Exposure of *S. cerevisiae* to pulsed magnetic field during chronological aging could induce genomic DNA damage

Silvia Mercado-Sáenz, Beatriz López-Díaz, Antonio M. Burgos-Molina, Francisco Sendra-Portero, Alejandro González-Vidal, Miguel J. Ruiz-Gómez *

Departamento de Radiología y Medicina Física, Facultad de Medicina, Universidad de Málaga, Málaga, España

*Corresponding author: Miguel J. Ruiz-Gómez. Laboratorio de Radiobiología, Departamento de Radiología y Medicina Física. Facultad de Medicina, Universidad de Málaga. Bulevar Louis Pasteur, 32, 29010 Málaga, España.

Tel.: +34-95-2131548; Fax: +34-95-2131630. E-mail: mjrg@uma.es (M.J. Ruiz-Gómez)

Scopus Author ID: 56962771400

URL: www.radiobiologia.uma.es

ORCID OF THE AUTHORS:

Silvia Mercado-Sáenz: 0000-0002-0002-8471

Beatriz López-Díaz: 0000-0001-5176-1852

Antonio M. Burgos-Molina: 0000-0001-5663-5134

Francisco Sendra-Portero: 0000-0001-9535-9806

Alejandro González-Vidal: 0000-0002-5544-6427

Miguel J. Ruiz-Gómez: 0000-0003-4630-7588

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Biographical notes on contributors

Silvia Mercado-Sáenz, MSc PhD, is an Instructor of Histology at the Department of Human Physiology, Human Histology, Pathology and Sports Physical Education, Faculty of Medicine, University of Málaga, Málaga, Spain. Her research interests include biological and health effects of electromagnetic fields. Other interests focus on cellular aging, DNA sequencing, genetic analysis and metagenomics.

Beatriz López-Díaz, MSc PhD, currently is a member of the research group at the Department of Radiology and Physical Medicine, Faculty of Medicine, University of Málaga, Málaga, Spain. Her research interests focus on experimental investigation of biological and health effects of electromagnetic fields.

Antonio M. Burgos-Molina, MEd MSc PhD, is an Instructor of Immunology and Immunopathology at the Department of Surgical Specialties, Biochemistry and Immunology, Faculty of Medicine, University of Málaga, Málaga, Spain. His research interests include biological effects of non-ionizing radiation. Other interests focus on proteomics and Immunology.

Francisco Sendra-Portero, MD PhD, is an Associate Professor of Radiology and Physical Medicine and Department Chair, at the Department of Radiology and Physical Medicine, Faculty of Medicine, University of Málaga, Málaga, Spain. His research interests include biological and health effects of non-ionizing radiation. Other interests focus on breast imaging, image processing and innovation in medical education.

Alejandro González-Vidal, MEd MSc, currently is a member of the research group at the Department of Radiology and Physical Medicine, Faculty of Medicine, University of Málaga, Málaga, Spain. His research interests focus on experimental investigation of biological and health effects of electromagnetic fields.

Miguel J. Ruiz-Gómez, MSc PhD, is an Associate Professor of Radiology and Physical Medicine and Head of the Radiobiology research group, at the Department of Radiology and Physical Medicine, Faculty of Medicine, University of Málaga, Málaga, Spain. His research interests include biological and health effects of non-ionizing radiation. Other interests focus on molecular radiobiology, cellular aging, proteomics, biological dosimetry and e-learning.

ABSTRACT

This study evaluates the DNA damage induced by pulsed magnetic field (MF) on *S. cerevisiae* cells exposed during chronological aging. Samples were exposed to 25Hz pulsed MF (1.5mT, 8h/day) while cells were aging chronologically. Clonogenic drop test was used to study cellular survival and the mutation frequency was evaluated by scoring the spontaneous revertant mutants. DNA damage analysis was performed after aging by electrophoresis and image analysis. Yeast cells aged during 40 days of exposure showing that pulsed MF exposure induced a premature aging. In addition, a gradual increase in spontaneous mutants was found in pulsed MF samples in relation to unexposed controls. An increase in DNA degradation, over the background level in relation to controls, was observed at the end of the exposure period. In conclusion, exposure of *S. cerevisiae* cells to pulsed MF during chronological aging could induce genomic DNA damage.

KEYWORDS: Aging; magnetic field; DNA damage; *S. cerevisiae*

Introduction

Research about magnetic fields (MF) damage has led to the publication of a multitude of articles in recent years that measure all kinds of cellular and molecular parameters. It is surprising that the possibility of undesirable effects induced by the non-thermal component of the MF is still no clear, so the controversy continues.

The current situation resembles a scale where more and more positive items and more negative items are placed on each plate. But what is necessary to clarify the effects? Firstly, the experiments must be replicated by other authors in other laboratories, which is difficult; and secondly, a clear and correct answer must be given to Hill's criteria, which examine: (1) a clear relationship between the risk of harmful effect and the level of exposure, (2) are there many consistent studies indicating the same risk?, (3) clear results in dose-response curves with different endpoints measurements and effects, (4) clear evidence of *in vitro* studies, and (5) are there plausible biological mechanisms that suggest that there should be a risk?. Up to now there is not totally clear answer to these questions.

Numerous articles have been published that demonstrate DNA damage caused by low intensity and frequency MF exposure. In this way, Ivancsits et al. (2003) reported DNA breaks by intermittent MF (1 mT, 50 Hz, 1-24 h), Wolf et al. (2005) after exposure to continuous MF (0.5, 0.75, and 1 mT, 50 Hz, 3–72 h), and Kim et al. (2010) by repetitive exposures (6mT, 60Hz; 30min/day, 3 days). In addition, DNA fragmentation was published by Focke et al. (2010) (1mT, 50 Hz), an increase in DNA breaks by pulsed MF (20Hz, 3mT, 60 min/day) (Crocetti et al. 2013), and recently Solek et al. (2017) and Sherrard et al. (2018) found DNA damage by pulsed MF exposures (0-10 mT, 2, 50, 120 Hz, 2 h) and (2 mT, 10 Hz, 15 min-96 h), respectively. In contrast, numerous authors reported the absence of effects on DNA damage by sinusoidal continuous, intermittent or pulsed MF exposure at similar frequencies, magnetic flux densities and time of

exposure (Ruiz-Gómez and Martínez-Morillo 2009, Giorgi et al. 2014, Su et al. 2017, Zeng et al. 2017 and Sun et al. 2018).

A previous work reports that 25Hz pulsed MF (1.5mT, 8h/day, 16 days) produces an enhancement of spontaneous DNA damage in *S. cerevisiae* after 16 days of exposure. The biological model used in this work was isolated genomic DNA and therefore there was no possibility of DNA repair (López-Díaz et al. 2014).

This work aims to assess the DNA damage induced by pulsed MF (low magnetic flux density and frequency) on *S. cerevisiae* cells exposed during chronological aging and therefore with active DNA repair mechanisms.

Materials and methods

Yeast strain and culture medium

The biological model used was the wild-type haploid WS8105-1C *Saccharomyces. cerevisiae* (ATCC® 200383™) strain (Genotype: MATalpha RAD HDF ade2 arg4-17 trp1-289 ura3-52). A rich media (Yeast extract–Peptone–Dextrose (YPD) 1:2:2 (%) ± 2% Bacto-agar) was used for vegetative growth (Siede et al. 1996; Friedl et al. 1998).

The process of chronological aging was studied in yeast cultures grown in a synthetic dextrose complete (SDC) medium (dextrose–yeast nitrogen base–ammonium sulphate–aminoacids mixture, 2:0.17:0.5:0.15 (%). The mixture of aminoacids was: adenine, arginine, tryptophan, and uracil (4X) (Fabrizio and Longo, 2003).

These reagents were purchased from Difco, BD, Co. Sparks, MD, USA)

Pulsed magnetic field

The equipment used and its characteristics have been previously described by Ruiz-Gómez et al (2002) and Ruiz-Gómez and Martínez-Morillo (2005). A Helmholtz type equipment (2 air core coils connected in series) (15cm x 10.5cm) was used in the generation of 25 Hz rectangular voltage pulses (Pulsatrón─CEM-84/J, J.&J. Electromédica, Málaga-Spain).

The waveform of the voltage applied at 25Hz consisted in groups of 15 square pulses (180μs width, 20μs gap, 1.5mT peak). Yeast cells were exposed 8h/day, 40 days. The equipment was used at its maximum potential generating a magnetic flux density similar to the values reported by other authors (Li and Chow, 2001.

Background magnetic field

The DC geomagnetic field in Málaga is 42.95μT (27.225μT horizontal, 33.20μT vertical; 3°5' Western (Data from the Institute of National Geographic, Madrid, Spain). The background MF in the laboratory was 0.68μT (López-Díaz et al., 2014).

Chronological aging

The first phase prior to aging starts with yeast cultures in flasks to reach the stationary phase in SDC medium. From fresh YPD plates, one loop of cells was inoculated in 1ml of SDC. After optical density measurement at 600 nm (OD600), 20 ml SDC was inoculated with 1,500,000 cells/ml in separate flasks. Then, they were cultured during 4 days at 30°C, 300 rpm to reach the stationary phase. When early stationary phase was reached, it was the moment when the aging process began really (second phase), considering this day as day 0 in the assay. To perform the second phase, yeast cultures were transferred to 15 ml test tubes. They were divided in two groups, located into the MF exposure systems and at control location, without continuous shaking. To minimize sedimentation of the cells and keep them in suspension as long as possible, they were

vortexed several times a day. MF exposed samples and controls (located 5 meters away) were maintained for 40 days at the same temperature conditions (23°C). The heat produced in the MF equipment was dissipated by a fan (Mercado-Sáenz et al., 2019). Viability was periodically assayed by clonogenic survival (Fabrizio and Longo, 2003).

Cellular concentration

Cellular quantification was calculated every day after OD600nm measurements (Helios-ε; Unicam; Cambridge-UK). OD600nm<1.0 has a linear relationship with the quantity of yeast cells (Amberg et al., 2005).

Clonogenic survival

Yeast survival was assayed by clonogenic drop test. Five 10-fold serial dilutions from each sample were prepared and 5 µl aliquots of each dilution were spotted onto YPD plates. Then, the plates were incubated for 3 days at 30°C. Then, the number of colonies grown was counted and the surviving fraction calculated. Dilutions with 10–30 colonies grown were scored and compared as representatives to calculate the surviving fraction (Umezu et al. 1998).

Microscopic analysis of the cell cycle

The different phases of the cell cycle were studied by optical microscopy (400X) (Nikon-Instech Co, Kanagawa-Japan). The analysis of cell morphology before chronological aging was made classifying yeast cells in four groups: (i) cells in G0/G1 phase (single cells with no buds), (ii) cells in S phase (with small buds), (iii) cells in G2+M phase (with large buds), and (iv) others (cells with more than one bud or aberrant) (Umezu et al. 1998).

DNA preparation and electrophoresis

After the end of the chronological aging and MF exposures, genomic DNA was isolated and purified by Phenol:Chloroform extraction and precipitation with ethanol, following the protocol described by Hoffman and Winston (1987). This technique, with some modifications, has been previously reported by López-Díaz et al (2014). Cells were lysed in Tris/Cl Ethylenediaminetetraacetic acid (EDTA) (10mM, pH 8.0), Triton X-100 (2 %), lauryl sulfate (SDS) (1 %), and NaCl (0.1M). After Phenol:Chloroform:isoamyl alcohol (25:2:1) and small glass beads addition, samples were vortexed and centrifuged (5 minutes, 15,300 g). The DNA, in the aqueous phase, was precipitated with absolute ethanol and RNA digested with RNase A (50µg/ml). Isolated DNA was maintained in a pH 8.0 buffer (Tris-EDTA). Agarose gel electrophoresis (0.8 %) were performed (80V, 90min) in Tris-Borate-EDTA buffer (0.5X), and subsequent staining with ethidium-bromide. The DNA preparation of all samples was carried out at the same time and under the same conditions, so it is assumed that the yield of DNA from each sample was similar. The same volume of sample was included in each well in the gels. Reagents were purchased from Sigma Aldrich Co, St Louis-MO, USA). Hyperladder-I (Bioline Ltd., London-UK) was used as DNA ladder.

Image analysis of agarose gels

The analysis of agarose gels was made from a photography (jpg file) with the software: ImageJ 1.52a (Wayne Rasband, NIH, USA (http://imagej.nih.gov/ij). The measurement of the signal intensity and the distance migrated by the DNA in each lane permitted to represent in a graph the DNA profile of each sample. From these profiles, the DNA content (measured in arbitrary units) was calculated automatically as the area under the curve. The DNA profile considered as undamaged was located at the location of the control band in the gel. A DNA ladder was used as a reference for DNA fragments size (López-Díaz et al. 2014).

Mutation frequency

The study of the spontaneous mutation frequency during chronological aging was possible due to the mutation in the *ade2* gene present in the genome of this strain which makes it auxotrophic and hence the colonies appear in red. When the genotype reverses to the wild type, the colonies turn to white and they are called “revertants”. During the aging period, 300 cells from controls and pulsed MF samples were spread on YPD and incubated at 30°C during 3 days. Then, the number of colonies was scored. The mutation frequency was calculated as the number of revertant colonies in relation to the number of total colonies grown (survivors) (Mercado-Sáenz et al., 2019).

Electric field, current density and Specific Absorption Rate (SAR) induced by pulsed magnetic field

The exposure to pulsed MF induces, within a cell, an electric field (E; in V/m) that depends on the radius of the yeast cell. Its value can be obtained from the magnetic flux density (B; in Tesla), its frequency (f; in Hz), and the cell radius (r; in meters) (Ruiz Gómez et al. 1999). As described in a previous work in the characterization of the pulsed MF (Ruiz-Gómez et al. 2002), in accordance with the Faraday’s Law of Induction and considering a circular contour parallel to the coils, also considering a homogeneous MF in the inner surface of the contour, the induced electromotive force (ξ) can be calculated and measured. At the low frequency used (25 Hz) the MF is proportional to the intensity of current that flows in the coils (Biot & Savart law). After the values of peak pulses (positive and negative) of ξ measurements, in an oscilloscope for pulsed MF, the corresponding peak values of E were obtained from the equation $E = \xi / (2 \pi r)$. The measurements were performed using circular pickup coils in air of different radii (r=2, r=3 and r=4cm). The values calculated for E were E=0.5, E=0.7 and E=0.8V/m, respectively, for the positive pulses and E=2.98, E=3.82 and E=3.98V/m, respectively, for the negatives. After plotting

the obtained values of E *versus* r for the pickup coils, a linear adjustment was made and the value of E for the mean radius of a yeast cell was extrapolated. Then, the electric current density (J; in A/m^2) was calculated from the values of E and the cytoplasm conductivity (σ; in S/m) using the equation: $J=\sigma E$. In addition, the value of Specific Absorption Rate (SAR; in W/kg) was obtained as $SAR=(\sigma E^2)/d$; being d=cytoplasm density, measured in Kg/m^3.

Statistical analyses

The normal distribution of data samples was assayed by the rankit plot of Wilk-Shapiro. Student's *t*-test and the Analysis of Variance (ANOVA) or Friedman were applied to study statistically significant differences ($p<0.05$). From 3–6 independent experiments were performed (2–8 samples each one) (n=6–48). Six replications of the DNA damage assay were performed.

Results

The effect of pulsed MF on genomic DNA integrity after chronological aging of yeast cells was evaluated. Samples were exposed to MF during the whole period of aging (40 days).

The exposure conditions of cells during 40 days of aging were 1.5mT, 25Hz pulsed MF, 8h/day. The mean radius of a single yeast cell is 3.75μm, and considering 1S/m the value of conductivity of the cytoplasm and 1.1029g/ml its density, for asynchronous yeast cells (Bryan et al. 2010; Sebastian Franco et al. 2013; Burgos-Molina et al. 2020); the values calculated for E, J and SAR, within a single cell, induced by the pulsed MF (for both positive and negative pulses) were for the positive pulses: E=0.21675625 V/m, J=0.21675625 A/m^2 and $SAR=4.25998x10^{-5}$ W/kg; and for the negative pulses: E=2.0934875 V/m, J=2.0934875 A/m^2 and SAR=0.003973787 W/kg.

No author has previously reported the value of these parameters within a single yeast, mammalian or bacterium cell. This is the first time that the values of these parameters are reported for pulsed MF, which is an important novelty of this work.

Chronological aging and exposure to magnetic fields

Previous to start with the aging process and therefore with the MF exposures, yeast cells were cultured during 4 days. During this period cell density and cell cycle were evaluated. As shown in Figure 1A yeast cultures reached the plateau phase at day 2 with a cell density of $8x10^7$ cells/ml. The cell cycle distribution at day 4 was: 51.03% in G1 phase, 31.96% in G2, 17.70% in S phase and 1.31% as other aberrant forms (Figure 1B). Then, cells were chronologically aged during 40 days.

Table 1 shows the chronological aging of yeast. Surviving fraction decreased with time for both control and pulsed MF exposed samples. The survival values observed at days 10 and 15 of aging showed the bigger differences between control (0.82 and 0.61) *versus* pulsed MF samples (0.52 and 0.25), respectively. Therefore, pulsed MF induced an acceleration of aging ($p<0.05$ ANOVA). Figure 1C shows the cellular concentration during the chronological aging. Along this period the cellular loss was minimal, maintaining all cultures a cell concentration around $6x10^7$ cells/ml. There were no statistically significant differences between control and pulsed MF samples ($p>0.05$ ANOVA).

Analysis of genomic DNA damage

The damage to the DNA was evaluated at the end of the period of chronological aging of cells to evaluate the possible accumulated damage induced by MF during this period. Genomic DNA was isolated and electrophoresed. The area on the gel that corresponds to the width and location of the DNA band in the not aged control sample (control-not aged lane) was considered as undamaged

DNA and the area in the gel below this band was considered as damaged DNA (Figure 2A). This band showed no degradation and was used as control in the analysis of profiles. As shown in figure 2A, the chronological aging (on day 40) in control aged yeast induced the appearance of a small degradation of genomic DNA (control-aged lane), which was revealed by the presence of a small DNA signal of less molecular weight. However, a bigger intensity fuzzy band of degraded DNA was found for pulsed MF treated yeast (PMF-aged lane) with DNA fragments of less molecular weight. This result indicates that pulsed MF induces a few randomly damage in DNA. The ladder pattern typical of apoptosis was not observed.

With the purpose of quantify the DNA content in each lane, an image analysis was performed to obtain the DNA profiles; that represent on the graph the signal intensity versus the migration distance (Figure 2B). DNA content was calculated by the ImageJ software measuring the area under the curve. In this way, the DNA not damaged was located in the profiles as the area under the curve that match with the width of the peaks in the not aged control samples (from pixel 325 to 450). The area under the curve of the profiles where DNA was over pixel 450 (in relation to not aged control) was established as DNA with increased damage. Figure 2B shows a bigger area under the curve for pulsed MF treated samples. This area match with the high intensity signal observed in the pulsed MF aged lane (fuzzy band in figure 2A).

Figure 2C shows the values of DNA content obtained from the analysis of profiles considering the not aged control cells as controls. The undamaged and damaged DNA content was estimated as the region under the curve indicated in figure 2B in different color. All data were normalized for comparison in relation to the total DNA content measured from the profiles and were expressed as percentage of control. As shown in figure 2C, the undamaged DNA was 96.48±10.08 % for not aged controls, 69.84±5.83 for aged controls and 49.08±4.52 % for MF treated samples. Differences were significant ($p<0.005$ Student's *t*-test). In addition, aged control and aged yeast exposed to pulsed MF showed a significant increase in damaged DNA (30.16±4.03 % and 50.92±6.21 %) respectively, in relation to the damaged DNA found for not aged controls

(3.52±0.43 %) (p<0.005 Student's t-test). These data suggest that aging and PMF exposure are both producing DNA damage.

Mutation frequency

Figure 3 shows the mutation frequency during chronological aging, measured as the number of revertant mutants per 100 survivors. At the beginning of aging it was 7.96±1.67 revertants/100 survivors. The mutation frequencies significantly increased in both groups (control and exposed to pulsed MF) during aging. The frequencies were significantly higher in the exposed cells (p<0.005 Friedman). At day 40 of aging they showed the biggest difference, reaching values of 19.95±6.8 revertants/100 survivors and 35.61±3.52 revertants/100 survivors for control samples and pulsed MF exposed samples, respectively. This result suggests that some type of damage is occurring at genomic level, which is consistent with the results obtained from DNA damage shown in figure 2, which contributes to support the results.

Discussion

In this study, we found that yeast cultures aged during the 40 days of exposure, observing a smaller surviving fraction in the groups exposed to MF in relation to controls. The survival values observed at days 10 and 15 of aging showed the bigger differences between both groups. Therefore, pulsed MF induced a premature aging, indicating that some type of damage is being induced. In this way, to study DNA damage, we considered as a more representative measure the effects at the end of the MF exposure time (day 40) when cells are aged, rather than during the exposure period. During this period, the cells are allowed to repair the damage produced in the DNA so that the accumulated damage is visualized. In our case, the repair period coincides with

the exposure during aging and the genotoxic activity measured at the final of the aging. Therefore, the differences between the treatments are better shown, with the assurance that the observed damages correspond to cumulative genotoxic damage not repaired.

The yeast populations in the current study lived much longer than yeast typically do in SDC medium during chronological aging. As reported by Fabrizio and Longo (2003) and Burtner et al. (2009) yeast in standard SDC medium reach ~20% viability after only 5-10 days. However, in the current study yeast cells reached ~20% viability at day 30. Possible reasons include that in the current study the yeast were aged at 23°C, they were not aged with continuous aeration, or both of those factors.

At the end of the exposure period, pulsed MF (1.5mT, 25Hz, 8h/day) affected the DNA integrity considerably increasing the level of DNA damage over the background level observed for controls. The net time of exposure to pulsed MF was 320 h. It is important to mention that the net exposure period for pulsed MF produced an energy absorption of SAR=4.25998×10^{-5}W/kg for the positive pulses and SAR=0.003973787W/kg for the negative ones. Articles published by other authors do not consider the energy absorbed at the cellular level in relation to the observed effects, the MF type applied and the exposure protocol.

In addition, pulsed MF induced an increment in the mutation frequency along the aging period in relation to controls. This result suggests a genomic alteration or damage, which is consistent with the premature aging observed and with the increment found in the genomic DNA degradation. We do not know what type of mutation is the *ade2* mutation present in this *S. cerevisiae* strain since it does not appear neither in the product sheet published by ATCC nor in other articles. To know what type of *ade2* mutation was reverted in this strain would help in the interpretation of the DNA damage effects. In this way, the mutation could be reverted by a simple error during DNA replication (such as incorrect replication of simple repeats), or could require recognition by a specific repair process and incorrect repair.

When red colonies lose their mitochondrial function they turn white and appear as petite colonies called “white petite”. These colonies have very little growth so they are very small and were not counted. Since the initial objective of this work was to evaluate the genomic damage, the frequency of mitochondrial mutation was not determined. Only normal-growing white colonies were counted as revertants. As a limitation of this study, it is highlighted that mitochondrial dysfunction can cause ade2 mutant colonies to appear white instead of red. This type of mutants were not assayed (Chatterjee and Singh 2001; Kim et al. 2002).

The results found suggest that exposure to pulsed MF during chronological aging, measured at the end of this period, induces the degradation of genomic DNA. The induced DNA degradation by pulsed MF, for the exposure period, the frequency and the magnetic flux density, could indicate a role of an interaction mechanism where the MF type is involved and/or the level of absorbed energy.

As previously reported by Burtner et al. (2009) the acidification of the extracellular medium, mainly due to the accumulation of fermentation products such as acetic acid, promotes the chronological aging of yeasts under standard conditions. In this way, despite the fact that the levels of acetic acid and pH were not evaluated throughout the aging process, it is logical to think that the extracellular conditions were similar to those described by these authors since the composition of the culture medium used was similar (2% dextrose, 0.17% yeast nitrogen base, 0.5% ammonium sulphate, 0.15% aminoacids mixture, 4X auxotrophic markers [ade, arg, trp, ura]) and also the aging method (Longo et al. 2012). The conditions of culture and manipulation were maintained equal for both controls and MF exposed cultures, so although acidification induces cellular aging, this could not be the cause for the difference found between both test groups.

The technique used in this study (agarose gel electrophoresis) to detect DNA damage is sensible enough according to the results reported by other authors in similar studies (Narita et al.

1997, Li and Chow 2001, Potenza et al. 2004, López-Díaz et al. 2014). The image analysis contributes to the improvement of the technique.

Although multiple studies about MF effects have been published during the last decade, there are no studies related to alterations in aging cells. Results reported by other authors use different biological models, manipulation protocols, type of MF and time of exposure. In relation to these parameters, biological and physical, the interaction mechanism between MF and living beings could be different (Luukkonen et al. 2014, Kesari et al. 2015, Wang and Zhang, 2017).

Our findings agree with those reported by Solek et al. (2017) (0-10 mT, 2, 50, 120 Hz, 2 h) and Sherrard et al. (2018) (2 mT, 10 Hz, 15 min-96 h). These authors found that pulsed MF produce more DNA damage (DNA strand breaks) with an increase in cellular aging, measured after the exposure period.

We hypothesize that the effects observed by pulsed MF exposure could be induced on the one hand by the efficacy in Reactive Oxygen Species (ROS) generation, and on the other hand by the level of absorbed energy in single cells. Both mechanisms could be explained by the theory of a differential enhancement of ROS levels during aging due to the exposure, with a higher DNA damage (Solek et al. 2017, Sherrad et al. 2018).

The relevance of the results lies in the relationship found between MF type, and especially the SAR value at the cellular level (novelty of this work), and the parameters measured that suggest DNA damage during chronological aging. The three observed effects (premature aging, increase in DNA degradation and increase in the mutation frequency) contribute to support the results and hence the conclusions.

Taking into account the Hill's criteria necessary to clarify the effects of MF, this work contributes to provide scientific evidence, in *in vitro* studies, of a harmful effect observed at low levels of exposure over a long period of time, such as chronological aging, not previously studied by other authors. The results found provide knowledge about the harmful effects of low intensity

and frequency pulsed MF, and contribute to lean the scale towards a greater perception of the associated risk.

Additional investigation could clarify the interaction mechanisms between MF and living beings and to stablish dose-response relationships.

Conclusions

Exposure of *S. cerevisiae* cells to pulsed MF (25Hz, 1.5mT, 8h/day) during chronological aging (40 days) showed a premature aging with an increase in genomic DNA degradation and in the mutation frequency. Therefore, under the experimental conditions assayed, the applied pulsed MF could induce genomic DNA damage during chronological aging.

This is the first time that the values of SAR for pulsed MF at cellular level are reported, which is a novelty of this work.

Acknowledgements

We wish to thank Dr A. Friedl (Department of Radiation Oncology, Ludwig-Maximilians-Universität München, Munich, Germany) for the supply of yeast cells. Thanks to Ms. L. Gil-Carmona (Universidad de Málaga, Spain) for her technical assistance.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This study was sponsored by: “Plan Andaluz de Investigación, Desarrollo e Innovación (PAIDI); Junta de Andalucía”, code CTS-181.

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Word count: 5996 (Including references and figure legends)

FIGURE LEGENDS

Figure 1. Growth, cell cycle and cell density. **A)** Growth curve of yeast in SDC in the phase previous to aging. **B)** Cell cycle distribution of yeast during the previous phase. **C)** Cellular concentration during the chronological aging ($p>0.05$ ANOVA). PMF: pulsed magnetic field. Mean±SD

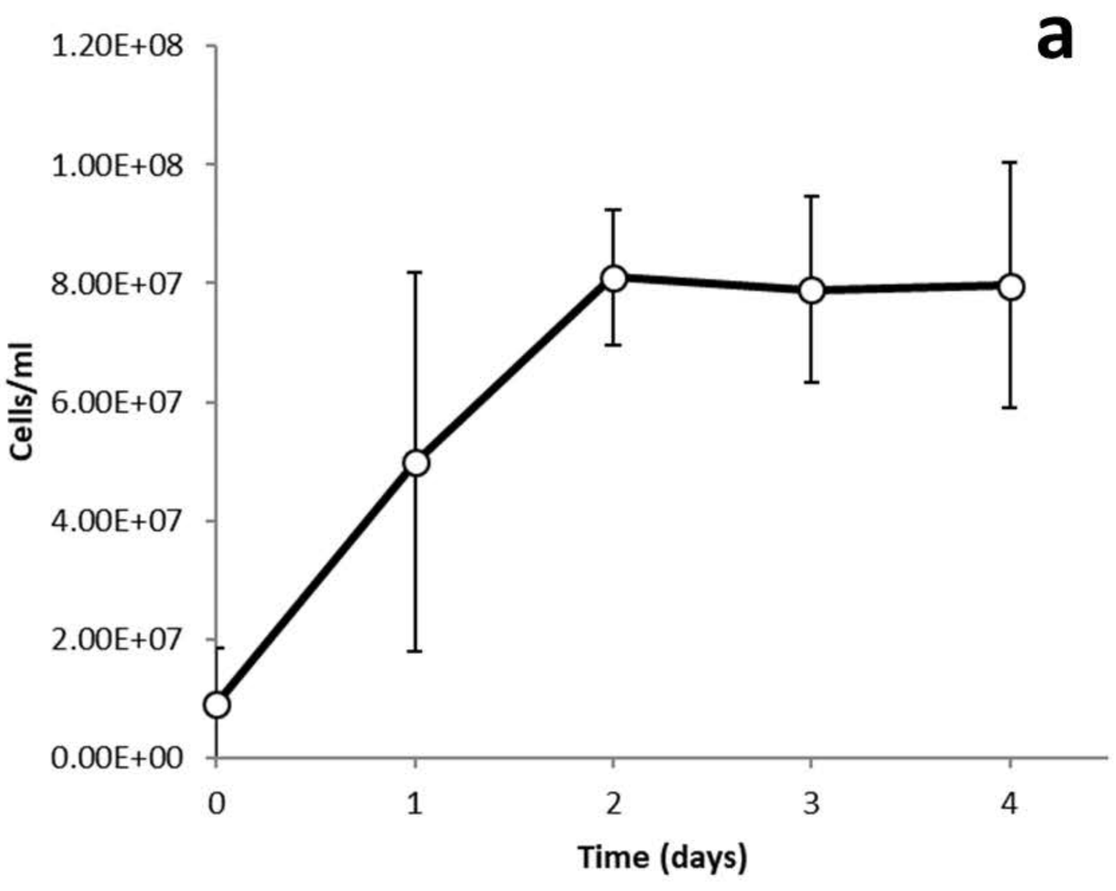
Figure 2. Analysis of genomic DNA damage. **A)** DNA pattern obtained after chronological aging of yeast exposed to pulsed magnetic field (PMF) and the respective unexposed aged and not aged controls. DNA samples were electrophoresed at the end of the chronological aging and therefore at the end of the exposure period (40 days). **B)** DNA profiles obtained for each electrophoresis lane in (A). **C)** DNA content obtained from the analysis of profiles calculated as the area under the curve of profiles in (B) and normalized in relation to the total DNA content. Mean±SD. *$p<0.005$ Student's *t*-test.

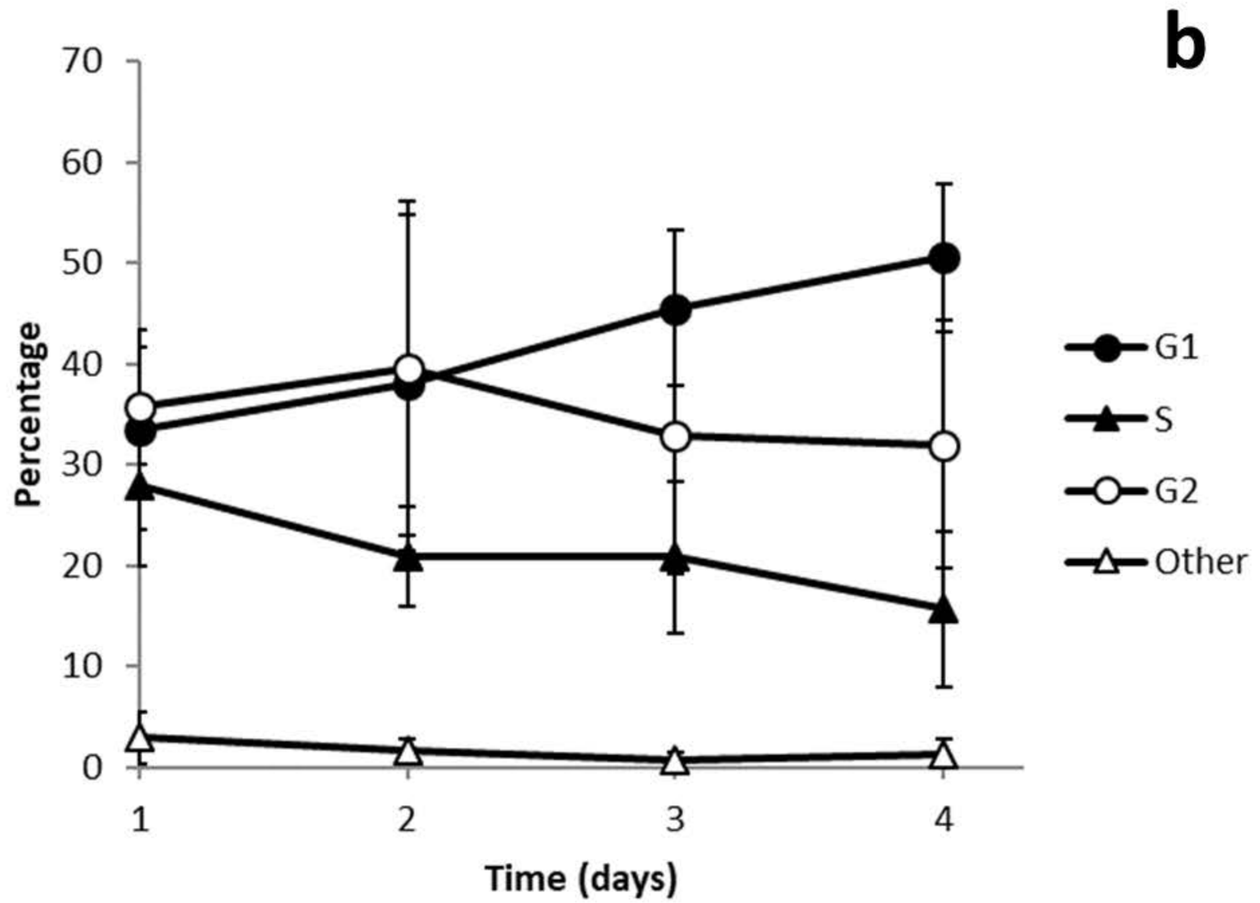
Figure 3. Mutation frequency during chronological aging. The mutation analyzed was the reversion of the *ade2* mutation present in the genome of the yeast strain. PMF: pulsed magnetic field. Mean±SD. $p<0.005$ Friedman.

Table 1. Chronological aging of yeast.

	Surviving fraction	
Aging (days)	Control	PMF
0	1.00 ± 0.01	1.00 ± 0.01
10	0.82 ± 0.19	0.52 ± 0.09
15	0.61 ± 0.15	0.25 ± 0.09
20	0.31 ± 0.21	0.23 ± 0.02
30	0.20 ± 0.04	0.07 ± 0.03
40	0.18 ± 0.04	0.09 ± 0.01

PMF: Pulsed magnetic field. Mean ± SD. $p<0.05$ ANOVA.


a
1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Cells/ml
0
1
2
3
4
Time (days)


b
70
60
50
40
30
20
10
0
Percentage
1
2
3
4
Time (days)
G1
S
G2
Other

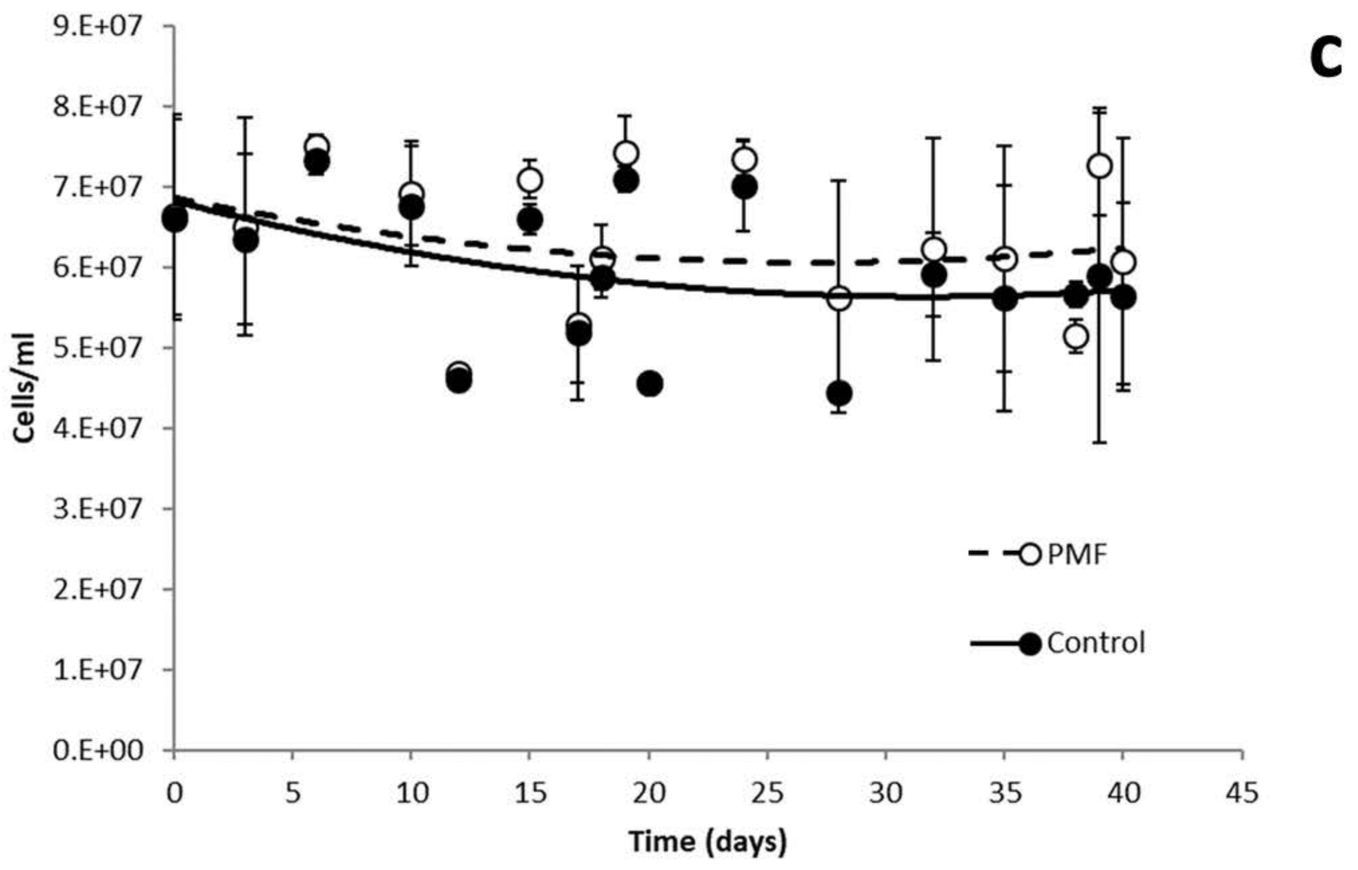
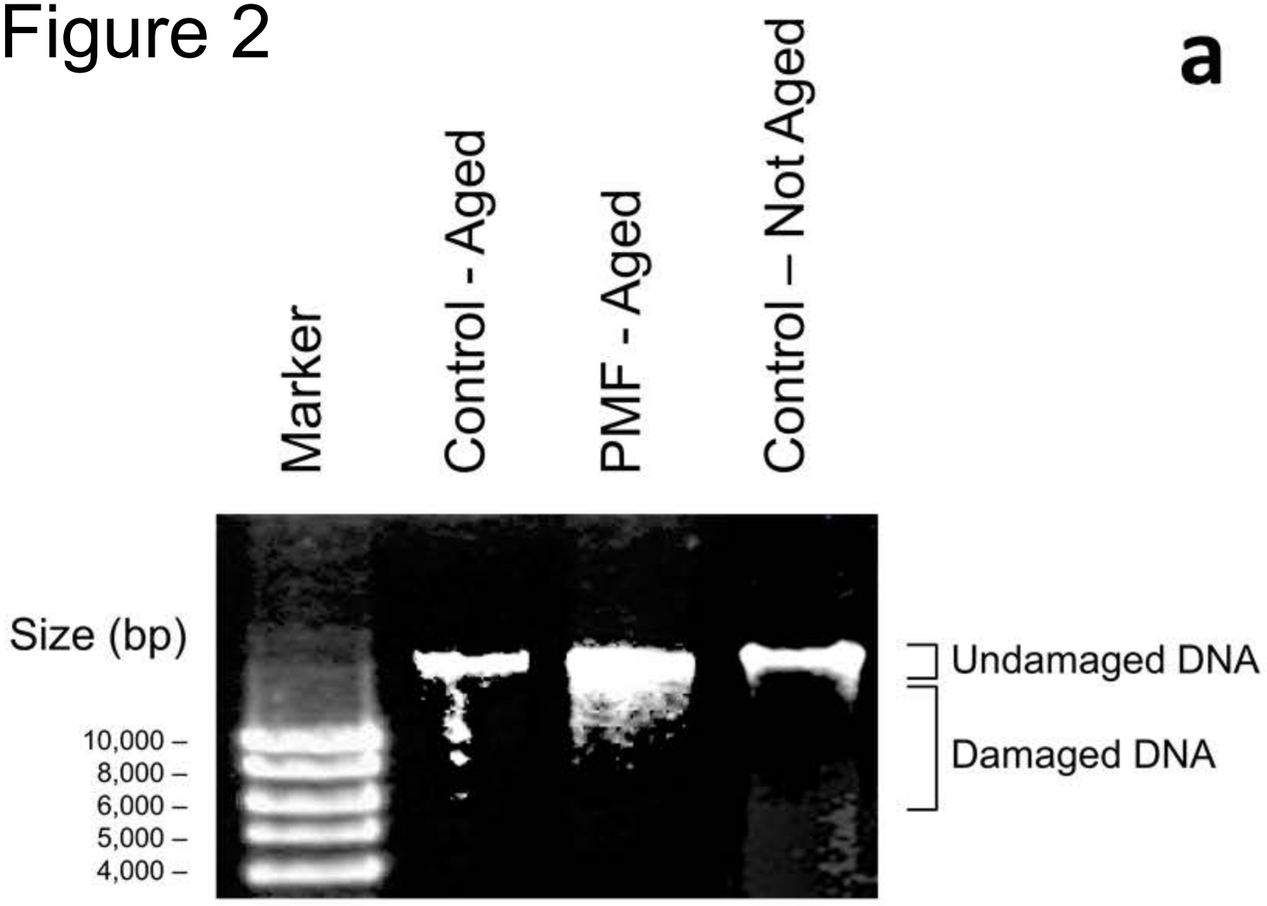
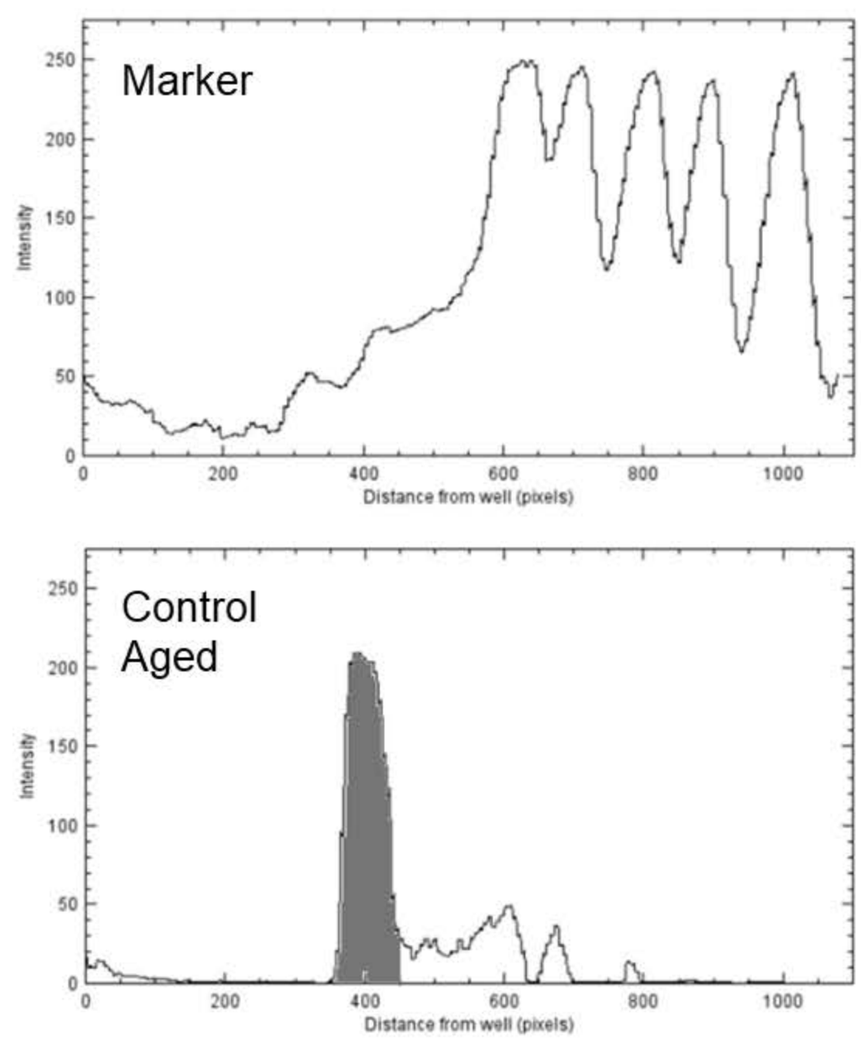

c
9.E+07
8.E+07
7.E+07
6.E+07
5.E+07
4.E+07
3.E+07
2.E+07
1.E+07
0.E+00
Cells/ml
0
5
10
15
20
25
30
35
40
45
Time (days)
PMF
Control

Figure 1

Figure 2


a
Marker
Control - Aged
PMF - Aged
Control – Not Aged
Size (bp)
10,000 –
8,000 –
6,000 –
5,000 –
4,000 –
Undamaged DNA
Damaged DNA


b
Marker
Intensity
Distance from well (pixels)
Control
Aged
PMF
Aged
Control
Not Aged
Undamaged DNA
Damaged DNA

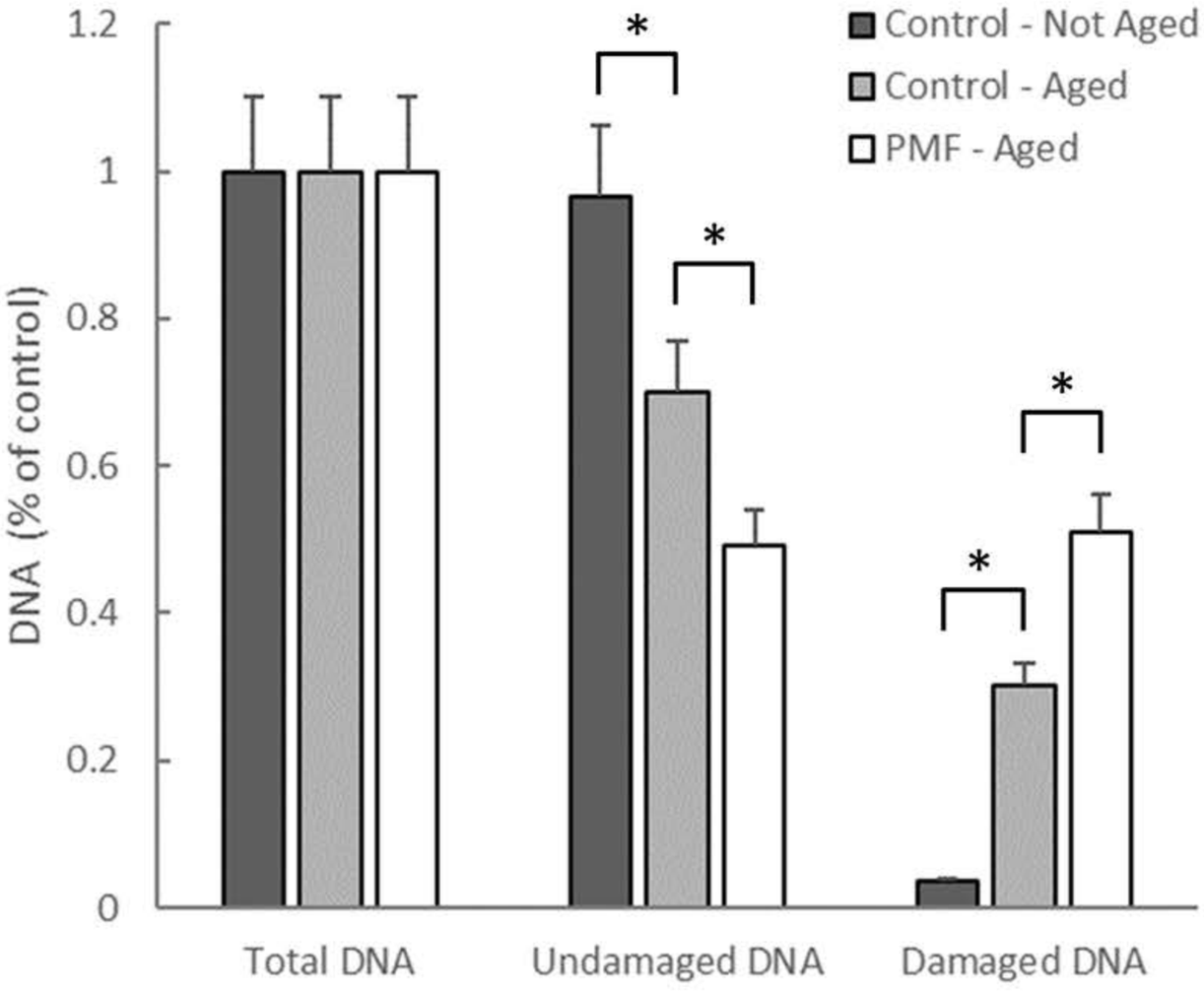
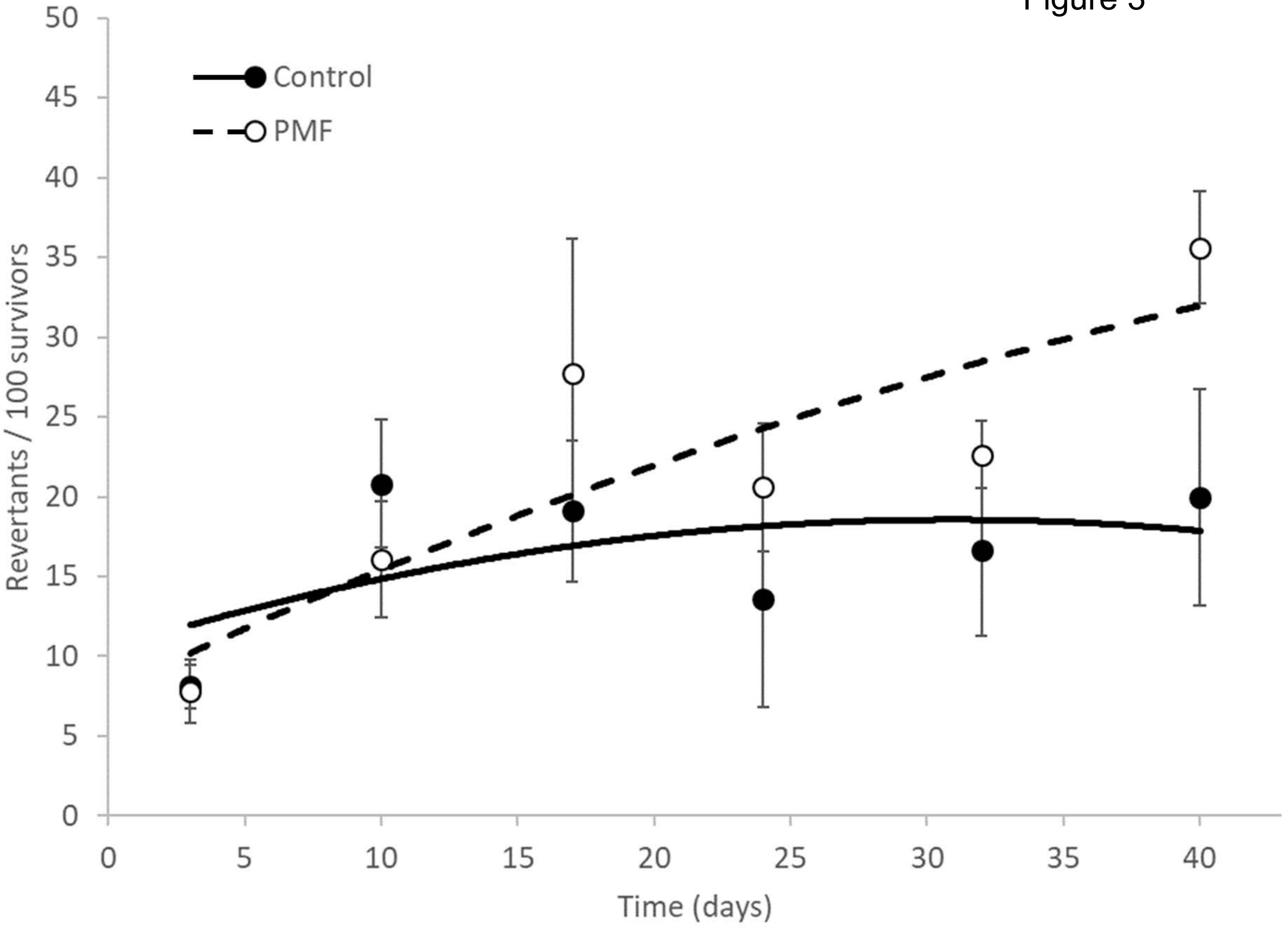

c
Control - Not Aged
Control - Aged
PMF - Aged
DNA (% of control)
Total DNA
Undamaged DNA
Damaged DNA
*

Figure 3


Control
PMF
Revertants / 100 survivors
0
5
10
15
20
25
30
35
40
45
50
0
5
10
15
20
25
30
35
40
Time (days)