Article

Mechanisms of the non-thermal exposure effects of non-ionizing Millimeter Waves radiation on Eukaryotic Cells for improving technological precision enabling novel biomedical applications

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Abstract: Nonionizing millimeter-waves (MMW) are reported to interact with cells in a variety of ways. Possible mechanisms of the inhibited cell division effect were investigated using 85-105 GHz MMW irradiation within the ICNIRP (International Commission on Non-Ionizing Radiation Protection) non-thermal 20 mW/cm^2 safety standards. ~1.0 mW/cm^2 exposure over 5-6 hours treatment on 50 cells/µl samples of *Saccharomyces cerevisiae* model organism, resulted in 62% growth rate reduction compared to control (sham). The effect was specific for 85-105 GHz range and energy dose and cell density dependent. Irradiation of wild type and *Δrad52* (DNA damage repair gene) deletion cells presented no differences of colony growth profiles indicating non-thermal MMW treatment does not cause genetic alterations. Dose versus response relations studied using a standard horn antenna (~1.0 mW/cm^2) and compared to that of a compact waveguide (17.17 mW/cm^2) for increased power delivery resulted in complete termination of cell division via non-thermal processes supported by temperature rise measurements. Combinations of MMW mediated Structure Resonant Energy Transfer (SRET), membrane modulations eliciting signaling effects, and energetic resonance with biomolecules were indicated to be responsible for the observations reported. Our results provide novel mechanistic insights enabling innovative applications of nonionizing radiation procedures for eliciting targeted biomedical outcomes.

Keywords: Non-ionizing Radiation; Millimeter waves; Novel biomedical applications; Yeast; Non-invasive devices

1. Introduction

The influence of millimeter wave (MMW) radiation on biological systems has gained prominence in recent years because of two important reasons: 1) to establish safety standards for the use of MMWs in communication devices, 2) to understand the mechanisms of interaction between MMW and living systems. These investigations have opened new avenues for potential applications of MMW in the field of biomedical devices like selective targeting of cancer cells. MMW in the range of 75-110 GHz (W-band) are classed as nonionizing radiation because of the low 0.3-0.4 meV range of energy in their photons.

Cancer considered to be one of the deadliest human diseases is challenging to diagnose at early stages [1]. Cancer is known to arise from accumulated mutations in oncogenes and tumor suppressor genes, leading to uncontrolled tumor cell growth [2,3]. Conventional radiation therapy in cancer treatment gives rise to many detrimental side effects [2] including the development of other more

dangerous cancers due to the ionizing radiation (involved in such treatments) resulting in mutagenesis [3]. Thermal ablation techniques have been employed as the other alternative but with restricted application in order to not burn normal cells/tissues during treatment. Our experiments demonstrated that non-ionizing MMW (75–105 GHz) exposure with a non-thermal power density of 0.2 mW/cm^2 can elicit morphological changes in H1299 human lung cancer cells [4] leading to targeted apoptosis and mortality [5] without harming normal cells under the same exposure conditions. MMWs are also reported to be helpful for detecting different types of cancers [6]. Further, such technologies involving non-ionizing radiation has shown promising applications in the treatment of other diseases [7, 8] like gastrointestinal disorders, wound healing, remote monitoring of wounds, non-invasive detection of glucose levels, pain relief, diabetes, dermatitis, etc. However, the exact mechanism of the therapeutic effects on biological specimens is not well understood hindering the wide-scale application of this technology.

Being the simplest eukaryotic organism with a nucleus, *Saccharomyces cerevisiae* yeast cells are a standard model organism for *in-vitro* studies of cell division applicable up to higher eukaryotes. Many essential cellular processes in yeast and humans are similar, making yeast a suitable system to study basic molecular processes [3, 9, 10]. About 23% of the yeast genome is conserved with human cells, including all the corresponding biological functions and biochemical pathways remaining the same [11]. Characteristics of tumor cell growth have been discovered using models of yeast cell division [3, 9, 10]. Yeast cells are a cheap laboratory model that are simple to grow, culture, and experiment making it easier to unravel molecular mechanisms of MMW interactions within cells. Previous studies involving irradiation of wild type *Saccharomyces cerevisiae* yeast culture in aqueous suspensions have been highly ambiguous [12, 13]. These studies reported either no change or increased/decreased rate of growth upon microwave irradiation of 42 GHz and 50 mW power [12, 13]. The authors reported the exclusion of thermal effects in such procedures by continuously monitoring temperature during the duration of exposure. On the other hand, MMW irradiation of yeast cells in the range of 41.650 - 41.798 GHz for 4 h and 20 mW power found frequency sensitive results with increased cell growth at some frequencies and reduced at other values [14]. Another study confirmed the increased growth rate of yeast upon irradiation with 968 MHz for 7 h at 17 dBm power [15]. Results of such studies on the interaction of millimeter waves with biological samples are often met with inconsistence and non-reproducibility due to missing investigations of deeper biological mechanisms like influence on genetic material, the involvement of free radicals on oxidative processes, other metabolic disturbances etc. to correlate with the observed effects [16]. Thus, understanding the mechanisms of action will enable better use of MMW technology for therapeutic applications.

Given the rising challenges of biochemical drug resistance and the adverse effects from ionizing electromagnetic spectra limiting the use of conventional radiation therapies [3], molecular mechanisms of non-ionizing radiation procedures can provide novel approaches to finding effective solutions. While non-ionizing radiation has mostly been used in thermal ablation procedures, we explored the effect of MMW (85-105 GHz) irradiation on the *Saccharomyces cerevisiae* yeast model of eukaryotic cell division in this article with the non-thermal safety standards set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) maintained for the stringency of analyses to unravel novel mechanisms. The MMW were propagated using a standard pyramidal horn antenna. Radiated power density and power distribution across the antenna aperture were analyzed and the influence of MMW exposure on yeast cells manifesting in the retarded cell growth effect studied. Irradiation of cells with deletion of the RAD52 gene (*Δrad52*), responsible for DNA damage repair was examined for radiation related genomic perturbations. Temperature rise measurements were conducted for analysis and maintenance of non-thermal exposure conditions. Non-ionizing radiation MMW absorption by cellular water content was investigated. Reactive Oxygen Species (ROS) effects, Structure Resonant Energy Transfer (SRET) mediated contributory effects, membrane modulations elicited signaling effects, and biomolecular resonance with electromagnetic spectrum effects were examined to provide molecular indications of non-thermal proteomic processes accountable for the inhibited cell division phenomenon. Dosage dependent effects to achieve

complete termination of yeast cell division were studied and compared involving a waveguide delivering higher energy; which presented novel avenues of microbial infection control. Our results provide mechanistic insights into the non-thermal biological effects of MMW exposure enabling novel biomedical applications for clinical settings and research experiments.

2. Results

2.1. MMW irradiation effects are frequency, energy dose and cell density dependent

Wild Type (WT) *Saccharomyces cerevisiae* cells cultured under standard physiological conditions were subjected to 85, 95, 105 GHz MMW exposures using a horn antenna and the effects compared with that of (sham) control. Cell densities (10000, 1000, 100 and 50 cells/μl) were calibrated to power dosages and treatment durations (1, 2, 3, 4, 5 and 6 hours) to unravel the threshold exposure conditions (ref. methods) required to elicit the inhibited cell division effect. Subsequently, six separate yeast colonies at the calibrated cellular density (50 cells/μl) and exposure duration (6 hours) were irradiated corresponding to each discrete frequency regime. The growth rate and division of treated yeast cells were then examined by incubating them under physiological conditions. 85 – 105 GHz MMW irradiation at ~1.0 mW/cm^2 for 6 hours of exposure affected the growth rate of (50 cells/μl) WT yeast cells for all the examined frequencies and reduced the rate of division by up to 62% as compared to sham (control) (Fig. 1 and Table 1). Delay in cell proliferation becomes significantly noticeable over 3-5 hours of physiological incubation post-irradiation treatment (Fig. 1a). Interestingly, although increasing the value of frequency correlated with a reduction in respective power densities (ref. Table 1); the effect on cell proliferation was similar for each discrete frequency. This suggests that anti-proliferative MMW radiation effects were specific for 85-105 GHz range with a constant cell density (50 cells/μl). And minimal power density of 0.83 ± 0.02 mW/cm^2 at 105 GHz (ref. Table 1) was sufficient to inhibit cell growth and division for 50 cells/μl samples of WT *Saccharomyces cerevisiae*.

Subsequently, the effect of different durations of irradiation (5 h versus 6 h) and power densities (5 dBm versus 6 dBm) was examined using constant frequency of 105 GHz. Using the same number of cells, the experiment demonstrates that the inhibited cell division effect was MMW energy dose dependent (Fig. 1b); being absent in the control (sham). We did not observe difference in the delay of the proliferation rate between 5 hours (with 6 dBm power) and 6 hours (with 5 dBm power) of irradiation as both exposure regimes involved the same amount of energy (i.e. product of time and power) (ref. Fig. 1b). Single frequency at 105 GHz was used as it affects WT yeast cell growth to the same extent as for all the other examined MMW frequencies (ref. Fig 1a); but provides the minimal threshold power densities (ref. Table 1) to elicit the targeted outcome. The agar layer thickness was kept constant throughout this and other repeated experiments under similar conditions. Power densities emitted by the horn antenna aperture are presented in Table 1. The experiments indicate the inhibited effect on cell growth and division were power, irradiation duration and frequency dependent for a fixed number of cells.

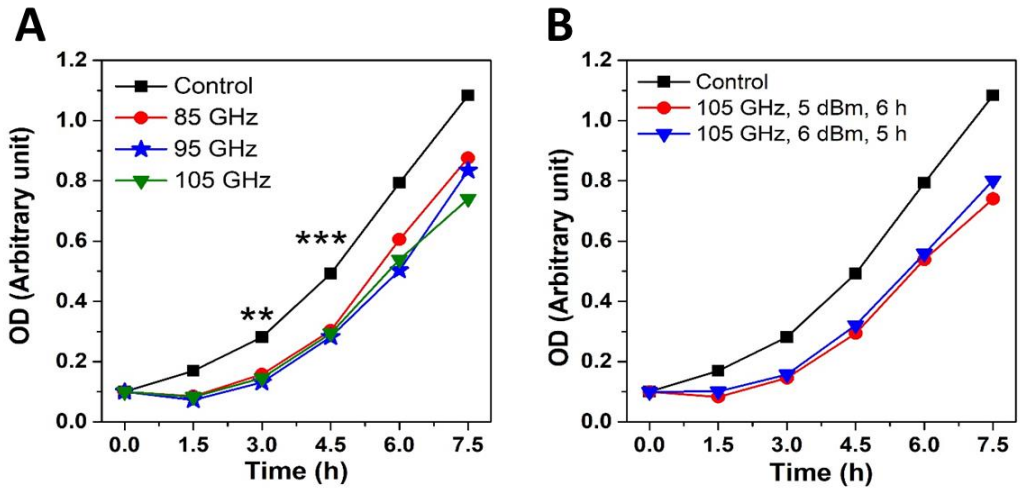


Figure 1. (**A**) Growth profiles of BY4741 *Saccharomyces cerevisiae* (50 cells/µl) cells measured in OD units after irradiation for 6 hours at 5 dBm. Plots indicate mean values of growth rate of six separate yeast colonies (n = 6) for each discrete frequency regime; Single Factor Analysis of variance (ANOVA) analysis (** indicates p-value <0.01 and *** indicates p-value less than <0.001) (**B**) Growth profiles of BY4741 *Saccharomyces cerevisiae* (50 cells/µl) cells subsequent to irradiations of different time durations and power densities for a constant frequency (and constant dose energy) at 105 GHz.

Frequency (GHz)	Power density (mW/cm^2)	Time of irradiation (h)
85	1.39±0.03	6
95	1.04±0.02	6
105	0.83±0.02	6
105	0.83±0.02	5

Table 1. Average energy flux at the aperture of the antenna for different frequencies.

2.2. Non-thermal MMW wave irradiation does not alter genetic DNA

The effects of MMW irradiation were observed to be persistent even beyond 3 – 6 hours after termination of MMW exposure on the irradiated cells across six separate experiments. Evidently, thermal effects can be ruled out as the same non-thermal threshold powers were involved as reported earlier [17]. Irradiations of MMW under 1 mW/cm^2 are deemed not to give rise to thermal effects in living cells [14]. As our experiments involved power densities around 1 mW/cm^2 placing the results in a non-thermal range, it is necessary to investigate other possible mechanism(s) responsible for the decreased growth rate of MMW irradiated cells. Therefore, the next step of the investigation was to check for the possible presence of genomic alterations arising due to radiation in the treated cells. In this direction, we examined for genomic DNA perturbations using the *Δrad52* deletion strain. Rad52 is a protein required to repair DNA double-strand breaks. Rad52p mediates Rad51p function in homologous recombinational repair (HRR) in both yeast *Saccharomyces cerevisiae* and in mammalian cells of mice and humans [18]. Double strand breaks (DSBs) are one of the most dangerous forms of DNA damage and are known to cause gross chromosomal rearrangements which are hallmarks of human cancers [19]. Yasuhara et al. highlighted the importance of human RAD52 for maintaining genome integrity [20]. In the absence of this protein in the *Δrad52* deletion strain, yeast cells reportedly die upon irradiation by DNA damaging electromagnetic spectra [21, 22]. Irradiation of WT and *Δrad52* at the minimal threshold powers (0.83 ± 0.02 mW/cm^2) and frequency (105 GHz) as indicated from the previous experiment did not yield any differences in colony growth profiles

between the two. To discount the possibility that low power at 105 GHz (ref. Table 1) was unable to unravel any differences in growth profiles both WT and *Δrad52* cells were subsequently exposed to 90 GHz MMW at 5dBm power (corresponding to ~1.0 mW/cm^2 surface power density) (ref. Table 1) for 6 hours and subsequently incubated under physiological conditions for analyses of colony growth profiles. It was observed that both types of cells showed similar colony growth profiles (Fig. 2) even after serial dilutions were made to unravel any low intensity differences which may have been overlooked; demonstrating that reduced cell growth is not accountable to genetic DNA damage of the treated cells. The absence of Rad52p didn't show any significant effect on the cells' growth after treatment indicating non-ionizing MMW radiation does not cause genomic alterations.

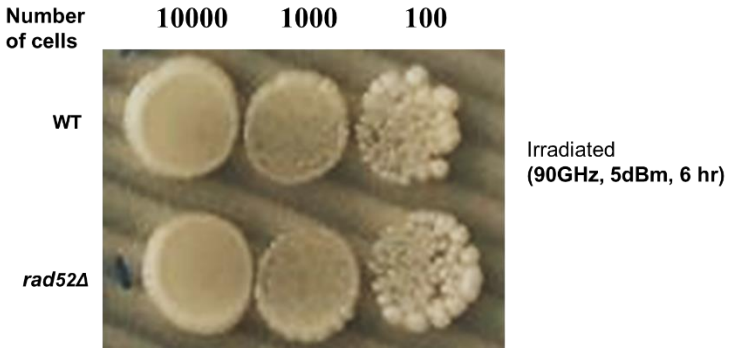


Figure 2. Colony growth profiles of WT and *Δrad52* cells with serial dilutions after (90 GHz) MMW irradiation at 5dBm for 6 hours. The cells were grown under physiological conditions at 30°C over 3-4 days post-treatment period. Number of cells after serial dilutions are as indicated. Five independent experiment were performed. No statistically significant differences were found between the two examined strains.

In general, non-ionizing radiations with low photon energies of 0.3-0.4 meV are not expected to biochemically alter physiological DNA structure or function. Edwards et al. proposed a mechanism of coherent frequency-specific deposition of microwave energy on DNA in water [23]. The resonance of DNA molecules with irradiated spectra can be calculated in terms of the absorption coefficient. 2734 base pairs (bp) supercoiled circular DNA, 2734 bp linear DNA, 1786 bp linear DNA, and 948 bp linear DNA were found to resonate with 2.55-8.75 GHz, 2.75-5.60 GHz, 4.10 GHz, and 2.65 GHz respectively [23]. This indicates that DNA polymer chain length determining the structural conformation and size (globular or linear, large or small) directly correspond to their respective resonant frequencies. Illustratively, a resonance shift occurs within the frequency range of 41-52 GHz upon changing the length of the haploid genetic material in E. coli [24]. Further, relative viscosity measurements showed that the resonance frequencies decreased proportionally to the enhancement of haploid genome length. These reports suggest that resonant interactions of MMW with genetic material can arise by structure based energetic coupling without causing biochemical genomic alterations (ref. Fig. 2) within the threshold powers of non-thermal exposure. Experimentally, irradiation of transformed Human Corneal Epithelial (HCE-T) and Human Lens Epithelial (SRA01/04) cell lines by 60 GHz at 1 mW/cm^2 over 24 hours of constant exposure found no statistically significant genotoxic effects on the nucleus [25].

2.3. MMW interaction with water as a factor for reduced cell growth

In the above section, we concluded that the MMW irradiation can affect cell growth/division without causing biochemical DNA damage. Radiation elicited perturbations of other metabolic processes like biomolecule synthesis, glycolysis and gluconeogenesis could also play a role in changes of cell proliferative activity. Besides the genome which encodes all the genetic information for hereditary purposes, the living characteristics of cells are manifested by the interplay of the proteome. Water is a significant constituent of the cell cytoplasm and the site of all biochemical reactions which give rise to biological functions of growth, division, and genetic inheritance in living organisms. Water is also known to absorb electromagnetic radiation in the microwave and infrared spectrum [26].

A biological cell is a compartmentalized structure separated from the surrounding environment by the lipid cell membrane containing transmembrane proteins. It has been reported that 65 GHz irradiation reduced the effects of heliogeophysical factors on yeast cells due to the destabilization of intracellular water structure [27]. Under physiological conditions, yeast cells are reported to bear 65% water by composition with about 20-30% of the volume inhabited by cytosolic proteins that participate in most metabolic process of the cells [28]. Biological functions at the cellular level are affected by proteins, and the functionality of proteins is, in turn, determined by their molecular structure. Proteins are polypeptide chains composed of sequentially joined amino acids folding into the lowest energy conformations in their physiological environment giving rise to three-dimensional structures. These structures are essential for the protein's biochemical interactions with other molecules which manifests in biological functions. Changes in the cytosol environment can therefore translate into changing the properties of biomolecules like proteins by affecting their charge densities in space and time.

Interactions of MMW irradiation with proteins in the cytosol could generate structural and/or functional changes which subsequently affects cell functionality. Structurally, water is a physical participant during the folding of the polypeptide chain in protein folding through hydrophobic collapse [29]. Thus, water interacts with proteins to affect their dynamics. Conversely, changes in the chemical composition of the aqueous environment can alter the three-dimensional structure of proteins. Molecular Transfer Model (MTM) predicts conformational changes in protein structures when pH changes occur in the vicinity in solution using calculated partition functions of polypeptides [30]. Illustratively, Nitrophorin 4 (NP4) is a protein that releases nitric oxide (NO) in a pH-sensitive manner. NP4 remains in a closed conformation and tightly binds NO at pH 5.5 [31]. At pH 7.5, deprotonation occurs, changing the conformation and releasing NO.

To unravel the contributions of MMW interaction with the cytosolic part of the cells; absorbed power over time represented by a ratio of incident and reflected powers for yeast cells spotted on SC agar was compared to that of plain water and blank SC agar without cells respectively. The irradiation conditions were kept constant at 85 GHz (5 dBm, at the maximal threshold powers corresponding to 1.39 ± 0.02 mW/cm^2) for stringency of analysis.

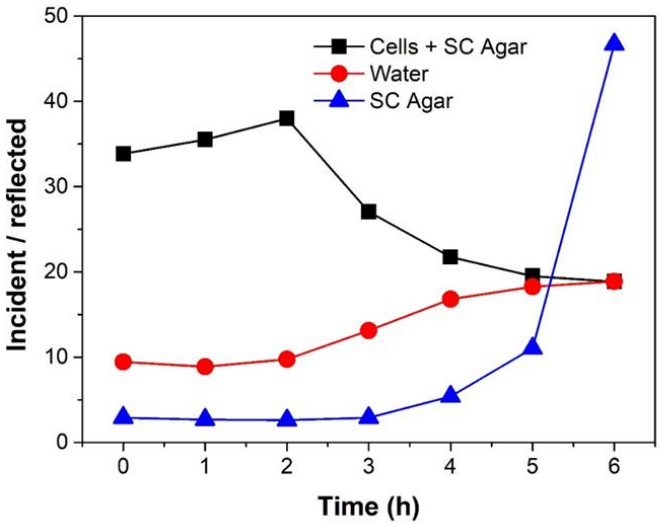


Figure 3. Absorbed power represented as a ratio of incident and reflected power from yeast cells spotted on Synthetic Complete (SC) agar compared to that of plain water and Synthetic Complete (SC) agar without cells respectively. Experiments were performed with a frequency of 85 GHz at an amplitude of 5 dBm (corresponding to 1.39 ± 0.02 mW/cm^2 surface power density).

The sample(s) absorb a part of the incident radiation and the remaining is reflected; with the ratio between the incident and reflected power values indicating the absorbed power of the sample under treatment. We find that the yeast cells spotted on SC agar absorbed more power (Fig. 3) as compared to plain water and blank SC agar without cells. Interestingly, plain SC agar reflected most of the incident power. The results suggest that non-thermal exposure of MMW could affect the proteins present in cytosol through interactions with water molecules and without involving heat shock proteins [28]. This could account for the observed phenomenon of cell growth inhibition without genetic perturbation (ref. Fig. 2). The experiment also indicates that cells have a high absorbance of MMW irradiation with a cytosol made mostly of water separated from the environment within a confined volume of membrane bound structures.

Therefore, in order to ascertain any relation behind MMW absorption and volume of sample treated as indicated from the previous experiment, different volumes of water were irradiated using the horn antenna and temperature rise monitored. Irradiation conditions of frequency, power and duration of exposure were kept constant as mentioned previously to maintain analytical stringency. A 2°C rise of temperature measured using a digital thermometer was associated with a 6 hours exposure duration at 1.39 mW/cm^2 from a horn antenna (Fig. 4a) on a large volume of water (6500 µl). Reduction in the size of the irradiated sample to a smaller volume of water (250 µl) led to a 1°C increase in the rate of temperature rise (Fig. 4b). Interestingly, exposure involving a waveguide (delivering a higher power density of 17.17 mW/cm^2) on the same small volume of water (250 µl) did not change the rate of temperature rise any further (Fig. 4c). Further, contrary to the expectation of the waveguide causing thermal ablation due to higher power density, the experiment demonstrates that thermal effects were practically absent in our irradiation setup. Therefore, under conditions of constant frequency, power and exposure duration; the radiation associated rise in temperature is inversely proportional to the volume of the sample irradiated to a certain extent beyond which it is likely to saturate. Finally, this experiment also confirms the hypothesis that biological cells exhibit high absorbance of MMW irradiation accountable to being constituted of water within a confined volume separated from the environment. The experiment demonstrates that MMW irradiation under the listed parameters raises temperatures up to 22 °C and is therefore not expected to elicit thermal stress on yeast cells which grow at physiological temperatures of 30 ℃.

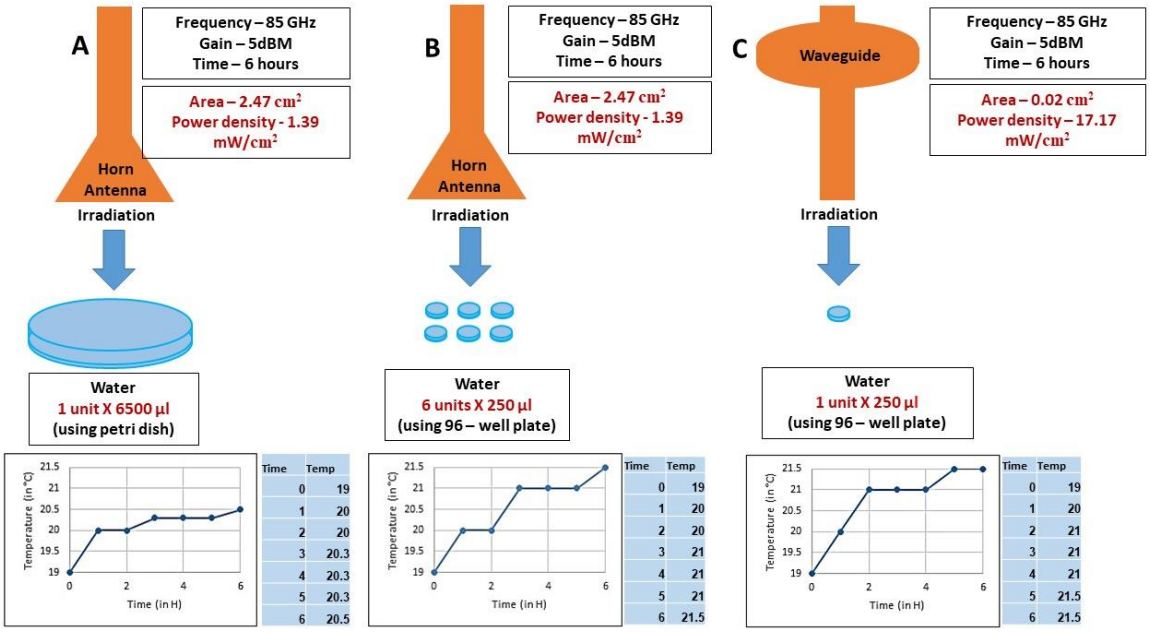


Figure 4. Comparison of temperature rise during 85 GHz MMW exposure at constant power and treatment duration, involving horn antenna and wave guide. Indicated volumes of water were irradiated as illustrated. (**A**) Temperature rise of 6500 µl water during MMW exposure at 1.39 mW/cm^2. (**B**) Temperature rise of 250 µl water during MMW exposure at 1.39 mW/cm^2. (**C**) Temperature rise of 250 µl water during MMW exposure at 17.17 mW/cm^2. Frequency, power and duration of exposure were kept constant at the values and specific experiments as indicated.

A wave-guide provides a focused beam of irradiation as compared to a horn antenna (ref. Fig. 4) allowing more precise energy delivery. To investigate potential applications of this exposure regime we performed MMW irradiation at 85 GHz and 5 dBm on BY4741 *Saccharomyces cerevisiae* WT yeast cells using an open-ended waveguide (Fig. 5). Single frequency at 85 GHz was used as it provides the maximal threshold powers and affects WT yeast cell growth to the same extent as for all the other examined MMW frequencies (ref. Fig 1a). The thickness of agar medium and the number of cells were kept the same (50 cells/µl) as those mentioned in the previous experiments. Cells were treated for 3 - 4 hours at the non-thermal exposure power density of 17.17 mW/cm^2 (ref. Fig. 4). Subsequent incubation of these cells under physiological conditions (at 30 °C for 2 days) did not yield any colony growth. The experiment demonstrates that MMW irradiation using an open-ended waveguide at ~12 times stronger power density than the one involving a horn antenna (ref. Fig. 1, 4 and Table 1) completely terminates cell growth and division.

3. Discussion

Since MMW are classed as non-ionizing radiation with low 0.3-0.4 meV range of photon energies, their direct exposure is not expected to directly generate Reactive Oxygen Species (ROS) by biochemical degradation unlike ionizing radiation therapies [2, 3], which involve higher photon energies and thereby provide only safety restricted applications to elicit therapeutic effects and limit the adversities. The phenomenon of Structure Resonant Energy Transfer (SRET) allows membrane bound spherical core – shell charge separated bodies (from 28 – 100 nm diameters) to exhibit dipolar coupling with incident electric fields [32] in the range of 12 – 45 GHz of the electromagnetic spectrum with an inverse relation between frequency and size dimensions. Such phenomena which occur at resonant frequencies determined by the structural dimensions and charge status can lead to rupture events [33], resulting in leakage of matrix contents depending on the exposure power conditions and duration of treatment. Cells are composed of multiple subcellular organelles bound by membranes which maintain distinct environments within the matrix separated from the cytoplasm outside. Peroxisomes and microbodies are a class of cell organelles which function as a source of Reactive Oxygen Species (ROS). ROS causes oxidative stress and DNA damage at high levels inhibiting cell

division [34]. Generally observed to be spherical in shape, peroxisomes resemble the membrane bound core-shell charge separated structures described above [32], raising the possibility that 85 – 105 GHz MMW irradiation may have resulted in peroxisome rupture with subsequent ROS leakage leading to inhibited cell division. However, colony growth profiles of the MMW irradiated WT and *Δrad52* (DNA damage repair) deletion strains did not show any significant difference between the two even after serial dilutions to ascertain power and cell density dependent relationships (ref. Fig. 2). Further, dipolar coupling at 85 – 105 GHz frequencies will correspond to charge separated spherical membrane bound structures of diameters around 11 – 14 nm [32]; which are much below the peroxisomes size range of 200 – 500 nm [35, 36], indicating that reduced cell division upon 85 – 105 GHz exposure is not accountable to ROS mediated mechanisms. However, other smaller vesicles with resonant dimensions covered by this bandwidth may contribute to the observed non-thermal effects by different non-ROS mediated pathways. Such processes could explain the ambiguous MMW treatment results reported from other studies [12, 13, 14] described above in the introduction.

As such, MMW radiation affecting cell signaling could also account for the observed effects. Translocation of signaling proteins into the cytoplasm across membranes which otherwise retain them confined within their respective storage sites inside organelles separated from the cytosol are known to activate events which can lead to induction of cell death [37]. MMW treatment is known to result in membrane depolarizations resulting in the loss of integral charge status and direction [8] at specific threshold power densities dependent on the number of cells under target, which could trigger such translocation events of signaling proteins activating the corresponding cascade processes. Sweeping frequency regimes over 75 – 105 GHz at 0.2 mW/cm^2 resulted in apoptosis of H1299 lung cancer cells [5] and is likely to have arisen from such electromagnetic modulation of charge separated membranes activating signaling cascade processes. Experimentally, irradiation at 35 GHz is reported to activate the apoptotic caspase pathway in A375 melanoma cells in vitro [38]. This suggests that herein the case of MMW treatment of 50 cells/µl of *Saccharomyces cerevisiae* resulting in reduced cell proliferation at 0.83-1.39 mW/cm^2 and complete termination at 17.17 mW/cm^2; the higher power densities are required for better penetration since yeast cells bear thick cell walls which were absent in the H1299 and A375 cells described above. Further, the observations suggest MMW radiation can be used to elicit targeted biochemical responses for therapeutic applications via physical perturbations of biological membranes.

Further, resonance absorption of MMW irradiation corresponding to different DNA polymer chain lengths [23] and found to be related to their structural conformation and size (globular or linear, large or small) as discussed above [ref. Results section 2.2] could allow certain irradiation regimes to exercise switch ON/Off control over genetic expression by affecting conformational changes which translate into specific modifications of the proteome determining the extent of cell division effects. Experimentally, resonance shifts occurring within the frequency range of 41-52 GHz corresponded to changing lengths of the genetic material in *E. coli* located directly in the cytosol of the cells [24]. In eukaryotes, the genetic material in the nucleus is distinguishable into heterochromatin (condensed and inactive) and euchromatin (actively directing protein synthesis). MMW irradiation regimes may be affecting conformational changes on chromatin which modulates the gene expression without causing genetic DNA damage (ref. Fig. 2). Such a mechanism will be analogous to the biochemical ON/OFF control of epigenetic regulation wherein specific biological molecules physically associate or dissociate with certain genetic sequences in the nucleus in response to external stimuli for either blocking or enhancing gene expression accordingly. In contrast, by involving a medium of radiation to enable non-biochemical control of genetic expression through conformational changes in the genome, MMW therapy can provide interesting applications of eliciting targeted biological responses for various purposes. Such effects are likely to be dose dependent and potential applications of this technique will require characterization of the genomic conformational changes possible for different exposure conditions correspondent with their respective biological outcomes. Such a mechanism of action can also explain the ambiguous cell proliferation results after MMW treatment from the other studies described above in the introduction [12, 13, 14].

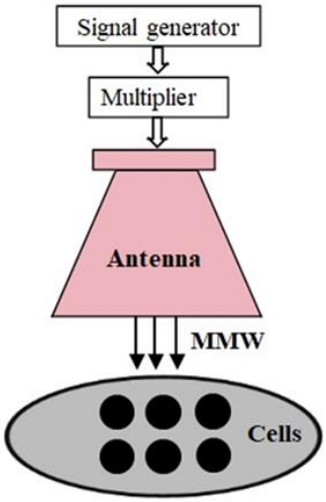
4. Materials and Methods

4.1. Conditions of Cell Culture

Wild type (WT) budding yeast BY4741 *Saccharomyces cerevisiae* (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and its *Δrad52* mutant strain BY4741 *Saccharomyces cerevisiae* (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RAD52::KanMX4)* available through EUROSCARF (Frankfurt, Germany) were used for irradiation. Cells were grown in standard synthetic complete (SC) liquid medium at a temperature of 30°C. The growth rate of both control and irradiated cells were measured using an absorbance plot at 600 nm measured by a standard spectrophotometer in units of optical density (OD). Cultures were adequately diluted to 0.1 OD using a standard absorbance plot at the start of experiment and incubated until they reached an OD value of 0.4 (the point at which cells initiate the logarithmic growth phase). Cultures at 0.4 OD were diluted to 10000, 1000, 100 and 50 cells/µl (to determine the optimal concentration of cells and energy dosage). 1-2 µl volume of those solutions were dropped onto SC agar plates. Six colonies were seeded in two replicates: one for irradiation and another for comparison as control (sham) for each discrete frequency regime. After irradiation, the cells were transferred to SC liquid medium and incubated under standard conditions. Growth rate of both irradiated and control (sham) yeast cells were measured regularly at intervals of 90 minutes over a period of ~8 h to assay the effect of MMW exposure on physiological growth.

4.2. Conditions of Irradiation

The schematic diagram of the experimental setup for MMW irradiation is illustrated in Fig. 5. Yeast cells were spotted on the Agar medium prior to irradiation. Cells were exposed to specific frequencies of 85 GHz, 95 GHz, and 105 GHz at 5dBm power for 6 hours respectively. The irradiation experiment for each frequency involved six distinct experiments at the same cell density. MMW (85-110 GHz) were generated using a signal generator (Keysight technology, N5183B, 9 kHz-20 GHz), and 6× active frequency multiplier (Quinstar Tech Inc., QMM-311220025). A standard gain pyramidal horn antenna (Quinstar Tech Inc., QWH-WPRROO) was used for MMW emission. The power of transmitted waves from the horn was measured using an identical horn antenna and digital storage oscilloscope (Agilent technology, DSO-X 2004A). Friis transmission formula was employed for the calculation of transmitted power in the far-field region. Distribution of relative energy across antenna aperture was measured using an open-ended waveguide (Quinstar Tech Inc., QWH-WPRROO) in the near field (ref. Fig. 6).



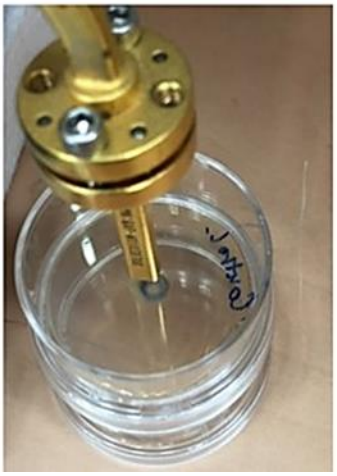


Figure 5. Block diagram of the experimental setup for irradiation and pictures of the pyramidal horn antenna and wave guide.

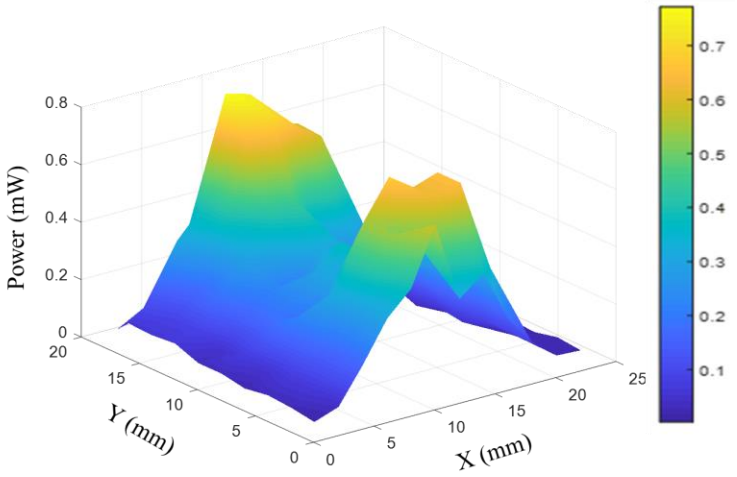


Figure 6. Relative distribution of power across horn antenna aperture.

5. Conclusions

In this study, we explored the mechanism of MMW (85-105 GHz) interactions with eukaryotic cells using *Saccharomyces Cerevisiae* yeast cells as a standard model of cell division. W-band (85 – 105 GHz) MMW irradiation at a minimum power density of ~1.0 mW/cm^2 for 5-6 hours exposure duration resulted in a 62% reduction in the growth rate of irradiated yeast cells at a density of 50 cells/µl. MMW radiation effects were found to be frequency, energy dose and cell density dependent. Experimental exposure conditions maintained within the ICNIRP (International Commission on Non-Ionizing Radiation Protection) non-thermal safety standard of 20 mW/cm^2 [39] emphasizes the analytical stringency of this study. Further, measurements of temperature rise demonstrated that effects were non-thermal in nature. A comparative analysis of changes in the growth profile of irradiated wild type and *Δrad52* (DNA damage repair) deletion cells revealed no detrimental effects on genomic stability arising from this non-ionizing radiation treatment. Analyses of dose dependent effects of MMW propagation and calibrations of cell density to exposure conditions revealed threshold parameters which can be used for targeted inhibition of infectious microbes. Specifically, using an open – ended waveguide delivering a higher power density of 17.17 mW/cm^2 for 3 – 4 hours exposure duration achieved complete termination of cellular proliferation in 50 cells/µl samples of *Saccharomyces Cerevisiae* yeast cells. The non-thermal effects are indicated to arise from a combination of MMW interactions with physical membrane modulations affecting vesicular structures and activating signaling cascades, MMW absorption by the cellular water content affecting proteomic changes and MMW resonant conformational changes of chromatin altering genomic expression accounting for the observed phenomena. Thus, the advantages in using non-ionizing MMW radiation enabling non-thermal control of targeted biological responses without requiring biochemical activators or giving rise to DNA damage has promising potential for innovative biomedical procedures and devices.

Invasive fungal infections remain a serious clinical problem. The current use of several classes of antifungal drugs have also increased the antimicrobial resistance of pathological species like *C. albicans* by accelerating the development of mutations, overexpression of multidrug efflux pumps [40], etc. Moreover, *C. albicans* as a pathogen depending largely on its ability to generate diversity at not only the genomic but also the morphological and physiological levels by its ability to switch from yeast to hyphal or pseudo hyphal forms [41, 42]; constitutes a major challenge in the development of new anti-fungal drugs and therapeutic strategies. A direct application of the MMW radiation effect mechanisms presented in this article for non-thermal exposure regimes can be used for treating

pathogenic fungal infections (via cell division inhibition effect, ref. Fig 1) which are common in dermatology [43] and reproductive health [44]; and cancer lesion/tissue treatments (via cancer cell mortality effect [4, 5]) by appropriately scaling the exposure conditions described here to match clinical cell densities; besides various other promising purposes. Further, recent advances in endoscopic probes [45] operating in the Super High Frequency (SHF) 3 – 30 GHz bandwidths and similar technological trends will enable novel avenues of adapting such non-thermal techniques to treat internal body sites overcoming the burn – hazard restrictions in application of conventional thermal ablation procedures. Our experiments demonstrate that non-thermal regimes of non-ionizing MMW exposure allows cells to retain biochemically undamaged genetic material, but produces a combination of physically derived bioelectromagnetic effects generating proteomic modifications accounting for the observed phenomena. Non-ionizing radiation biological effects are frequency, energy dose and target density dependent and our results provide insights into novel molecular mechanisms which can be used for innovative biomedical applications.

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