Eur Biophys J (2004) 33: 117–120
DOI 10.1007/s00249-003-0352-5

ARTICLE

B. Steel · M. M. Bilek · C. G. dos Remedios
D. R. McKenzie

Apparatus for exposing cell membranes to rapid temperature transients

Received: 16 April 2003 / Accepted: 18 August 2003 / Published online: 24 September 2003

Abstract We seek to determine whether cell membranes contain sensors that trigger a downstream response to temperature excursions. To do this, we have developed a novel apparatus for exposing a cell membrane to an extremely rapid temperature excursion in the nanosecond range. Cells are plated on a gold surface that is back-heated by a pulsed laser and cooled by conduction of heat into the glass substrate and the liquid medium. Analysis using the heat diffusion equation shows that the greatest temperature rise is localized within a region tens of nanometres thick, suitable for specifically heating a cell membrane without heating the remainder of a cell. We refer to this device as a nanosecond hotplate.

Keywords Heat diffusion equation · Laser temperature jump · Thermal modelling

Introduction

Recently (Steel et al. 2002), we reported on a technique to produce a microsecond temperature excursion in a sample placed on a silicon surface. This technique was novel in allowing fast temperature excursions of a region of the sample up to one micrometre thick. Gold nanoparticles have also been used to expose bound protein to a nano- or picosecond temperature excursion (Radt et al. 2001). We combine elements of both systems in designing the nanosecond hotplate capable of transiently heating a large surface (on a biological scale) such as a cell membrane, or as an alternative method for investigating protein unfolding on a nanosecond timescale.

Production of a fast temperature excursion relies on very rapid heating and cooling of a sample. Lasers are a convenient method of rapidly delivering controlled amounts of power through the absorption of energy from a laser pulse. Lasers are readily available with pulse lengths as short as femtoseconds, although pico- and nanosecond lasers are more common. Very rapid cooling of samples can only be achieved by heat conduction. Since cooling time is related to the square of the dimensions involved, rapid cooling can be achieved by confining the temperature rise to less than a micrometre in at least one dimension.

Likewise, any confined temperature rise must be of short duration to avoid continually conducting heat to neighbouring regions. Because of this, any system designed for localized heating must involve heating over a time scale shorter than the characteristic time for heat conduction out of the system.

Most biological samples of interest are stable in an aqueous environment. This includes cells, proteins and DNA. However, in solution these samples do not absorb light strongly enough to localize heating within a micrometre. A strongly absorbing medium must be used to absorb laser light, and heat conduction relied upon to first heat, then cool the sample. Gold is ideal for this purpose as it is a strong absorber, good thermal conductor and is non-toxic to most biological specimens.

Gold nanoparticles have been used for transient heating of attached proteins (Radt et al. 2001), and the thermal characteristics have been studied for a range of particle diameters above 4 nm (Hu and Hartland 2002). Gold films can be readily manufactured in the 10–1000 nm thickness range using deposition techniques, such as magnetron sputtering. When such a film is coated on glass or another clear substrate, a laser pulse can pass through the glass to heat the film, allowing an

This paper was submitted as a record of the 2002 Australian Biophysical Society

B. Steel (✉) · M. M. Bilek · D. R. McKenzie
School of Physics, University of Sydney, 2006 NSW, Australia
E-mail: bsteel@physics.usyd.edu.au
Tel.: +61-2-93515980
Fax: +61-2-93517725

C. G. dos Remedios
Institute of Biomedical Research, University of Sydney, 2006, NSW, Australia

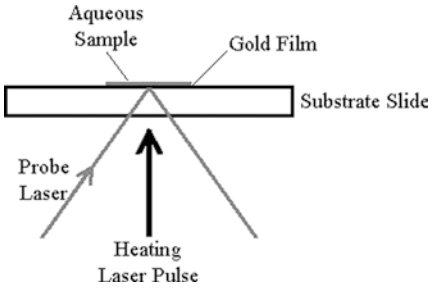

Fig. 1 Diagram of the nanosecond hotplate modelled in this paper

aqueous sample to be placed on the other side of the film and rely on conduction both to heat, then subsequently cool, the sample. We name this setup a nanosecond hotplate and it is presented in Fig. 1.

Sufficiently thick gold films ($>\sim$75 nm) block the laser beam from passing through the sample, removing the potential for any photoeffects. The reflectivity of gold decreases with temperature, allowing the temperature of the gold/glass surface to be monitored using reflected light in a method similar to that reported by Chen and Hui (1999).

Methods

Thermal modelling

Laser light is absorbed by electronic transitions in the gold. Electron–electron, electron–phonon and phonon–phonon coupling in gold is known to occur on timescales shorter than 50 ps (Ahmadi et al. 1996; Hu and Hartland 2002). Modelling of processes occurring over significantly longer timescales than this can use thermal conductivities derived from steady-state techniques without loss of accuracy.

A typical gold film for modelling was considered to be approximately 100 nm thick, and exposed by laser over an area of at least 1 mm^2. Since the ratio of thickness to width for the hotplate is around 10^4, it was considered appropriate to use a one-dimensional model of the heat flow in the system.

A finite-element analysis technique was used to model the temperature distribution. The system was broken into thin slabs, each with a fixed thickness and composition, and variable temperature, thermal conductivity and heat capacity.

The temperature change for each slab during an iteration of time Δt was:

$$T_i(t+\Delta t) = T_i(t) + \sum_{j=i\pm1} \left(Q_i + 2k_i k_j (T_j - T_i)\Delta t\right)/\left(k_i k_j + k_j l_i\right)/l_i c\rho \tag{1}$$

where i, j denote adjacent slabs, T is the temperature, k the thermal conductivity, l the slab thickness, c the heat capacity per unit mass, and ρ the density. Q denotes the energy per unit area absorbed within the slab from the laser pulse, and was calculated by using the software TFCalc to determine the absorption profile within the gold film. Optical constants for gold at room temperature were used interpolated for 532 nm light (Palik 1985).

Because the thermal properties of all materials vary significantly with temperature, thermal conductivity and heat capacity were recalculated each iteration for every slab, using a linear interpolation between published values for the appropriate material (Kaye and Laby 1995; Vargaftik et al. 1996; Lide 1999). Bulk values were used for conductivities.

For gold layers less than 100 nm thick, the gold was divided into five slabs of identical thickness for modelling. For 100 nm and thicker gold films, each slab was set to 16.7 nm thickness and the number of slabs adjusted accordingly. The substrate was divided into 50 slabs of staggered thickness, with thin slabs closer to the gold and thicker slabs away from the gold. Total thickness of the substrate modelled varied from 0.05 μm to 25 μm, depending on the substrate and gold thickness used; total width modelled in all cases was set to between two and four times the distance at which 0.1% of the surface temperature change occurred in the time taken for the surface temperature to cool to half its maximum value. Sixty slabs of water were modelled, the 10 closest to the gold of uniform thickness, followed by 50 slabs of staggered thickness.

The laser energy was assumed to be deposited uniformly over a set period of time, either 5 ps, 1 ns or 5 ns. Δt was set to 0.08 fs for gold film thicknesses of 100 nm and above, and shortened as required for thinner gold films.

The calculations were performed in MATLAB. Convergence of the finite element analysis method was checked for a number of models by doubling the number of slabs and shortening time steps by a factor of 10. No significant differences were detected.

The thermal properties of cells, proteins or DNA were assumed to be similar to water; however, the numerical details presented here will be slightly modified by the presence of lipids, proteins or other macromolecules close to the gold surface. However, we are not aware of accurate measurements of the thermal properties of membranes or proteins; even with such data, modelling would be further complicated by the uncertainties involved in including changes of state. After modelling more and less conductive water layers, we do not expect that including these elements would make a significant difference to the trends in the results presented here, and with the ability to measure the gold temperature during exposure it is possible to adjust the parameters used in modelling to give accurate numerical results.

Results

The effect of various substrates on the temperature profiles, for three lasers with square pulses of length 5 ps, 1 ns or 5 ns, was examined. A range of values for c (heat capacity), ρ (density) and k (thermal conductivity) were chosen for the substrate, with the model assuming these values were not temperature dependant. A gold layer 100 nm thick was modelled, followed by 1 μm of water in 60 layers of staggered thickness, 2 nm thick in layers close to the gold and up to 40 nm thick for distant layers. The hotplate was assumed to initially be at a temperature of 20 °C, with a laser flux of 4.0 mJ cm^{-2} absorbed by a gold layer (corresponding to an incident intensity of 14 mJ cm^{-2} at 532 nm, or 7 mJ cm^{-2} at 400 nm). It was quickly observed that temperature profiles in the gold and water layers depended only on the product $c\rho k$, a result that can also be shown analytically.

To describe the temperature profile, three parameters were selected. First, T_{max}, the maximum temperature reached by the gold/water interface, was determined. ϵ is the temperature efficiency of the hotplate, defined as the temperature rise divided by the fluence $(T_{max}-T_{initial})/F$. τ_{50} is our measure of the speed of the hotplate and is the time the interface spends above $(T_{max}+T_{initial})/2$, half the maximum temperature rise. Lastly, the maximum temperature reached by every point in the water was determined. Δ_{50} measures the distance from the gold

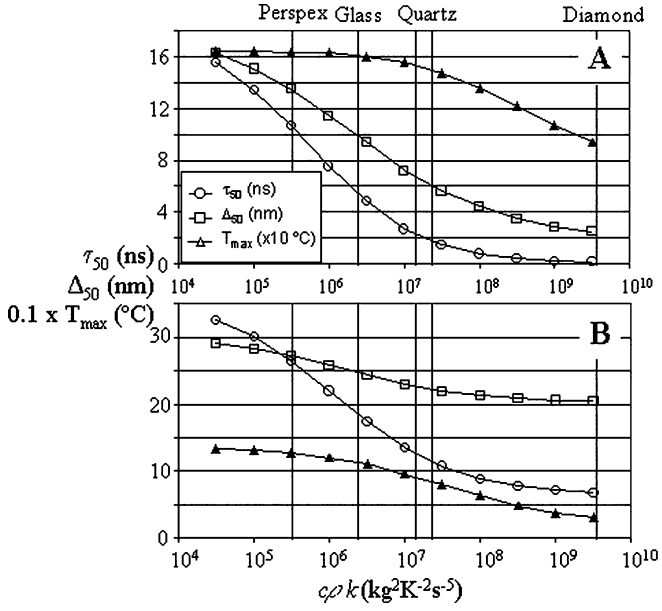

Fig. 2A, B Plot of the calculated timescale (τ_{50}), spread (Δ_{50}) and maximum surface temperature reached as a function of $c\rho k$ following an absorbed pulse of 4 mJ cm^{-2}. Part **A** models a 5 ps laser pulse, while a 5 ns laser pulse was used in **B**. The initial temperature was 20 °C, the gold film thickness 100 nm and the substrate was glass. Values of $c\rho k$ for Perspex, glass, diamond and quartz (two orientations) are marked with *vertical lines*

surface to the point where the maximum temperature reached was $(T_{max} + T_{initial})/2$. This is our measure of the size of the heated region.

Figure 2A and Fig. 2B show how T_{max}, τ_{50} and Δ_{50} vary for values of $c\rho k$ from 10^5 to 10^{10} kg^2 K^{-2} s^{-5} for a 5 ns and a 5 ps pulsed laser, respectively.

The effect of varying the thickness of the gold film was investigated in a series of simulations. Hotplates were modelled with gold layers from 10 nm to 300 nm thick, placed on one of three substrates: glass, quartz and diamond. These were simulated for 5 ps, 1 ns and 5 ns laser pulses.

Figure 3 plots the efficiency (ϵ) against the timescale (τ_{50}) of the hotplate. Glass, quartz and diamond lie on visibly separate portions of this graph. For very thin gold layers, the hotplate speed is close to the laser speed for 1 ns and 5 ns laser pulses, though only the diamond substrate responds fast enough to approach a 5 ps timescale for a 5 ps laser. The temperature efficiency falls considerably for thick gold films as the energy has a larger volume of gold to heat, and also falls slightly for very thin gold films as some of the laser energy is transmitted through the gold layer.

Discussion

Figures 2 and 3 demonstrate the time regime in which the nanosecond hotplate operates: roughly 0.1 ns to 100 ns. Although both faster and slower timescales are possible, there are limitations which make this time range the sensible limit. At the faster end of the spectrum, a highly conductive medium (diamond) has been shown (Fig. 3) to reduce τ_{50} below 10 ps, although the accuracy of this figure is doubtful, given the modelling limitations discussed previously for events shorter than 50 ps. However, using diamond in the hotplate is impractical, and there is a shortage of possible transparent substrates with higher thermal conductivity than quartz. Also, these timescales can only be achieved by reducing the gold thickness below its optical depth of 35 nm. In any experiment this immediately presents the possibility of photoeffects masking temperature-dependent effects.

Figure 2 plots values for Δ_{50}, our measure of the localization of the temperature pulse. Although Δ_{50} is much less sensitive to hotplate construction than τ_{50}, at the fastest timescales it is decreasing below 10 nm. Such a localized temperature pulse would also be of limited value.

Timescales slower than 100 ns are also possible, either with increased thickness of a gold film, or with a less conductive substrate such as Perspex. However, as Fig. 3 shows, using thicker gold films is comparatively inefficient. It would be sensible to use Perspex or another low-conductivity substrate in this time region, and also to use an absorbing medium with lower thermal conductivity than gold.

Δ_{50} is an important parameter which must be kept in mind in any experimental design. Clearly, large objects such as cells cannot be heated in their entirety using this method. However, proteins, DNA or other macromolecules can be tethered to a gold surface using current techniques, and cells can adhere to such surfaces. Proteins have been shown to be affected by similar temperature excursions when attached to gold nanoparticles (Radt et al. 2001). Using cells, this exposure

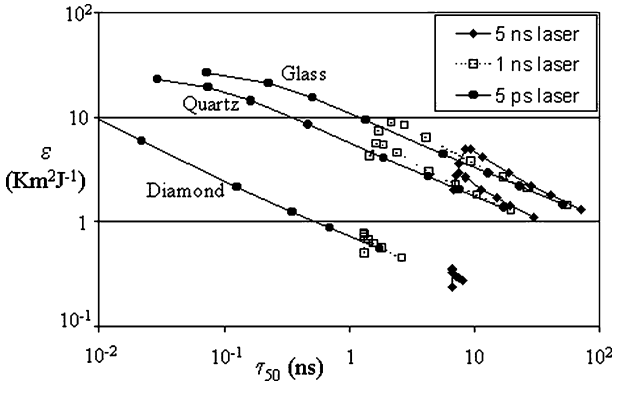

Fig. 3 Plot showing the relation between the temperature efficiencies (ϵ) and timescales (τ_{50}) of hotplate designs with three different laser pulses and three substrates. Substrates modelled are glass (*upper curves*), quartz (*middle curves*) and diamond (*lower curves*). Each substrate was modelled for a 5 ps, 1 ns and 5 ns laser pulse. All curves plot eight points corresponding to film thicknesses of (*left to right*) 10, 20, 30, 50, 100, 150, 200 and 300 nm. The 10, 20 and 30 nm points for diamond with a 5 ps laser do not lie within the timescales plotted and are hence not shown

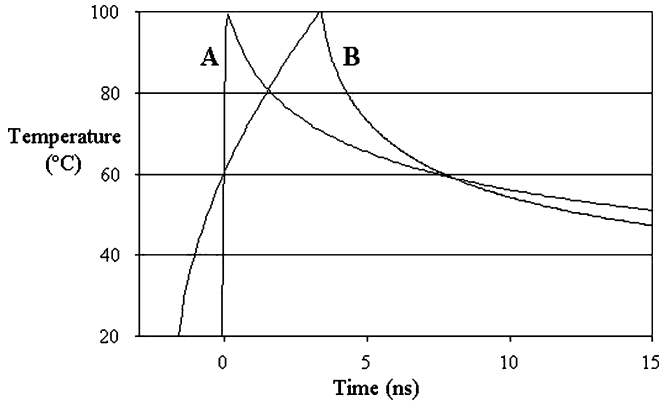

Fig. 4 Surface temperature of the nanosecond hotplate plotted against time for two setups with similar values of τ_{50}. Curve *A* models a 5 ps laser heating a gold film of thickness 200 nm, curve *B* a 5 ns laser with a 30 nm thick gold film. In both cases the substrate was quartz

would allow the heating of the cell membrane for a nanosecond period, without the temperature affecting the rest of the cell.

It should be noted that although both ϵ and τ_{50} provide measures for the efficiency and timescale at which the hotplate operates, neither offers a complete description of the system, and it is possible for two systems with the same value of τ_{50} to have different temperature profiles. Figure 4 presents the interface temperature as a function of time for two points plotted in Fig. 3 with similar values of τ_{50}. Large values of τ_{50} can be due to two effects, with speed limited by the design or by the laser pulse. In the former case, the temperature profile is a quick rise followed by a gradual decay, and is relatively insensitive to small changes in laser pulse shape or time. In the laser-limited case, the temperature profile is flatter, with a more gradual temperature rise and faster decay, and is more prone to effects from laser pulse time and shape. Laser-limited systems also spend more time near the maximum temperature for similar τ_{50}.

Both these situations could be advantageous. Laser-limited hotplates can use laser pulses of tunable shape and length to produce a nearly constant temperature profile for a short length of time such as 5 ns. Tuning this system could allow exposure of a sample to a nearly square temperature pulse of any amplitude or duration within the limits of the laser.

Design-limited hotplates can be used when a square temperature pulse is not required, and a fast laser is available. If the system is the slowest element, the laser is fast enough to allow the system to operate near its fastest possible speed. Because of this, the fastest temperature excursions ($\tau_{50} < 0.1$ ns) would be achieved in the "design-limited" domain. In this case, the shape of the temperature pulse has two features: it is almost independent of the laser pulse shape, determined only by the constructs of the system, and the system spends only a fraction of the τ_{50} time close to its maximum temperature. This further shortens the time that target molecules spend at high temperatures. The main limitation at these speeds is the confinement of the temperature rise, with Δ_{50} typically $\sim$2 nm for models with τ_{50} values near 0.1 ns.

Conclusions

The nanosecond hotplate described here offers new opportunities to impose temperature-jump pulses on biological systems. It provides an alternative method to heat-tethered molecules for nanosecond time periods, and to observe the effects of such transient heating. It will also permit us to examine the effects of very large temperature excursions on cell membranes without bulk heating of cellular contents.

References

Ahmadi TS, Logunov SL, El-Sayed MA (1996) Picosecond dynamics of colloidal gold nanoparticles. J Phys Chem 100:8053–8056

Chen G, Hui P (1999) Pulsed photothermal modelling of composite samples based on transmission-line theory of heat conduction. Thin Solid Films 339:58–67

Hu M, Hartland GV (2002) Heat dissipation for Au particles in aqueous solution: relaxation time versus size, J Phys Chem B 106:7029–7033

Kaye GWC, Laby TH (1995) Tables of physical and chemical constants. Longman, Harlow, UK

Lide RD (ed) (1999) CRC handbook of chemistry and physics, 79th edn. CRC Press, Boca Raton, USA

Palik ED (1985) Handbook of optical constants of solids. Academic Press, Orlando, USA

Radt B, Serbin J, Lange BI, Birngruber R, Huettmann G (2001) Laser generated micro- and nanoeffects: inactivation of proteins coupled to gold nanoparticles with nano- and picosecond pulses. Proc SPIE 4433:16–24

Steel BC, Bilek MM, McKenzie DR, dos Remedios CG (2002) A technique for microsecond heating and cooling of a thin (submicron) biological sample. Eur Biophys J 31:378–382

Vargaftik NB, Vinogradov YK, Yargan VS (1996) Handbook of physical properties of liquids and gases. Begell House, New York