry (Figure 2) was based on the design employed in infrared radiation sensors incorporating an insulating layer of air between the sensor and bulk material.16 The radial design of the thermopile (Figure 2a) maximized the uniformity of the temperature gradient running from the center of the structure to the bulk substrate. By removing the bulk material and suspending the sensor on a thin membrane (Figure 2b), and by taking advantage of the low thermal conductivity of air, thermal insulation both from within the sensor and from the ambient air was improved. Positioning all the reference junctions of the thermopile on to the bulk substrate (Figure 2a,b) held the reference temperature to that of the ambient environment, leaving the sensing junctions free to sense small changes of heat in the center of the membrane only. Thermocouples of gold and nickel were chosen as the thermoelectric transducer since they are self-generating offset-less devices, which require no external power source and thus, importantly, do not dissipate heat into the sample.

The nanocalorimetric sensors were batch fabricated in a 9 × 9 array on a 380μm-thick single crystalline 3-in. silicon wafer with (100) lattice orientation precoated with 300-nm Si3N4.

Level 1: The first fabrication level defined the markers used for alignment of patterns in subsequent fabrication steps. The wafer was spin coated with UV-3 resist and patterned directly on the polished surface using electron beam lithography. The markers were defined in a 10 135-nm-thick layer of titanium and gold, which produced adequate contrast between the marker and silicon bulk substrate.

Levels 2 and 3: The wafer was then spin-coated with PMMA resist to define the two metal layers of gold (level 2) and nickel (level 3), respectively (for thermopile fabrication). The metals were deposited in 50- and 100-nm-thick layers respectively by electron beam evaporation, resulting in a 10-junction thermopile from the serial connected thermocouples. Flash coating of a 10-nm adhesive layer of NiCr was required to bond the gold and nickel to Si$_3$N$_4$. The Seebeck coefficient was approximated to 220 $\mu$V K$^{-1}$, and the internal resistance was measured as 450 $\Omega$. A thin-film resistive heater of nickel was simultaneously patterned with the thermopile.

Level 4: The bonding pads of 200-nm gold (protected by a 30-nm sacrificial layer of NiCr) were patterned using photolithography and S1818 resist.

Level 5: The whole wafer was covered with an electrical insulating layer of 500-nm plasma-enhanced chemical vapor-deposited (PECVD) Si$_3$N$_4$, bringing the total thickness of Si$_3$N$_4$ to 800 nm. The pads were exposed using an etching mask of S1828 photore sist prior to dry etching with C$_2$F$_6$ at 18 mTorr, 100 W, and 20 standard cubic centimeters per minute (SCCM).

Level 6: A layer of 30-nm NiCr, used to assist the adhesion of the polyimide film, was defined at level 6, with S1818 resist and photolithography.

Level 7: A 500-nm-thick layer of PECVD Si$_3$N$_4$ was then deposited on the reverse (unpolished) side of the wafer, which functioned as an additional wet etching mask at the final fabrication stage (see level 11).

Level 8: The wafer was patterned on the reverse (unpolished) side by photolithography with the aid of front-to-back side alignment using infrared light. Protecting the front side with S1828 photore sist, the Si$_3$N$_4$ (800 nm) on the reverse side was selectively removed from the pattern by dry etching with C$_2$F$_6$.

Level 9: The 200-µm large circular cell chamber was defined in a 23-µm-thick film of polyimide (Durimide 7020), thus giving the chamber a total volume of 720 µL. The polyimide film, defined by photolithography, was cured at 300 °C for 90 min to increase the adhesion properties and the chemical robustness of the polymer.

Level 10: The sacrificial NiCr layer protecting the bonding pads was removed by the use of S25 Chrome Etchant, thus releasing any residual layer of polyimide.

Level 11: The wafer was finally clamped in a custom-made wafer holder of high-grade stainless steel equipped with Kalrez rubber O-ring seals which protected the front side pattern upon immersion in 8.1 M KOH solution. The 300 × 300 µm$^2$ trapezoidal cavity formed below the reaction vessel released the 800-nm-thick membrane bilayer of pure Si$_3$N$_4$ from the bulk silicon, thus reducing the overall thermal mass of the device.

The wafer was finally diced to yield 80 individual 5 × 5 mm$^2$ large chips (Figure 2c), which were individually attached with epoxy resin to 1-mm-thick glass chips, sealing off the trapezoidal cavity and creating the thermally insulating air pocket below the reaction vessel. Electrical connection to a chip carrier was achieved using a wire bonder with 25-µm-thick gold wires.

**Low-Noise Amplifier.** Low-noise, chopper-stabilized operational amplifiers, CS3001, were incorporated into the low-noise preamplifier. The input voltage noise with the thermoelectric sensor connected was 55 nV/$\sqrt{Hz}$ from dc to 1 Hz, corresponding to a spectral density of 9.2 nV/$\sqrt{Hz}$. The gain was set at 10$^5$, and the offset voltage was ~250 nV with respect to the input in all experiments. The sensitivity of measurements, determined as three standard deviations above noise (27.5 nV), corresponded to an ability to resolve a 0.125 mK temperature change. Experimental averaging further increased the sensitivity to 12.5 nV or 0.06 mK ($n = 5$), which is the resolution of the trace in Figure 5.

**Experimental Setup.** The sensor chip used in the experiment (Figure 2c) was connected to the custom-made low-noise preamplifier, which was positioned within 1 cm of the sensor chip in order to reduce attenuation of the voltage signal and minimize interference. The chip carrier was connected to the amplifier through a gold-plated 0.1-in.-pitch printed circuit board (PCB) edge connector (gold was used consistently in all contacts to avoid the creation of additional thermocouple junctions between the sensor output and the amplifier input). A transparent chamber enclosed the sensor from the surrounding air to eliminate rapid temperature changes. Water (37 or 23 °C), maintained at a constant temperature, was circulated through a 1-mL chamber integrated as part of the copper base plate. Both amplifier and sensor were positioned under a stereomicroscope for the manipulation and positioning of cells, injection of drugs, and visual observation throughout the experiment. The attachment of cells on the thermopile was enhanced by pretreating the sensor surface with poly(L-lysine), adsorbed from a 0.01% solution in buffer.

The cell chamber was first covered with a 0.5 µL drop of culture medium with a subsequent addition of 15 µL of liquid spectroscopic grade paraffin (mineral oil) in order to prevent evaporation of the medium. Resuspended adipocytes (or cardiomyocytes) were positioned on top of the sensing junctions using a micropipet and micromanipulator (Figure 2c). Excess medium was removed from the titer chamber leaving a total volume of 700 µL. The sensor chip was then connected to the amplifier, sealed by the polystyrene chamber, and placed under the microscope. Three back-filled glass capillaries containing culture medium, 100 µM NE, and 1.0 mM rotenone, respectively, were, in turn, mounted on the micromanipulator and inserted into the cell chamber for microinjection. Rotenone was insoluble in aqueous solutions at these relatively high concentrations, and the compound was consequently introduced in acetonitrile. Appropriate control experiments were performed to show that the thermal response was not due to mixing of the aqueous and organic solvents. Likewise, for cardiomyocytes, a capillary containing 1 mM CCCP, dissolved in ethanol, was used with a comparable series of controls to ensure the attribution of the recorded response to changes in cellular activity.

The device optionally incorporates dielectrophoresis (DEP) electrodes, Figure 2c, that, together with various surface modification procedures, were used to assist with the placement of cells.

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**RESULT AND DISCUSSION**

**Sensor Modeling and Calibration.** Finite element modeling of the temperature distribution was based on the heat diffusion equation, which considered heat transfer by conductance on the...
micrometer scale due to the Nernst diffusion layer. The cylindrical coordinate system was applied for reconstructing the device in two dimensions with respect to r and z. The heat diffusion equation was considered under steady-state conditions, due to the small time constant and long time duration of the experiments:

\[
\frac{\partial}{\partial t} \left( k \frac{\partial T}{\partial r} \right) + \frac{1}{r} \frac{\partial}{\partial r} \left( k r \frac{\partial T}{\partial r} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right) + S = 0
\]

where the symbol \( k \) is the thermal conductivity, \( T \) absolute temperature, and \( S \) the heat-generating source. A two-dimensional cross-sectional model was constructed and the thermal conductivity of the materials used (including their location in the system, determined prior to integration around the center point for \( \phi = 2\pi \), thereby creating a model in three dimensions). This same analysis of heat flow was also used to optimize sensor design prior to construction.

The cross-sectional temperature distribution from a modeled cluster of 10 idealized mammalian cells (brown adipocytes) is shown in Figure 3. Each cell was treated as individual hemispheres with a radius of 10 \( \mu \)m and a geometric volume of 2.1 pl (although isolated cells may range from 5 to 20 \( \mu \)m in radius). The cells are located in the center of the cell chamber in close contact with the sensing junctions of the thermopile. The signal output from the sensor was proportional to power (heat input) and could be expressed as \( nV = 2.54 \times 10^{-4} nW \).

The modeled result was further corroborated experimentally by biochemical titrations into the 700-\( \mu \)L titer chamber, using the exothermic enzyme-catalyzed activity of catalase in the presence of hydrogen peroxide. For a known calibrant heat input, the sensing junctions were found to give a linear voltage response over a dynamic range up to 200 \( nW \) (\( nV = 2.12 \times 10^{-4} nW \), \( r = 0.97 \)), enabling the use of the device in a range of systems including enzymic titrations as well as individual cells and tissue measurements. The results were in good agreement with the modeled response.

The unfiltered time constant to thermal equilibrium, with the sensor filled with ~720 \( \mu \)L of water covered with 500 \( \mu \)m liquid paraffin, was determined by activating the integrated calibration heater to produce a step response in generated power. The corresponding signal, \( f(t) \), recorded when the heater was switched off, conformed with an exponential decay as a function of time, t (ms):

\[
f(t) = e^{-0.086t}
\]

From these experimental data, the microsystem was found to have a very good temperature sensitivity of 0.125 \( mK \), an unfiltered time constant of 12 ms, a heat conductance of 104 \( nW \) \( mK^{-1} \), and a low thermal mass, giving a heat capacity of only 1.2 \( nJ \) \( mK^{-1} \). The device had a detection limit, defined as the signal three
standard deviations above the mean of the background, enabling measurements with a resolution of 13 nW from either enzymatic or cellular systems. The time constant after signal amplification was limited to 200 ms by the 1-Hz low-pass filter. The loss of biological information at this bandwidth was thought to be negligible, as rapid changes in source temperature were not anticipated from the cellular assays.

**Brown Adipocytes.** Cell-based measurements initially focused on brown adipocytes, which play an important role in thermoregulatory heat production in “nonshivering thermogenesis” in mammals. Brown fat cells are unique in that they generate heat from uncoupled mitochondria by expressing mitochondrial uncoupling protein, UCP, which decouples respiration from oxidative phosphorylation and which ensures that the cells maintain a high respiratory capacity and heat production. The clinical investigation of brown adipose tissue is important in the study of obesity and a variety of pathologies, including fever and trauma. This cell type is also of particular interest as it has previously been used in a pharmaceutical toxicity screen for assessing the action of new pharmaceuticals on mammalian cells.

We have been able to use cells isolated directly as primary cultures (in this example, immature differentiated brown adipocytes) for use in this biological sensor. These cells have the advantage that they have a similar thermogenic capacity and a lower lipid content, when compared with fully matured adipocytes. The lower buoyancy of the immature cell (whose density is greater than that of the culture medium) facilitates their attachment to the sensor and enables a good thermal contact (Figure 4a).

Measurements of heat output from cells (Figure 4) were made with the cell chamber filled with 720 µL of culture medium and overlain with a layer of mineral oil to prevent evaporation. The device was held at a constant temperature of either 23 or 37 °C. Thermogenic responses of BAT cells were observed at decade changes of concentrations of NE (Figure 5) and were compared with cellular basal heat production (not quantified) prior to the injection of NE. In the absence of cells, there was no response from the thermopile to the injection of 10 µL of culture medium or to 10 µL of culture medium containing a final concentration of NE. In the presence of cells, the injection of NE resulted in a thermogenic response that was observed as an increase in the temperature of the culture medium, as recorded by the thermopile. The response was quantified by the change in the temperature of the culture medium, as recorded by the thermopile, and was expressed as a change in the power output of the thermopile.

![Figure 4. Photomicrograph of mammalian cells. (a) Cluster of 10 isolated brown adipocytes in the microfabricated sensor. The cells (numbered) were tightly packed and located on and around the sensing junctions of the thermopile to maximize the temperature rise and the detection of heat. Pretreatment of the substrate with 0.01% poly( L-lysine) improved attachment of the cells to the sensor. (b) A single cardiomyocyte positioned in the nanocalorimetric chamber. (c) Twenty cardiomyocytes, microinjected into a single ultra-low-volume chamber. Scale bar, 50 µm.](image)

![Figure 5. Thermogenic response from a group of 10 brown adipocytes exposed to the following: (a) pure culture medium (control, first and second arrows) followed by 1.4% acetonitrile (solvent for rotenone, third arrow); (b) culture medium (first arrow) followed by NE 0.14 µM (second arrow) and rotenone 14 µM (third arrow); (c) culture medium (first arrow) followed by NE 1.4 µM (second arrow) and rotenone 14 µM (third arrow); (d) culture medium (first arrow) followed by NE 14 µM (second arrow) and rotenone 14 µM (third arrow). All injections were of 10 µL. The open circles represent the standard error of the mean (n = 3).](image)


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1.4 μM NE; see Figure 6a. Exposure to 1.4 μM NE in the presence of clusters containing ~10 cells resulted in a rapid rise in heat production (Figure 6b), with the maximum value recorded within ~2 min. The slow temperature response was dependent on the thermogenic response of the cells when compared to the time constant of the low-pass filter. The magnitude of the response was consistent with published values from populations of cells, in which above-threshold levels of NE activation induced a 10-fold increase in oxygen consumption.

Heat output from NE-stimulated cells appeared to be sustained for at least 20 min (Figure 6b). Mitochondrial activity was then inhibited by adding rotenone to the chamber at a final concentration of 14 μM. There was a 3-min delay, reflecting the time for dissipation of the mitochondrial proton gradient, before the temperature response returned to basal level within 5 min. The observed delay could also be explained due to an increased metabolic activity from other major catabolic pathways such as glycolysis, supplying the cell with additional energy in response to ATP depletion. Thus, the return of metabolism to the basal level would only occur upon glucose depletion. Cell death, observed by extensive blebbing and lysis of the cell membrane, occurred between 1 and 5 min later, but no further fall in heat production was detected. The overall change in signal magnitude compared to the start of the experiment can be attributed to voltage drift, due to the absence of cellular metabolism after the death of the cells. The thermogenic responses from groups of 10 NE-stimulated cells corresponded to an average power output of 16.3 ± 7.3 nW or 1.63 ± 0.73 nW per cell (n = 5). Again, this is consistent with published values from tissues, which range from an averaged heat production of 0.4 nW per cell, measured using microcalorimeters, to 5 nW per cell, based on the oxygen consumption of tissue (10^6 cells g^-1 wet wt) in vivo.

**Cardiomyocytes.** To demonstrate the generic nature of the assay, measurements were also made of the heat output from isolated cardiomyocytes, challenged with the mitochondrial uncoupler CCCP. Un coupling of the mitochondria is believed to be important in cardiac pathology, and this technique is an established protocol for developing an in vitro model for the ischemic cell. Experiments were carried out using a variety of cell numbers ranging from single cells to groups of ~20 cells (Figure 4b,c). Accordingly, rabbit ventricular cardiomyocytes, exposed to CCCP (final concentration of 11.3 μM), showed a mean change in signal of 0.136 mK within 3 min, equivalent to the production of 1.71 ± 0.94 nW per cell (n = 5). This value is comparable with published data on uncoupled isolated cardiomyocytes and multicellular heart biopsies, values for which ranged from 1.1 to 4.7 nW per cell.

**Mitochondria.** Again, to emphasize the generic nature of the assay, investigations of the metabolic response from suspensions of isolated rat liver mitochondria were performed. The protocol was similar to that of the cardiomyocytes. Most of the energy generation and heat dissipation in the organelle takes place in the respiratory chain, the function of which is affected in most mitochondrial diseases and in some neurodegenerative disorders. Thus, the use of isolated mitochondria provides a method for direct correlation between the heat production and respiratory chain function without any interfering catabolic processes in the cell. Experiments were performed on highly concentrated mitochondrial suspensions of 20 mg mL^-1, which upon exposure to 12.6 μM CCCP generated 0.12 ± 0.9 mW mg^-1 power (n = 3). The corresponding oxygen consumption of 23.6 nmol of O_2 min^-1 mg^-1 was calculated from the additional energy released in the presence of CCCP, considering a 50% efficiency of the electron transport chain in synthesizing ATP per unit O_2 under standard conditions. The calculated oxygen consumption was only 9.3% higher than the measured oxygen consumption of the rat liver mitochondria of 21.6 nmol of O_2 min^-1 mg^-1.

**Conclusion.** Temperature measurements offer a generic approach to primary cell-based assays and have a capability to screen bioactive compounds, particularly where the cellular actions, signaling pathways, or suitable probes are either not known or not available. In this context, we have demonstrated the feasibility of implementing a novel method for monitoring temperature changes in isolated mammalian cells, presenting data that consistently show good correlations with experimental values obtained using multicellular preparations. The technology has been implemented in a microsystems format with low-volume
assays of extreme sensitivity, fast response time, and ease of manufacturing with a demonstrated ability to be integrated in a lab-on-a-chip methodology using processes adapted from the semiconductor industry. It has the clear potential to be used in multiple screens of cells isolated from a single organ or biopsy, thereby reducing inherent variations between animals and enhancing the quality of data sets.

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