The Institute of Cancer Research

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Radiotherapy Coupled with Hyperthermia

In vitro system for ultrasound hyperthermia (and radiotherapy) of tumour spheroids

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Introduction

High intensity focused ultrasound (HIFU) hyperthermia (<50°C for ~minutes):

- Suitable for non-invasive clinical and pre-clinical use, however *in vitro* ultrasound studies are challenging:
  - *In vitro* 2D and 3D culture-ware acoustically opaque
  - 3D spheroids better predictors of clinical outcomes than 2D
    - Hard to target and hold in place in a HIFU beam
    - Easily moved by radiation force
    - Dosimetry is challenging
- Many *in vitro* studies performed using simpler water bath or PCR heating
- Tight HIFU focus produces non-uniform heating

Aims and objectives

To develop a HIFU hyperthermia delivery system with:

- more uniform heating over a wider area
- enough energy to induce hyperthermic temperatures

To develop an *in vitro* system for US hyperthermia that:

- Is better than simple water bath or PCR heating, as they omit the effect of ultrasound on cells
- Is a tissue-like holder that heats to hyperthermia temperatures
- Can hold spheroids in a way that minimises the risk of cavitation and radiation force damage
- Provides real-time dosimetry from measured temperatures

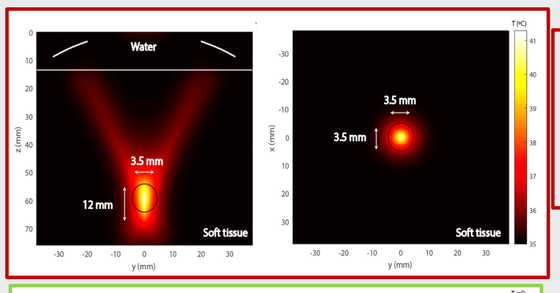
Advantages of an *in vitro* system:

- Reduces the need for in vivo experiments (3Rs: replacement)
- Wider range of assays available *in vitro* than in vivo

Lens design and fabrication

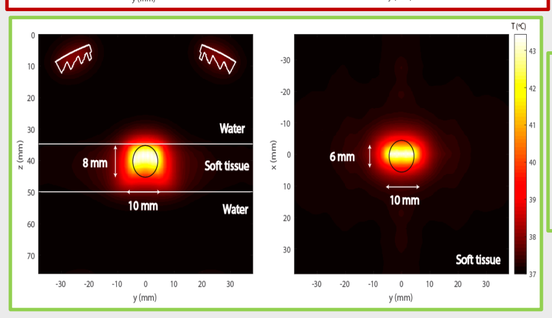
Widening and shortening the HIFU focus:

- Collaboration with UPV (University Polytechnique Valencia)
- Holographic acoustic/thermal simulations used for lens design



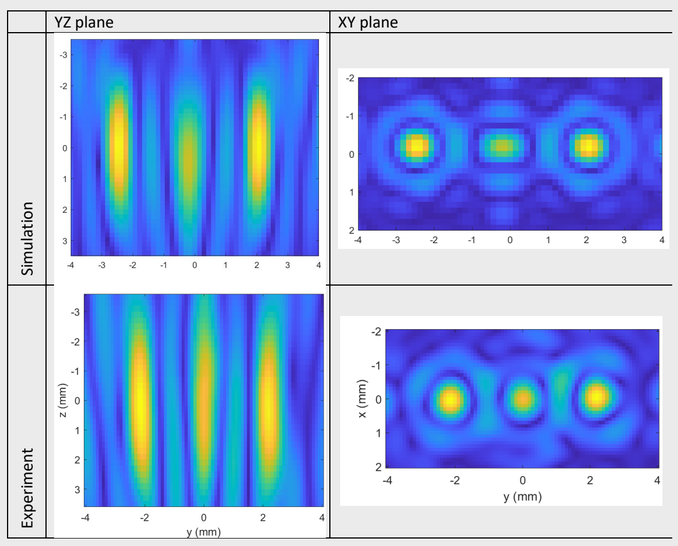
HIFU temperature
Start: 37oC, HIFU: 1 MPa

Hot spot length (Z) x height (X) x width (Y):
12 mm x 3.5 mm x 3.5 mm

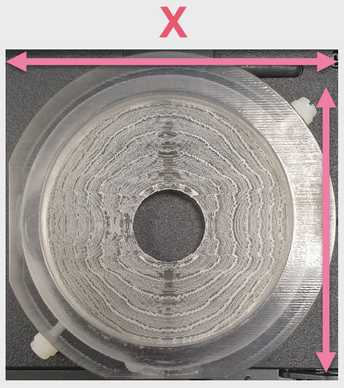


3D lens hyperthermia temperature
Start: 37oC, HIFU: 1 MPa

Hot spot length (Z) x height (X) x width (Y):
10 mm x 6 mm x 8 mm



3D printed lens



2D data (left) show widening in Y and shortening in Z close prediction

FWHMP	X/mm	Y/mm	Z/mm
HIFU - 1 focus	1.36	1.3	12.3
Lens - 3 foci	~0.85	5.25	~6.2

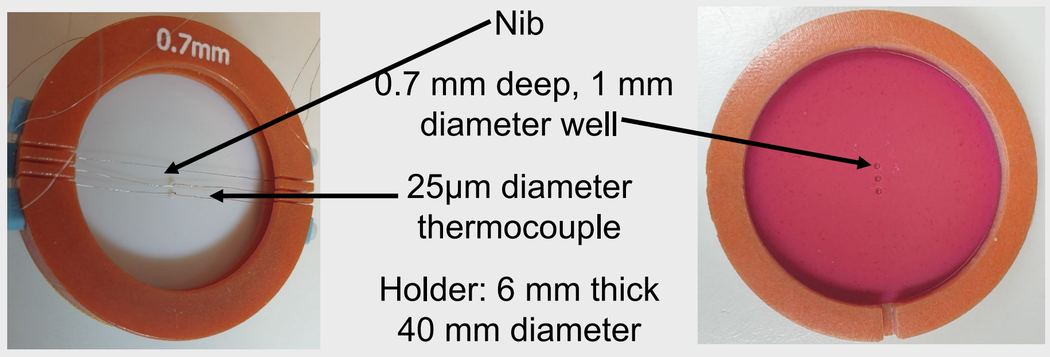
Spheroid exposure system

Holder (phantom) requirements:

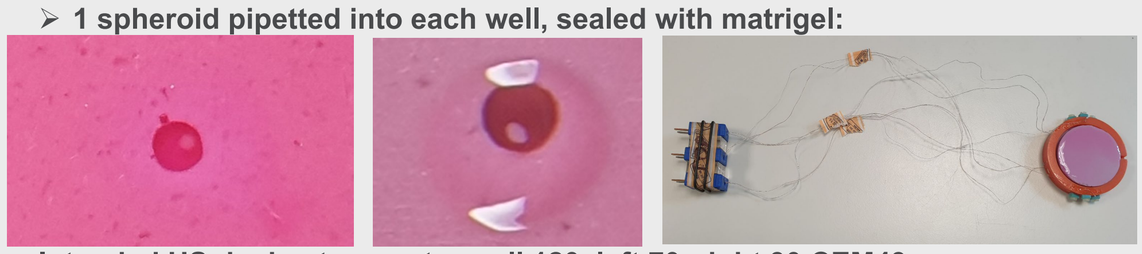
- Tissue mimic (IEC standard phantom + 1% phenol red for contrast with spheroids):
  - non-toxic
  - absorbs ultrasound, heats reproducibly (i.e. not too thin)
  - heats to 45-50°C without melting
  - holds spheroids without movement and mechanical damage from radiation force
- Provides spheroid targeting
- Geometric targeting between repeats
- Real-time temperature (thermal dose) monitoring

Spheroid holder

3D printed mould, 3 nibs produce 3 wells for spheroids
Thermocouples for localisation and dosimetry

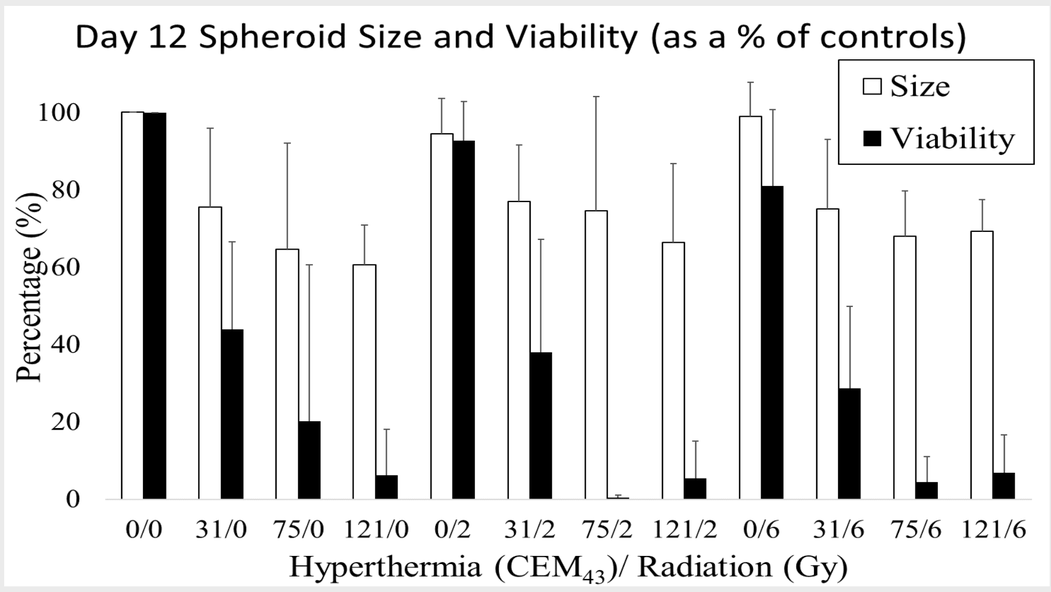


➢ **1 spheroid pipetted into each well, sealed with matrigel:**



Intended US dosimetry: centre well 120, left 70, right 30 CEM43
Radiation: 0, 2 and 6 Gy single fraction

Results



- 110/117 TUS exposed spheroids successfully harvested
- Delivered dose (CEM43): 121 ± 1%, 75 ± 11%, 31 ± 23%
- RT alone: little effect on viability or size
- US alone (≥ 31 CEM43): viability of <50%; little effect on spheroid size
- US+RT: enhanced effect with viability of <10%

Conclusions

Novel phantom/system for *in vitro* HIFU hyperthermia

- 94% spheroid harvest rate
- Dose delivered at least ± 23%
- Viability and size data obtained despite non-sterile conditions
- Preliminary results are promising

Contact

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