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Sucrose modulation of radiofrequency-induced heating rates and cell death

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Abstract

Background—Applied radiofrequency (RF) energy induces hyperthermia in tissues, facilitating vascular perfusion. This study explores the impact of RF radiation on the integrity of the luminal endothelium, and then predominately explores the impact of altering the conductivity of biologically-relevant solutions on RF-induced heating rates and cell death. The ability of cells to survive high sucrose (i.e. hyperosmotic conditions) to achieve lower conductivity as a mechanism for directing hyperthermia is evaluated.

Methods—RF radiation was generated using a capacitively-coupled radiofrequency system operating at 13.56 MHz. Temperatures were recorded using a FLIR SC 6000 infrared camera.

Results—RF radiation reduced cell-to-cell connections among endothelial cells and altered cell morphology towards a more rounded appearance at temperatures reported to cause *in vivo* vessel deformation. Isotonic solutions containing high sucrose and low levels of NaCl displayed low conductivity and faster heating rates compared to high salt solutions. Heating rates were positively correlated with cell death. Addition of sucrose to serum similarly reduced conductivity and increased heating rates in a dose-dependent manner. Cellular proliferation was normal for cells grown in media supplemented with 125 mM sucrose for 24 hours or for cells grown in 750 mM sucrose for 10 minutes followed by a 24 h recovery period.

Conclusions—Sucrose is known to form weak hydrogen bonds in fluids as opposed to ions, freeing water molecules to rotate in an oscillating field of electromagnetic radiation and contributing to heat induction. The ability of cells to survive temporal exposures to hyperosmotic (i.e. elevated sucrose) conditions creates an opportunity to use sucrose or other saccharides to selectively elevate heating in specific tissues upon exposure to a radiofrequency field.

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Graphical abstract

Keywords

radiofrequency; sucrose; hyperthermia; conductivity; cancer; serum

Introduction

Therapeutic approaches to increase drug accumulation in the tumor or enhance the efficacy of chemotherapy or radiation include applied hyperthermia.[1] Hyperthermic techniques include delivery via internal/external energy sources, perfusion of organs or limbs, irrigation of body cavities, or whole body hyperthermia.[2, 3] Elevated cell death in solid tumors during whole body heating has been attributed to the tumor microenvironment where the chaotic vasculature and dense matrix result in hypoxia and low pH, increasing cell sensitivity.[3]

Hyperthermia-induced alterations in tumor neovasculature perfusion and permeability has been demonstrated *in vivo* using near-infrared [4, 5] and radiofrequency (RF) [6] energy. Using an integrated microscopy and clinically non-invasive RF system, we previously demonstrated that mild temperature elevations to 41°C, as monitored using an infrared camera, increase tumor perfusion of Alexa Fluor 647 albumin and FITC dextran in mice bearing 4T1 breast tumors. At temperatures near 44°C, blood coagulation and tumor vessel deformations occurred.[6] Using chick embryos, others have demonstrated that even mild temperature increases of 3-4°C cause gaps between endothelial cells, causing vascular leak similar to that achieved with inflammatory factors.[7]

A critical parameter in ohmic (joule) heating is electrical conductivity.[8] Beyond the impact of RF-mediated hyperthermia on the morphology of the vascular endothelium, this study explores the impact of altering the conductivity of biologically-compatible solutions on heating rate, thermal dose and cell death in a RF field. For a nonhomogeneous material, such as tissue, conductivity is a critical parameter in determining the heating rate. As an example,

in addition to sugar, fat and protein, blood plasma contains 0.15 M NaCl. The ability of ions (Na^+ , Cl^-) to orient water molecules around them (i.e. strong hydrogen bonds) decreases their ability to adjust in an applied electric field, producing less heat.[9] Conversely, sucrose and urea hold water molecules by weak hydrogen bonds, destructuring the hydrogen bond network [10, 11] and enabling water to more easily rotate in an oscillating field of electromagnetic radiation, facilitating heat production based on dielectric polarization.

In this study, we modulate the conductivity of biologically-relevant solutions using NaCl and sucrose, and that of serum through supplementation with sucrose in order to study the relationship between conductivity, heating rate and cell integrity/death. To explore off-target effects and the translational potential of using sucrose to direct RF-mediated heating to pathological tissues, we monitor cellular responses to hyperosmotic solutions containing 125 to 750 mM sucrose. In addition to cell viability, cellular structure and integrity of the cytoskeleton are monitored immediately following exposure to hyperosmotic conditions or after a recovery period in normal media.

Experimental Section

RF system configuration

A Thermo LLC (Erie, PA, USA) capacitively-coupled RF device operating at 13.56 MHz was used to irradiate samples. The external RF generator, equipped with adjustable output power (0-2000 Watts), was connected to a high-Q coupling system equipped with a transmitter head (focused end-fired antenna circuit), receiving head and water chiller. Temperatures were recorded using a FLIR SC6000 infrared (IR) camera (FLIR Systems, Boston, MA, USA), with a thermal sensitivity, or noise equivalent temperature difference, of 20 mK at 30°C and a range of -20°C to 350°C.

Thermal dose calculations

Thermal doses, expressed as cumulative equivalent minutes at 43°C, were calculated using data from time-temperature plots for sucrose/FBS solutions exposed to RF radiation at 13.56 MHz for 2 minutes in a quartz cuvette using the following equation:

$$\text{CEM } 43^\circ\text{C} = \Delta t * R^{(43-T)}$$

Wherein Δt is the time interval, T is the average temperature, and R is a constant at 0.25 for $T < 43^\circ\text{C}$ and 0.5 for $T > 43^\circ\text{C}$. [12]

RF-mediated heating of solutions

Sucrose solutions at 125-1000 mM were prepared using 18.2 MΩ distilled water, fetal bovine serum (FBS), or cell culture media. A quartz cuvette housing 1.3 of the solution was placed in a custom Teflon holder. Using an electronically driven device, the cuvette was placed at 5/16ths of an inch from the RF transmission head in the central region of the field with a 10 cm air gap between the transmitting and receiving heads. Samples were exposed to a high voltage RF field operating at 13.56 MHz and 900 Watts. Temperatures were recorded

using an FLIR SC6000 IR camera, with irradiation halted if temperatures approached 50°C or sparks were generated. Each measurement was repeated in triplicate.

Cellular RF hyperthermia

HeLa human cervical carcinoma cells were purchased from ATCC (Manassas, VA, USA). Cells were cultured in Eagle's Minimum Essential Medium containing 10% fetal bovine serum and 100 I.U./ml Penicillin and 100 µg/ml streptomycin. Cells were seeded into the end wells of a 12-well plate in 2 ml of media per well at a density of 1×10^5 cells/ml. For serum studies, prewarmed media containing 0-25% FBS was added to cells, and cells were exposed to RF energy at 900 Watts for 5 minutes. Cells were then returned to conventional media containing 10% serum for 24 hours prior to evaluating viability.

For all studies, the cell culture plate was positioned between the transmitting and receiving heads on a Teflon holder 3.0 cm from the transmitting head, and cells were exposed to RF energy at 13.56 MHz at 900 watts for the indicated duration. Temperatures were monitored in all wells containing media and cells using the FLIR IR camera. Viability was evaluated 24 hours after RF exposure by propidium iodide uptake in detached and adherent cells, measured using a BD Biosciences LSR flow cytometer (San Jose, CA, USA).

Isolation, RF exposure, and imaging of porcine inferior vena cava

Pigs were put on the operating table in the supine position. General endotracheal anesthesia was administered and a midline sternotomy was performed. The left and right pleural spaces were opened, followed by opening of the pericardium with exposure of the heart. The left pleural reflection was dissected free from the inferior vena cava (IVC), the esophagus and the diaphragm. Then the abdominal cavity was entered just below the pericardial-diaphragmatic attachment. Heparin was given and the IVC was cut right at its hepatic junction in order to exanguinate the pig. Using a no-touch technique, the IVC was then cut at its diaphragmatic passage and at its junction with the right atrium. The IVC samples were immediately stored in DMEM/F12. All animal procedures were approved and are stated in the Baylor College of Medicine protocol AN-3346.

For transmission electron microscopy, dehydrated tissue was embedded in Epon resin, sectioned using a Leica U6 Ultramicrotome, stained with uranyl acetate, and imaged using a Hitachi H-7500 Transmission Electron Microscope.

Dielectric measurements

Permittivity measurements were acquired with an Agilent 85070E high temperature coaxial dielectric probe with an Agilent E4991A impedance analyzer (Agilent Technologies, Santa Clara, CA). The calibration was done with a four-point open, short, 50Ω load and low loss capacitor. The probe was calibrated with air, a shorting block, and Milli-Q water. Air calibration was used between samples. Corrected permittivity values were determined using published equations in MatLab.

DC conductivity measurements

Solution DC conductivity was measured using an InLab 731 conductivity probe manufactured by Mettler Toledo, Inc. (Columbus, OH, USA). The probe was calibrated with 84, 1413, 12880 $\mu\text{S}/\text{cm}$ standards. Data presented are the average of three measurements.

Fluorescent microscopy imaging of cells following exposure to hyperosmotic conditions

For confocal imaging, cells were seeded onto collagen-coated glass coverslips in 6 well plates at a density of 2×10^5 cells per well. The following day, cells were exposed to 125, 250 or 750 mM sucrose for 10 or 30 minutes, or 24 hours. Cells were then washed with PBS, fixed with 4% paraformaldehyde, and permeabilized with 0.1% triton X-100 for 15 minutes. PBS containing 1% BSA was used as a blocking agent prior to incubation with 200 nM Alexa Fluor 555 conjugated phalloidin (Invitrogen, Carlsbad, CA, USA) and anti-tubulin FITC conjugated antibody (Abcam Inc., Cambridge, MA, USA). Coverslips were then washed and mounted on glass slides using Prolong® Gold Antifade Reagent with DAPI (Fisher Scientific, Grand Island, NY, USA). Samples were imaged using either a Nikon upright or inverted fluorescent microscope equipped with either a 20 or 40 $\times$ dry objective.

Atomic force microscopy analysis of cell topography and volume

HeLa cells were grown to a 50% confluence in 60 mm polystyrene petri dishes coated with 50 $\mu\text{g}/\text{ml}$ rat tail collagen I to enhance cell attachment. Cells were then treated with 250 or 750 mM sucrose in 1 $\times$ PBS for 10 or 30 minutes at 37°C, followed by immediate fixation in 4% paraformaldehyde. AFM was conducted at the UTHealth Science Center AFM Core Facility using a BioScope II™ Controller (Bruker Corporation; Santa Barbara, CA, USA). The image acquisition was performed with the Research NanoScope software version 7.30 and analyzed with the NanoScope Analysis software version 1.40 (copyright 2013 Bruker Corporation). This system was integrated to a Nikon TE2000-E inverted optical microscope (Nikon Instruments Inc.; Lewisville, TX) to facilitate bright field imaging of the cell cultures. Random cells were selected, for each treatment, with the aid of optical microscopy (20 $\times$). Never dried cells were scanned in PBS using contact mode and MLCT cantilevers ($f_0=4\text{--}10$ kHz, $k=0.01$ N/m, ROC=20nm) Bruker Corporation (Santa Barbara, CA, USA).

The topography of the cell membrane was determined using contact mode operated in liquid to a scan rate of 0.5 Hz. Images of the cell structure were captured to a scan area of 50 to 80 μm^2 depending of the cell size. Recovery images were also captured of HeLa cells treated with 250 and 750 mM sucrose in media for 10 minutes at 37°C, followed by a recovery phase of 2 hours at 37°C in 1 $\times$ PBS. The height or thickness of the cell was determined by the difference between the highest point on the cell and the lowest point over the substrate (Figure 7 B,C) using the section analysis application of the Nanoscope Analysis software. The Volume of the cells was calculated using the bearing analysis application in the same software (data not shown).

Results

Effect of RF radiation on the morphology of the vascular endothelium

In this study, we explore the physical impact of RF radiation on tumor endothelium using an *ex vivo* porcine inferior vena cava vascular model. The Thermo LLC RF system, operating at 13.56 MHz (Figure 1a), was used to irradiate tissues immersed in PBS. Temperatures were monitored using a FLIR infrared camera focused on the outside three wells of the plate, two containing PBS controls and one containing the tissue specimen suspended in PBS (Figure 1b). All wells heated at the same rate (Figure 2b, lower), with final temperatures having mean values of 35.8 ± 0.6 °C based on the IR reading through the plastic plate. Transmission electron micrographs (Figure 1c) of control and RF-treated tissue support a 'rounding' of luminal endothelial cells following 14 min RF exposure, with loss of tight adhesion between neighboring endothelial cells.

As previously reported [13], IR temperature measurements of bulk media housed in plastic plates increase approximately 4 degrees when the outer rim of the plastic dish is removed. While we acknowledge that a discrepancy exists between actual temperatures and those measured by the IR camera,[14] we chose to use the IR camera based on its rapid response time, which enabled us to closely monitor samples subject to rapid heating to reduce risks due to electrical arcing.[14] Considering that actual temperatures of media housing the vessel likely exceeded 40°C, the resulting structural changes influencing cell-to-cell contact within the endothelium could account for reports of increased vessel perfusion *in vivo* and extravasation of macromolecules reported by others at similar temperatures. [6]

Effect of serum and RF dose on heating and cell viability

Macromolecules, such as proteins, cause charge dispersions at frequencies in the alpha (low frequency) and gamma (microwave) range. In this study, we explored the impact of serum and its associated proteins on heating rates of cell culture media at 13.56 MHz. Based on IR monitoring of temperature, sample replicas displayed identical heating curves (Figure 2a) and no significant differences in the heating rate of media alone or media containing 5-20% serum were observed (Figure 2b). When adherent HeLa cells were present in the serum solutions, no differences in cell death were observed at 24 hours post RF treatment based on a dose duration of 5 minutes (Figure 2c).

To examine the relationship between RF dose duration, temperature and cell death, adherent HeLa cells, seeded into 12-well plates in triplicate, were exposed to radiowaves for 4-8 minutes at 900 Watts. Linear fit of the temperature versus time data yielded a strong correlation coefficient of $R^2 = 0.87$ (Figure 2d). Cell death 24 hours after exposure was similar for cells treated for 0 and 4 minutes, but increased by 5-15% for cells treated for 5-6 minutes, and by 20 and 45% for cells in an RF field for 7 and 8 minutes, respectively (Figure 2e). The relationship between cell death and maximum temperature had a weak correlation coefficient of $R^2 = 0.44$, indicating that high temperature alone was not responsible for cell death.

To study the impact of heating rate on cell viability, isotonic solutions of varying conductivity were prepared using various concentrations of NaCl and sucrose, and

maintaining MgCl_2 at a constant 1 mM. RF-induced heating rates increased as the concentration of sucrose in the solution increased and as NaCl decreased (Figure 3a). During RF irradiation of solutions containing high sucrose, elevated heating and rapid condensation resulted in the accumulation of sucrose crystals on the lids of the cell culture plates and electrical arcing. Heating for all samples was terminated when the media reached a final temperature ranging from 32–36°C, based on IR detection through the plastic plates. Cell death, as measured by uptake of propidium iodide 24 hours post exposure, ranged from 30–80%, with no correlation between maximum temperature in the selected range and cell death (Figure 3b). Interestingly, there was a strong positive correlation ($R^2 = 0.925$) between relative heating rate and cell death (Figure 3c).

Heating rates of the three isotonic solutions in an RF field were repeated in triplicate using a quartz cuvette to confirm relative heating rates in the absence of the plastic dish. Elevated heating rates for solutions with high sucrose and low NaCl were confirmed (Figure 4a,b). In addition, the conductivity of the solutions was found to decrease as the concentration of sucrose was increased and NaCl was decreased (Figure 4c,d), supporting the link between solution conductivity and heating rates in the RF field.

Effect of sucrose on the RF-induced heating rate of serum

To explore the impact of supplemental sucrose on RF-induced heating rates of serum, sucrose ranging from 125 to 1000 mM was added to FBS and temperatures were measured as a factor of time in an RF field. Serum heating rates increased with elevations in sucrose (Figure 4e), and conversely, the conductivity of serum decreased incrementally with increasing volume fraction of sucrose (Figure 4f).

The influence of electric field frequency on permittivity is shown in Figure 5a for the range 10–10,000 MHz. The greatest variability occurs at the low end, with the measured and corrected permittivity at 13.56 MHz shown in Figure 5b. As the concentration of sucrose in serum is increased, the permittivity decreases. The positive relationship between permittivity (dielectric loss) and conductivity for the samples is displayed in Figure 5c, and the frequency of conductivity crossover, near 2.4×10^9 , for the samples is shown in Figure 5d. The relationship between sucrose concentration in serum and thermal dose in an RF field, the latter expressed as CEM 43°C, was linear with a correlation coefficient of 0.96.

Effect of sucrose on cell size, viability and recovery

The impact of sucrose on cell morphology and biophysical properties was examined using atomic force microscopy (AFM). AFM measures of cell morphology, surface roughness and stiffness have been shown to be indicative of apoptosis.[15] HeLa cells treated with 250 and 750 mM sucrose in PBS for 10 or 30 minutes displayed flattening in cell structure, more prominent nuclear visualization, with a bumpy appearance on the cell membrane and exaggerated filopodia projecting from the cell (Figure 6a). When exposed to sucrose for 10 minutes, followed by recovery in $1 \times$ PBS for 2 hours, cells treated with 250 or 750 mM sucrose regained normal cell features, with reduced filopodia, smoother surface, and more body throughout the cell (Figure 6b). When cells were given a full 24 hours in cell culture media to recover from the 10 minutes of sucrose treatment, normal rates of proliferation

were observed for cells exposed to 125-750 mM sucrose (Figure 6c), supporting the ability of cells to recover from temporary exposure to hypertonic solutions. Cells treated with RF and sucrose were loosely or no longer adherent to the cell culture device, making them unavailable for imaging by AFM.

In addition to surface topography (Figure 7a), atomic force microscopy can be used to measure cell height (Figure 7b-d), volume (Figure 7e,f), binding affinities, elasticity and surface roughness. When integrated with an optical microscope, bright field imaging is used to locate cells (Figure 7g). Cell height was compared for HeLa cells following 10 or 30 minutes exposure to 250 and 750 mM sucrose, with and without a 2-hour recovery period (Figure 7d). While cell height was similar for control cells and cells treated with 250 mM sucrose, there was a significant reduction in cell height after both 10 and 30 minutes of treatment with 750 mM sucrose. Cells permitted a 2 hour recovery phase after exposure to either 250 or 750 mM sucrose regained normal cell height supporting physical recovery.

To gain further insight into the impact of sucrose on cellular integrity, we examined the actin cytoskeleton 30 minutes or 24 hours after the introduction of sucrose at 125-1000 mM. Fluorescent images of HeLa cells incubated with sucrose for 24 hours and stained with rhodamine phalloidin are shown in Figure 8. Reorganization of actin stress fibers with uneven distribution of actin filaments is seen after treatment with 125 mM sucrose, and the chaotic actin organization is further enhanced after treatment with 250 mM sucrose. The few cells that remained after 24 hours in 500-1000 mM sucrose displayed a dramatic decrease in cell volume, with complete breakdown of cell structure (data not shown). Based on analysis of 3D tomograms of cellular actin obtained using confocal microscopy, cell volume was similar for cells after 24 hours in control or 125-250 mM sucrose. In addition to studying actin filaments, anti-tubulin antibody was used to explore the impact of hypertonic solutions on the microtubular network. Confocal micrographs displayed at the bottom of Figure 8 show increased disorganization of both microtubules (green) and actin (red) after just 30 minutes exposure to 750 mM sucrose as compared to 250 mM.

Discussion

Herein, using a porcine inferior vena cava model, we demonstrated that RF irradiation disrupts the vascular integrity of the luminal endothelium, leading to 'rounding' of endothelial cells and reduced cell-to-cell adhesion. This is consistent with reports by Hayat and Friedberg[16] showing that heat causes gaps between endothelial cells due to alterations in the integrin-cytoskeleton network leading to cell shape changes.[17] Kong *et al.* [18] further showed that temperatures below 42°C cause vessel alterations leading to increased extravasation of nanoparticles which he attributed to disaggregation of the endothelial cytoskeleton, including morphological changes that appeared to 'shrink' the cells.

When cells are placed in a radiofrequency field, frequency dispersions, called β -dispersions, occur at the cell membrane. The dispersions are largely due to Maxwell-Wagner [19] relaxation caused by polarization effects at the aqueous hydrophilic interface, which for cells is the phospholipid membrane.[20] While macromolecules, such as proteins, cause

dispersions at frequencies in the alpha and gamma range, addition of proteins, in the form of serum, to media did not influence RF-induced heating rates or impact cancer cell viability.

The ability of water molecules to rotate in an oscillating field of electromagnetic radiation contributes to heat through electric polarization.[22] Herein, biological solutions containing high sucrose and low salt displayed low conductivity and elevated heating rates in an RF field. The heating rates were linearly and positively correlated with cell death. In 2003, Dewhirst *et al.*[21] similarly reported that the rate at which cells and tissues heat can have a profound effect on the degree of cytotoxicity.

In contrast to ions, sucrose forms weak hydrogen bonds in fluids, freeing water molecules to continually rotate in an oscillating field of electromagnetic radiation and thereby contribute to heat induction. In other words, the chaotropic (structure-breaking) properties of sucrose in water [23] contribute to dielectric loss or heat production.

To explore the translational potential of using sucrose to accelerate RF induced heating rates in vivo, we supplemented serum was supplemented with incremental increases in sucrose and then exposed samples to RF radiation. The resulting dose-dependent decrease in conductivity was correlated with increases in the RF-induced heating rate. Higher concentrations of sucrose (low conductivity) support higher kinetic energy of associated water molecules and thereby dissipation or loss of energy through dielectric heating. Herein, decreases in conductivity mirrored increases in sucrose and were proportional to decreases in the imaginary permittivity (dielectric loss). The decrease in permittivity was dramatic at the investigated 13.56 MHz, but became less distinct at red-shifted frequencies. The crossover at which the impact of sucrose on conductivity reversed occurred near 2.4×10^9 Hz.

Sucrose, uncharged and bulky, does not freely cross the cell membrane, creating a hypertonic solution. When exposed to hyperosmotic stress, cells undergo shrinkage due to efflux of water and cytoskeletal rearrangements. Renal medullary cells frequently experience hypertonic fluxes with elevations in NaCl and urea. To compensate for shape and fluid changes, cells activate protective repair mechanisms, in a process known as regulatory cell volume increase (RVI).[25] Roger *et al.*[25] demonstrated that cell shrinkage (65% initial volume) with 600 mM sucrose was maximal at 10 minutes and that cells recovered 82% of their initial volume within 30 minutes. Using AFM to monitor changes in cell topography (e.g. height), we similarly demonstrated that cells exposed to 750 mM sucrose experienced a reduction in cell height that was recovered by return of cells to isotonic solution.

Yamamoto demonstrated that HeLa cells in hypertonic solution (250 mM sucrose) reorganize the actin cytoskeleton within 2 minutes of exposure, with maximal effects occurring within 10 minutes.[26] In the current study, HeLa cells exposed to 750 mM sucrose demonstrated similar structural changes in both actin and microtubule networks, with cells able to recover from short duration (10 min) exposures with normal proliferation 24 hours post exposure. Recovery of the cytoskeletal network is extremely important as it impacts cellular functions, including adherence, mobility and invasion.[15] While the overarching goal of this study was to determine if sucrose can be used to direct heat to

pathological tissues, such as tumors, it also sought to insure the vitality of normal tissues and avoidance of alterations to cancer cells that augment cancer cell invasion.

Conclusions

A critical parameter in ohmic heating is electric conductivity. For a nonhomogeneous material, such as tissue, fluid conductivity is a critical parameter in determining the heating rate. We demonstrated that application of sucrose to biological solutions causes temporal, reversible changes in cell structure, and results in faster ohmic heating and higher rates of cell death in an RF field. Clinical application of sucrose, or other saccharides, to biological lesions could therefore potentially support localized hyperthermia using RF energy. Therapeutic applications include localized vascular effects, including vessel shut-down, enhanced drug delivery through increased vascular permeability, and localized induction of cell death.

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Figure 1. Impact of radiofrequency (RF) hyperthermia on luminal endothelial cell structure
 Porcine inferior vena cava (IVC) was used to study RF-induced morphological changes to the endothelium. A) Photograph of RF system (top) and a 12-well cell culture plate placed on a teflon stage between the RF transmitting and receiving heads (bottom). B) Heat map generated using an infrared camera focused on the 3 end wells of a 12-well plate (top). Graph of temperature (°C) versus time for 3 wells, 2 containing PBS and one with PBS containing IVC tissue (bottom). C) Transmission electron micrographs of control and RF-treated (900 watts for 14 min) IVC.

Figure 2. Relationship between RF exposure, heat and cell death

A) Graph showing heating versus time for triplicate wells containing media with 10% fetal bovine serum. B) Adherent HeLa cells in 12-well plates were put in RPMI media containing 0, 5, 10, or 20% fetal bovine serum and then exposed to RF radiation at 900 watts for 5 min in triplicate. The bar graph shows the calculated heating rate for each condition. C) After RF exposure, cells were returned to media with 10% FBS and incubated for an additional 24 h at 37°C. Shown graphically is cell death based on flow cytometric analysis of propidium iodide uptake. D) Adherent HeLa cells in 12 well plates with 2 ml of cell culture media per well were treated in triplicate with RF for 4-8 min. The maximum temperatures per well are graphed as a function of exposure time. Linear fit of the data resulted in a strong correlation coefficient $R^2 = 0.87$. The percent dead cells are presented as a function of dose duration (E).

Figure 3. Heating rate is directly related to cell death

A) Heating rates of isotonic solutions containing variable concentrations of NaCl and sucrose in 1mM MgCl₂ exposed to RF at 13.56 MHz and 900 W in 12 well plates containing 2 ml per well (*p<0.05). B) Percent cell death (propidium iodide uptake) 24 h after RF as a function of maximum well temperature. C) Percent cell death as a function of heating rate.

Figure 4. Sucrose accelerates the heating rate of physiological solutions in a dose-dependent manner

A) Heating rates of NaCl/Sucrose/MgCl₂ solutions placed in a quartz cuvette and exposed to RFH at 13.56 MHz, 900 w. B) Heating rates of solutions shown as a function of NaCl or sucrose concentration. C) Conductivity of NaCl/sucrose/MgCl₂ solutions determined using an InLab 731 conductivity probe. D) Conductivity of solutions shown as a function of NaCl or sucrose concentration. E) Heating rates of serum samples supplemented with sucrose ranging from 125-1000 mM. D) Conductivity of serum-sucrose solutions.

Figure 5. Impact of sucrose on serum conductivity and permittivity (ϵ')

a) Permittivity (AC capacitance) of serum containing 0-1000 mM sucrose from 10-10,000 MHz. b) Permittivity (measured and corrected) of FBS-sucrose solutions at 13.56 MHz. c) Dielectric loss (ϵ'') and conductivity of serum supplemented with 1-1000 mM sucrose. d) Conductivity crossover for serum containing 0-1000 mM sucrose. e) Thermal dose for sucrose solutions in FBS expressed as cumulative time at 43°C (CEM43). A positive relationship exists for thermal dose and heating rate with a correlation factor of 0.95.

Figure 6. Impact of sucrose on cell morphology and proliferation

A) Atomic force micrographs of HeLa cells showing surface topography following treatment with PBS containing 250 or 750 mM sucrose for 10 or 30 min at 37°C. B) Surface topography of cells treated with 250 or 750 mM sucrose for 10 minutes followed by incubation in PBS for 2 h at 37°C. C) Alamar blue proliferation assay of cells incubated with 125-750 mM sucrose in media for 24 h or for 10 min followed by 24 h recovery in conventional cell culture media. AFM images were acquired in contact mode in PBS following fixation.

Figure 7. Atomic force microscopy analysis of cell height and volume

Cells were scanned using contact mode operated in liquid using a scan rate of 0.5 Hz. A) Topography of a HeLa cell after 250 mM sucrose treatment for 10 min at 37°C. B,C) The height or thickness of the cells was determined by the difference between the highest point on the cell and the lowest point over the substrate using the section analysis application of the Nanoscope Analysis software. D) Comparison of cell height after 10 or 30 min incubation in PBS containing either 250 or 750 mM sucrose. Data shown in green represents cells treated with sucrose for 10 min followed by 2 h recovery in PBS at 37°C. E,F) Cell volume was calculated using the bearing analysis application in the same software. G) Bright field image taken with the integrated optical microscope (cell in previous measurements is highlighted with a blue box).

Figure 8. Hypertonic solutions induce reorganization of cellular cytoskeletons

Confocal micrographs of HeLa cells stained with rhodamine phalloidin (red) and DAPI (blue) following 24 h incubation with media supplemented with 125 or 250 mM sucrose (top), or of cells stained with phalloidin (red) and anti-tubulin antibody (FITC; green) after 30 min incubation with media containing 250 or 750 mM sucrose (bottom).