Acoustic holograms for homogeneous hyperthermia over several tumor spheroids

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INTRODUCTION

High intensity focused ultrasound (HIFU) hyperthermia (<50 ºC for ~minutes) is used as a chemo- or radio- sensitizer in cancer treatment. It can increase temperature locally in a non-invasive way. Nevertheless, the focus of an ultrasound transducer is tight and produces non-uniform heating. In-vitro and in-vivo studies on tumor models are needed to understand how ultrasound hyperthermia affects these cells. 3D tumor spheroids are better predictors of clinical outcomes than 2D cultures [1].

Aims and objectives

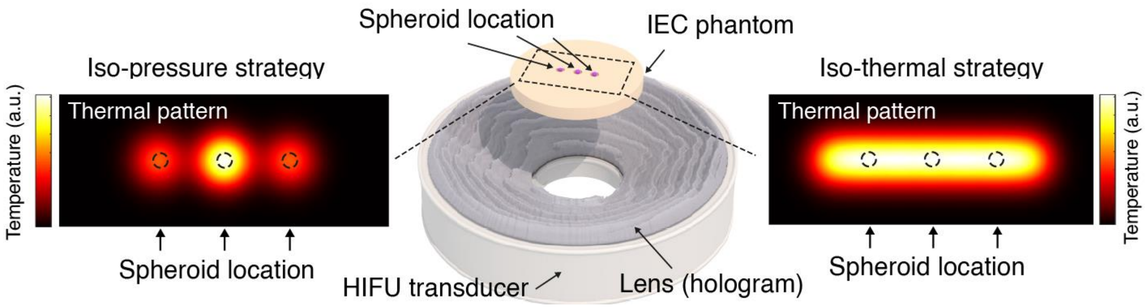
In this work we use **acoustic holographic lenses** coupled to an ultrasound transducer to produce a **region of uniform hyperthermia**. Also, a **system to hold spheroids** in place and allow **real-time dosimetry** was designed.

The aim is to study how ultrasound-delivered hyperthermia affects tumor spheroids in-vitro and compare their response to those treated using PCR heating.

METHODS

Holographic lens design

Holographic lenses were designed with time reversal methods using pseudo-spectral time-domain simulations to be coupled to a focused ultrasound transducer (1.66 MHz, 63.2-mm radius of curvature, 64-mm outer diameter and 23-mm inner hole diameter). Two strategies were investigated: an **iso-pressure strategy** with 3 foci located at the spheroid's location and an **iso-thermal strategy** using 7 virtual sources with tuned amplitude and phase. Acoustic and thermal simulations were performed to characterize the performance of both lenses. Lenses were 3D-printed using stereolithography.

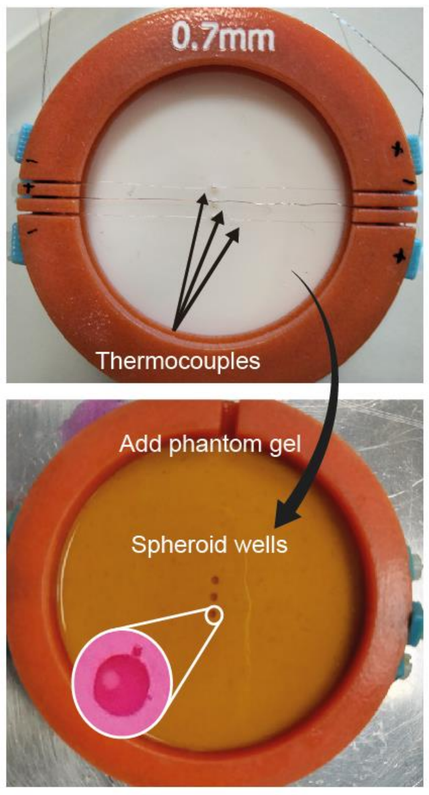


Spheroid holder

The spheroid holder was made of IEC ultrasound-absorbing tissue mimicking phantom with three wells to hold the spheroids. Underneath each well is an embedded thermocouple for **real-time dosimetry**. Above, a lid of phantom material was used to ensure no movement of the spheroids.

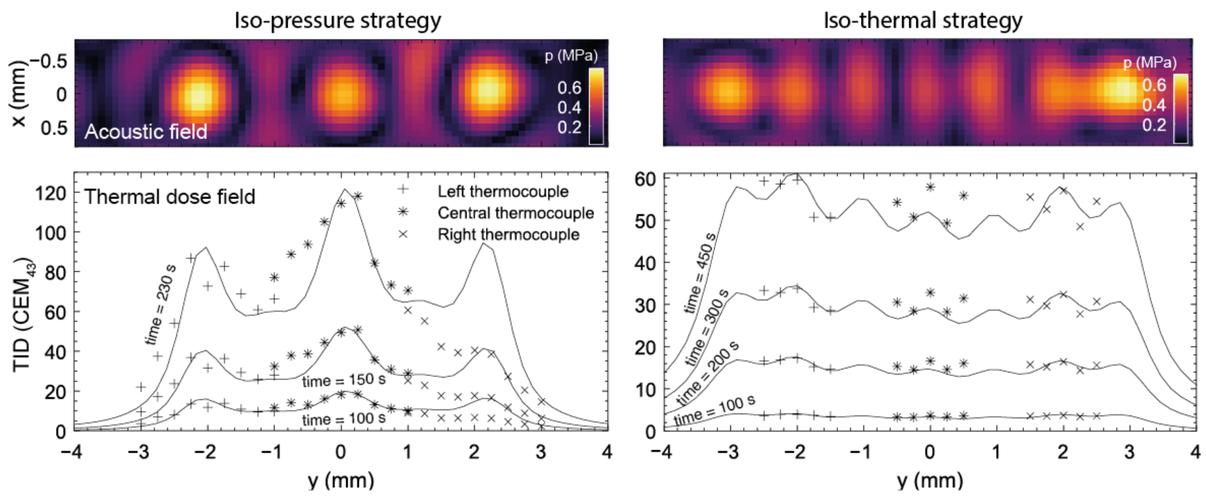
Experiments

Acoustic field characterization was performed in degassed water with a hydrophone for both holographic lenses. Thermal characterization and spheroid exposure was performed in a continuously degassed water tank held at 38ºC. Temperature and thermal dose were recorded in real-time. Spheroids were exposed to thermal iso-effective doses (TIDs) of 0, 7.5, 30, 60 and 120 CEM_{43} with either the **ultrasound-heating system** or a **polymerase chain reaction (PCR) thermocycler**. To study their response, growth (microscopy) and viability (Cell Titer Glo 3D assay) studies were performed over 10 days.



RESULTS

Acoustic and thermal fields

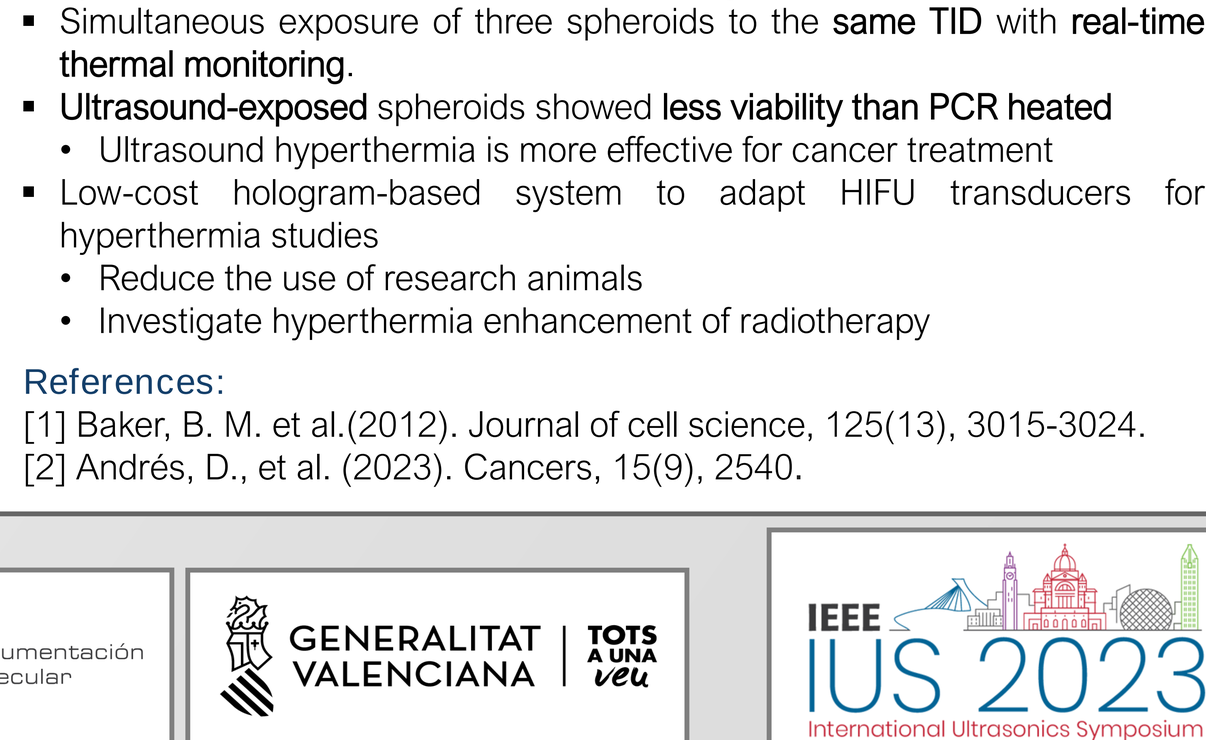


For the **iso-pressure** lens we obtained a maximum difference in acoustic pressure among foci of 15%, with maximum foci deviation of 0.2 mm. Thermal profiles were recorded at each thermocouple. At each well, mean TIDs were 73, 103 and 38 CEM_{43} from left to right, with maximum difference of 63% in thermal dose.

For the **iso-thermal** strategy we obtained a maximum difference in acoustic pressure among foci of 40%, while all the foci appeared at the desired location. At each well, mean TIDs were 56, 54 and 54 CEM_{43} from left to right, with maximum difference of 4% in thermal dose.

Spheroids response

To study spheroids response to ultrasound-delivered hyperthermia we used the iso-thermal lens. Sham- and 7.5 CEM_{43} ultrasound-exposed spheroids increased their size by approximately 40% over 10 days. Spheroids exposed to **120 CEM_{43} contracted by 15%** while no difference in size was seen at 30 or 60 CEM_{43}. **PCR-heated spheroids presented no shrinkage** even at the highest thermal dose. A statistically significant **reduction in spheroid metabolic activity** was seen when ultrasound exposed to TIDs of 30, 60 and 120 CEM_{43}, compared to sham exposed spheroids. PCR-heated spheroids showed no significant viability changes between TIDs



CONCLUSIONS

- Tuned **"iso-thermal" acoustic holograms** (lenses) to obtain uniform hyperthermia inside an ultrasound-absorbing phantom
- Simultaneous exposure of three spheroids to the **same TID** with **real-time thermal monitoring**.
- **Ultrasound-exposed** spheroids showed **less viability than PCR heated**
  - Ultrasound hyperthermia is more effective for cancer treatment
- Low-cost hologram-based system to adapt HIFU transducers for hyperthermia studies
  - Reduce the use of research animals
  - Investigate hyperthermia enhancement of radiotherapy

References:

[1] Baker, B. M. et al.(2012). Journal of cell science, 125(13), 3015-3024.
[2] Andrés, D., et al. (2023). Cancers, 15(9), 2540.

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