In vivo proton range verification with ultrasound imaging using injectable radiation sensitive nanodroplets: a feasibility study

Bram Carlier¹,², Sophie V. Heymans¹,³, Sjoerd Nooijens⁴, Yosra Toumia⁵, Marcus Ingram⁶, Gaio Paradossi⁷, Emiliano d’Agostino⁶, Uwe Himmelreich⁷, Jan D’hooge⁴, Koen Van Den Abeele³ and Edmond Sterpin²

¹ These two authors contributed equally
² Department of Oncology, KU Leuven, Leuven, Belgium
³ Department of Physics, KU Leuven, Kortrijk, Belgium
⁴ Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium
⁵ Department of Chemical Sciences and Technology, University di Roma Tor Vergata, Rome, Italy
⁶ DoseVue, Philips Open Manufacturing Campus, Turnhout, Belgium
⁷ Department of Imaging & Pathology, KU Leuven, Leuven, Belgium

E-mail: edmond.sterpin@kuleuven.be

Abstract

Technologies enabling in vivo range verification during proton therapy are actively sought as a means to reduce the clinical safety margins currently adopted to avoid tumor underdosage. In this contribution, we applied the semi-empirical theory of radiation-induced vaporization of superheated liquids to coated nanodroplets. Nanodroplets are injectable phase change contrast agents which can vaporize into highly echogenic microbubbles to provide contrast in ultrasound images. We exposed nanodroplet dispersions in aqueous phantoms to monoenergetic proton beams of varying energies and doses. Ultrasound imaging of the phantoms revealed that droplet vaporization occurred in regions proximal to the proton Bragg peak. The statistically significant contrast increase in irradiated regions of the phantoms is proportional to the proton fluence and was observed for doses as low as 2 Gy. The absence of enhanced response in the vicinity of the Bragg peak, combined with theoretical considerations, suggests that droplet vaporization is induced by high-LET recoil ions produced by nuclear reactions with incoming protons. Vaporization profiles were compared to non-elastic cross-sections and LET characteristics of oxygen recoils. Shifts between ultrasound contrast drop and $R_{80}$ values showed a submillimeter reproducibility. These early findings confirm the potential of superheated nanodroplets as a novel tool for in vivo range verification.

Keywords: proton therapy, range verification, dosimetry, ultrasound, nanodroplets

1. Introduction

The increasingly growing fleet of proton therapy facilities, owing to substantial cost reduction and compactness improvements over the past ten years, has contributed in making proton therapy accessible to a variety of clinical indications (Thariat et al 2013). The favorable depth dose distribution of protons implies that excellent dose conformity and therefore healthy tissue sparing can potentially be achieved. However, taking full advantage of the physical selectivity of protons, e.g. to preserve organs-at-risk, is hampered by uncertainties on their in vivo range, which arise from inaccuracies of the stopping power calculation from CT scans, imaging artefacts, setup errors, patient motion and...
anatomical changes throughout the treatment (Paganetti 2012, Knopf and Lomax 2013). Additionally, to avoid tumor underdosage due to these range uncertainties, substantial safety margins (up to several millimeters), conservative planning strategies (sub-optimal choice of beam angles), or large value of the range uncertainty parameter used in robust optimizers (around 3%) have been adopted in most proton therapy facilities (Paganetti 2012). In order to reduce these margins and gather more insight into the influence of different factors on the range, an urgent need for accurate in vivo range verification techniques exists.

Several methods have emerged and reached different stages of development. However, none of them is routinely employed in the clinic. The most mature technology for in vivo range verification relies on PET imaging of positron-emitting isotopes activated by proton nuclear interactions (Paganetti 2012). The measured activity distribution is correlated with the actual proton range through Monte Carlo simulations. PET imaging can be performed offline (Parodi et al 2007), in-room (Min et al 2013) or in-beam (Kraan et al 2014, Helmbrecht et al 2012). While in-room and in-beam acquisitions benefit from shorter scan time and higher resolution compared to offline PET imaging, they require bespoke detectors and may affect the throughput in the treatment room (Paganetti 2019).

Alternatively, prompt gamma imaging (PGI) makes use of gamma rays emitted by nuclei excited by the incoming proton beam for real-time range verification (Jongen and Stichelbaut 2003, Min et al 2006). PGI has been recently tested to assess range shifts in a patient with brain cancer and demonstrated a shift retrieval precision of 2 mm (Xie et al 2017), but its translation towards clinical applications is hampered by technological limitations and detector cost (Knopf and Lomax 2013, Rohling et al 2017). Range probe (1D) and proton radiography (2D/3D) require high energy protons completely traversing the body in the low-dose plateau (Knopf and Lomax 2013, Mumot et al 2010, Plautz et al 2014). The stopping power of the body tracks is then determined from the residual ranges of the transmitted protons. However, this technique results in undesired additional dose to the patient and involves high energy protons, therefore increasing the cost and footprint of the proton facility (Paganetti 2019).

Detection of ionizing radiation by means of the vaporization of superheated droplets was achieved in the 1950s by Donald Glaser (Glaser 1952), who was awarded the Nobel Prize for his invention of the bubble chamber. Since then, the use of superheated emulsions significantly expanded to different fields such as space applications, medical physics, neutron dosimetry or dark matter search (Roy 2001). These detectors typically feature superheated drops of dimensions ranging from tens to thousands of microns, embedded in a compliant polymeric or aqueous matrix (D’Errico 2001). Upon exposure to radiation, the drops vaporize into bubbles which can be detected either by visual inspection, volumetric measurement or acoustic readout (Sarkar et al 2006). Two decades ago, Apfel (Apfel 1998) envisioned the use of injectable superheated emulsions as in vivo dosimeter, but to our knowledge, the idea has never been pursued.

Nanodroplets, or phase change contrast agents (PCCAs), have become increasingly popular over the past ten years as versatile contrast agents for ultrasound imaging and therapy (Sheean and Dayton 2012). They are made of a perfluorocarbon liquid core surrounded by a stabilizing lipidic or polymeric shell, whose diameter typically ranges from hundreds of nanometers to a few microns (Lea-Banks et al 2019). Nanodroplets can be injected intravenously and circulate inside the patient’s vasculature, where the smallest sizes (< 200 nm) also extravasate. Additionally, the nanodroplet shell can be functionalized to target tissues of interest, making them suitable for molecular imaging and targeted therapy (Deshpande et al 2010). Localized nanodroplet vaporization can be achieved with ultrasound waves of moderate to high intensities (Sheean et al 2012) or through laser heating (Dove et al 2014), yielding micrometer-sized echogenic bubbles readily imaged with Contrast Enhanced Ultrasound Imaging (CEUS). In order to minimize potential tissue damage from cavitation or heating, droplet vaporization should be achieved with moderate amounts of acoustic or thermal energy (Sheean et al 2011). Therefore, the droplet liquid core is generally kept in a metastable superheated state.

In this report, we evaluated for the first time the applicability of the radiation-induced nucleation theory to nanometer-sized superheated droplets. Particularly, we upgraded the naked superheated emulsions used in dosimetry towards injectable nanodroplets, similar to the ones employed for CEUS. First, we briefly review the generally accepted theory of superheated droplet vaporization induced by ionizing radiation. Then, we prove the existence of a radiation response and assess the suitability of these radiation sensors for in vivo non-invasive proton range verification. To this aim, tissue-mimicking phantoms with entrapped nanodroplets were irradiated with varying proton energies and the relationship between the resulting ultrasound signals and the predicted proton range was investigated. Finally, the potential sensitivity to dose is examined and the feasibility of the presented approach at clinically-relevant doses is shown.

2. Materials and methods

2.1 Ionizing radiation induced nucleation theory

The nucleation of bubbles along particle tracks in a metastable liquid is a complex physics problem involving time and length scales covering several orders of magnitude and different fields such as thermodynamics and radiation physics, for which a complete analytical description is still lacking. The most widely accepted semi-empirical model combines the
thermal spike theory developed by Seitz (Seitz 1958) with the isothermal spontaneous nucleation thermodynamics. In Seitz’s theory, the kinetic energy of charged particles is transferred to the medium by a multitude of highly localized temperature spikes forming along their track (West 1998, Apfel 1979). The thermal spikes occur within a time scale so small compared to thermal diffusion that the liquid literally explodes into vapor embryos (D’Errico 2001, Sarkar et al 2006) along the particle track, which then acquire a spherical shape that can grow indefinitely provided that the initial bubble size exceeds a critical radius value, given by the thermodynamics of phase equilibrium,

\[ R_c = \frac{2\sigma}{(p_e - p_l)(1 - \rho_v/\rho_l)} \]  

(1)

where \( \sigma \) is the surface tension of the superheated liquid, \( p_e \) is the saturation pressure, \( p_l \) the pressure inside the superheated liquid drop, and \( \rho_v \) and \( \rho_l \) the densities of the vapor and liquid phases, respectively.

The energy required to nucleate a critical vapor embryo is obtained from homogeneous nucleation theory, with additional terms specific to radiation-induced nucleation (D’Errico 2001).

\[ W_{tot} = \frac{16\pi\sigma^3}{3(p_e - p_l)(1 - \rho_v/\rho_l)} + \frac{\Delta H}{2\Delta x} \left[ 1 + \frac{2D}{(p_e - p_l)(1 - \rho_v/\rho_l)} - \frac{\Delta T}{T}\right] + W_{irr} \]  

(2)

\[ W_{irr} = 2\pi\rho_lR_c^2\dot{R}^2 \]  

(3)

\[ \dot{R} = \frac{4D(\rho_l/\rho_v)^3}{R_c} \]  

(4)

\[ D = \frac{k}{\rho_l c_p} \]  

(5)

where \( \Delta H \) is the latent vaporization heat of the fluid, and \( W_{irr} \) accounts for the irreversible energy losses from the action of viscous forces and the transfer of kinetic energy to the surrounding liquid (D’Errico 2001). \( \dot{R} \) is the vapor wall velocity, \( D \) is the thermal diffusivity of the liquid, and \( k \) and \( c_p \) are its thermal conductivity and specific heat, respectively.

In order to nucleate a vapor embryo of dimensions larger than the critical radius, the energy deposited by the charged particle along an effective path length (\( L_{eff} \)) must exceed \( W_{tot} \) (D’Errico 1999). The effective path length is often assumed proportional to the critical radius, yielding \( L_{eff} = \alpha r_c \). However, a single value of the proportionality constant, also called nucleation parameter, is insufficient to describe the behavior of superheated drop detectors for all degrees of superheat (D’Errico 1999). Moreover, the linear relationship between the effective length and the critical radius remains questionable (Andrews et al 2006). Nevertheless, most authors assume a constant value of the nucleation parameter that typically ranges from 2 to 13 (Ing et al 1997).

The energy transferred by a charged particle per unit track length in a medium is given by its linear energy transfer (LET) (Paganetti 2019). The nucleation condition writes:

\[ \int_0^{L_{eff}} \frac{dE}{dx} \geq W_{tot} \]  

(6)

and can be further expressed in terms of track-averaged LET (Ing et al 1997):

\[ \frac{dE}{dx} \geq \frac{W_{tot}}{\alpha r_c} \]  

(7)

The left hand side of the equation is only dependent on the energy deposition characteristics of the radiation, while the right hand side obeys the thermodynamic properties of the superheated fluid. Since the nucleation energy drops by increasing the superheat, the LET threshold of superheated drop detectors is inversely proportional to the degree of superheat. The “reduced superheat” parameter is commonly used to describe the operating point of a superheated liquid with respect to the temperature boundaries of the superheated state (D’Errico 1999), and is defined as:

\[ s = \frac{T - T_b}{T_c - T_b} \]  

(8)

\( T_b \) is the boiling temperature at atmospheric pressure, and \( T_c \) is the critical temperature of the fluid above which the liquid phase can no longer exist. By appropriately tuning the degree of superheating of the liquid core, one can tailor the droplet sensitivity to different types of radiation. Typically, neutron dosimeters operate at \( s = 0.2 \), as the droplets are vaporized by high-LET secondary charged particles produced by nuclear reaction products (D’Errico 2001). To sensitize bubble detectors to low-LET radiation such as photons and protons, higher degrees of superheat are required, which comes at the cost of decreased droplet stability. The practical limit of superheat is reached for \( s = 0.65 \), when the metastable liquid spontaneously vaporizes (Porteous and Bland 1975, D’Errico et al 2000). Detection of proton radiation in the vicinity of the Bragg Peak was reported by several groups (Green et al 2005, D’Errico and Egger 1994, Guo et al 2002), indicating that the threshold for proton detection lies between \( s = 0.35 \) and \( s = 0.42 \), corresponding to a LET threshold of 70-90 keV/\( \mu \)m, typically reached by protons at the end of their range. Proton irradiation of bubble detectors with lower degrees of superheat revealed that high-LET nuclear reaction products (heavy recoils) induce uniform vaporization tracks (Green et al 2005, D’Errico et al 1997, Miller et al 2018, Takada et al 2004).

### 2.2 Nanodroplets synthesis and characterization
2.2.1 Nanodroplets composition and synthesis. The nanodroplets employed in this study are comprised of a perfluorobutane (C\textsubscript{4}F\textsubscript{10}, boiling point of -2°C) liquid core encapsulated in a fatty acid monolayer of 10,12-pentacosadiynoic acid (PCDA). The complete nanodroplet synthesis is described elsewhere (Toumia et al 2019). Briefly, decafluorobutane was fluxed for a few seconds into an empty glass vial sealed with a rubber septum and immersed in liquid nitrogen to ensure liquefaction. Afterwards, injection of 6 ml of PCDA aqueous suspension (1 mM) in the vial, followed by a 10-minute sonication in an ultrasound bath at room temperature, yielded a milky suspension of nanodroplets. Non-encapsulated decafluorobutane vaporized during sonication, filling the headspace of the glass vial. After addition of surfactant (Pluronic F127) and photoinitiator solutions (Irgacure 2959), nanodroplets were exposed to 352 nm UV-light (UV lamp model ENF-260C, Spectroline Corporation, Westbury, NY) for 30 minutes, to polymerize the PCDA shell, resulting in enhanced nanodroplet stability. The vial was stored at 4°C for three days before use.

2.2.2 Nanodroplet size and concentration. The size distribution of the nanodroplets was measured by Dynamic Light Scattering (DLS), as described in (Toumia et al 2019). The mean diameter was found to be 500 nm (polydispersity index xxx). The concentration of nanodroplets was evaluated using \textsuperscript{19}F NMR spectroscopy (400 MHz Avance II, Bruker Biospin GmbH, Rheinstetten, Germany) referenced against 5 mM fluorocytosine.

2.3 Phantom synthesis

Gelatin matrices were chosen to fix the droplets as they are easy to prepare, have a low gelling temperature and tissue-mimicking ultrasonic properties (Culjat et al 2010). The gelatin powder (6% vol., ITW Reagents) was added to deionized water at room temperature to prevent flocculation and then boiled to ensure complete dissolution and removal of entrapped air bubbles. Afterwards, the mixture was poured in rectangular phantom containers (inner dimensions L = 54 mm, W = 26 mm, D = 31 mm, 43.5 ml in volume, figure 1). Different volumes of nanodroplets were added (see table 1) using an 18G needle when the gelatin solution reached 32°C to prevent spontaneous vaporization due to large injection pressure and temperature fluctuations. After manual sample homogenization, phantoms were placed immediately in an ice box to solidify before nanodroplet sedimentation occurred. Due to the observed limited stability of the nanodroplets in gelatin over time, the phantoms were always made within three hours before irradiation.

2.4 Irradiation protocol

2.4.1 Irradiation setup. Proton irradiations were carried out at the Centre de Ressources du Cyclotron (Université
catholique de Louvain, Louvain-la-Neuve, Belgium), an experimental research facility. The cyclotron (Cyclone 110) produced a monoenergetic, passively scattered proton beam at 62 MeV. The proton range was modulated in discrete steps by inserting different thicknesses of degrader material in front of the irradiated sample. A brass aperture of 40 mm diameter was positioned in front of the phantoms to limit the field size. The phantoms were fixed in a water tank heated to 25°C, as illustrated in figure 1(a-b). Each phantom was irradiated twice, first in the configuration of figure 1(a), and then the phantom was flipped by 180° to irradiate the other side (figure 1(b)). Since the proton range was shorter than half of the phantom length, we assumed each irradiation independent of the other. In both configurations, the protons travelled a certain depth before penetrating the gelatin phantoms. Due to the asymmetric design of the phantom containers, the path travelled by the proton beam before penetrating the gelatin differed for the forward and reverse positions. This difference, as well as the presence of PVC material in the beam path (water tank entrance window and PVC phantom container walls), was accounted for when estimating the Bragg Peak position in the phantom. The impact of a sub-millimeter thin acoustic window sheet on one side of the phantom container (figure 1) was assumed to be negligible.

2.4.2 Absolute range measurement. Two beam energies were employed during the experiments (62 MeV and 46.8 MeV). For each energy, we performed an absolute measurement of the proton range with a bespoke setup consisting of a water tank equipped with a thin 23 µm polyethylene terephthalate entrance window. An automated 1D linear stage was employed to move a dosimetry diode (1.33 mm WET, model PR60020, PTW, Freiburg, Germany) along the depth of the proton beam with a step size of 1 mm. The measured depth-dose curve was used to determine the range (defined here as the distal 80% dose point, R50, as recommended in REF) and the skin-to-peak dose ratio for the dose calculation. To account for the PVC layers of the water tank and phantom container, we measured the beam profile with and without a 5-mm thick PVC plate in front of the water tank. Relying on these range measurements and the phantom geometry, we calculated the R50 values for both forward and reverse positions (figure 1(c)).

2.4.3 Irradiation conditions. The irradiation conditions of each phantom are listed in table 1. Three phantoms with 25 µM PCDA droplets were irradiated with 62 MeV protons on both sides, 10 Gy in forward position and 20 Gy in reverse. A gelatin phantom without nanodroplets was also irradiated with the same parameters to verify that the gelatin matrix itself does not exhibit any dose responsiveness detectable via ultrasound imaging. Three phantoms with the same nanodroplet concentration were not irradiated and acted as controls. These phantoms were made simultaneously with their irradiated counterparts, and immersed in a separate water tank at 25°C for ten minutes to mimic the exposure conditions of the irradiated phantoms. Additionally, three phantoms received a dose of 10 Gy at a different energy (46.8 MeV) in forward position, and a clinically-relevant dose of 2 Gy at 62 MeV in reverse. We doubled the nanodroplet concentration for these phantoms. The reported doses and dose rates are evaluated at the Bragg Peak, and dose calculations were performed assuming a peak-to-skin dose ratio equal to five, in agreement with the measured beam profiles. The proton entrance flux was measured by a calibrated ionization chamber present in the beam path.

### Table 1: Irradiation conditions

<table>
<thead>
<tr>
<th>Phantom composition</th>
<th>Number of phantoms</th>
<th>Forward phantom position</th>
<th>Reverse phantom position</th>
<th>Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% Gelatin No NDs</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>6% Gelatin 25 µM NDs</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>6% Gelatin 25 µM NDs</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6% Gelatin 50 µM NDs</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>46.8</td>
</tr>
</tbody>
</table>

2.5 Ultrasound imaging

Each phantom was immersed in water at room temperature and imaged with an experimental ultrasound scanner (DiPhAS, Fraunhofer IBMT, Germany) driving a 7.5 MHz linear array (L7-Xtech, Vernon, France). The ultrasound probe was mounted on a 1D linear stage and moved manually to scan the phantom parallel to the proton beam direction, yielding up to eleven images per phantom. Because the probe’s lateral field of view (38.5 mm) was smaller than the sensitive zone (54 mm), we acquired three different views of each phantom (figure 1(d)) aligned with either end or the center of the sensitive matrix. Low pressure, plane wave imaging was employed, which was verified not to cause acoustic droplet vaporization.
All phantoms except the last three phantoms in table 1 were imaged twice, before and after forward and reverse irradiation (or immersion in 25°C tank for controls), with identical imaging parameters.

2.6 Image processing

Ultrasound images of the phantoms were divided in three zones: the region irradiated in the forward configuration, the one irradiated in the reverse configuration, and the space in between, distal to both forward and reverse irradiations (figure 1(c)). For each zone, a rectangular isometric region of interest (ROI) was defined of size 7 mm parallel to the proton beam and 17 mm parallel to the phantom depth. An in-house developed bubble-counting algorithm, based on peak detection and thresholding on the pixel grey value, was employed to estimate the number of microbubbles in each ROI. Thresholding was made depth-dependent to compensate for the decrease of the mean grey value due to attenuation of the ultrasound wave (especially in regions with high bubble density).

Lateral bubble intensity profiles along the proton beam path were derived from the ultrasound images as follows. Each microbubble detected by the bubble-counting algorithm was assigned to a single pixel (brightest spot). Then, all bubbles in a vertical bin with a size of seven pixels were counted and the resulting count was assigned to the central pixel position. Consecutively, the vertical bin was moved pixelwise along the lateral axis (parallel to the proton beam direction) and the resulting bubble counts at each position were averaged over the 11 frames per phantom. To translate the pixel position into an actual position in the phantom, the middle of the non-vaporized zone in the phantoms (distal to Bragg peaks in both forward and reverse direction, see figure 1(c)), defined as the center point between the two 50% intensity drops, was aligned with the position equidistant from the forward and reverse range (R_{50}) values (figure 1(c)). Finally, to provide full coverage of the lateral view, intensity profiles derived from the three different probe alignments were combined via polynomial weighting. The proton range in the phantom was then compared with the end of the vaporization zone, defined as the position at which the bubble count drops by 50%.

2.7 Statistical analyses

Statistical data were calculated as mean ± standard deviation. Differences in bubble count between the three ROIs in phantoms of the same condition (irradiated or control) were examined using two-tailed Student’s t-tests. Increases in bubble density between images acquired before and after irradiation were assessed for control and irradiated samples using one-tailed paired Student’s t-tests on corresponding ROIs (alternate hypothesis μ_{post} > μ_{pre}). Finally, the difference in bubble count increase between irradiated and control groups was evaluated with a one-tailed Student’s t-test. All tests were performed in Matlab (The Mathworks, Natick, MA, USA) with a significance level (α) of 0.05.

3. Results

3.1 Analytical evaluation of the experimental conditions

In this study, the radiation response of nanodroplets with a decafluorobutane core at 25°C was evaluated. The necessary physical quantities to estimate the required vaporization energies are listed in table 2. Applying these values in equations 1-5 results in a nucleation energy W_{tot} of 66 keV and a critical radius R_c of 89 nm. In order to compute the LET threshold from the semi-empirical nucleation theory, we set the nucleation parameter equal to two, in agreement with the experimental findings of d’Errico (D’Errico 1999) for moderate (<< 1 MeV) values of the critical nucleation energy W_{tot}. This led to a calculated track-averaged LET threshold of 370 keV/μm. At 25°C, the reduced superheat value of the nanodroplets dispersion was 0.23.

| Table 1. Physical quantities of C_{10}F_{10} at 25°C |
|---------------------------------|----------------|-------------------|
| Quantity                        | Symbol | Value [unit]       |
| Surface tension                 | σ      | 7.19*10^5 [N/m]   |
| Saturation pressure             | p_s    | 2.68*10^5 [Pa]    |
| Latent vaporization heat        | ΔH     | 8.75*10^5 [J/kg]  |
| Liquid pressure                 | p_l    | 1.01*10^5 [Pa]    |
| Heat conductivity               | k      | 4.27*10^5 [W/(m*K)]|
| Gas density                     | p_g    | 2.89*10^5 [kg/m³] |
| Liquid density                  | ρ_l    | 1.50 [kg/m³]      |
| Specific heat capacity          | σ_h    | 1.08*10^5 [J/(kg*K)]|

3.2 Radiation response of the nanodroplet formulation

Examples of ultrasound images acquired along the lateral axis of the phantom container (parallel to the proton beam) and aligned to the center of the phantom are displayed in Error! Source du renvoi introuvable. (a,d). Before irradiation, the two phantoms are similar, with only a few visible microbubbles (figure 2(a,b)). This background signal is caused by spontaneous vaporization of a small fraction of the superheated droplets into microbubbles, either already in the nanodroplet vial or during addition to the gelatin solution at 32°C. The microbubbles appear bright due to the large acoustic impedance mismatch between the surrounding water-equivalent matrix and the microbubble gaseous core. On the contrary, the liquid core of the nanodroplets is invisible on the ultrasound images. As microbubbles are too small to be resolved by ultrasound imaging, their shape on the image is dictated by the Point Spread Function (PSF) of the ultrasound system. The irradiated phantoms (Error! Source du renvoi introuvable. (c)) exhibit spatially confined zones of higher bubble density inside the primary proton beam path and lower
bubble density beyond the Bragg peak (middle zone). Bubble density is higher for the 20 Gy region compared to 10 Gy. After immersion at 25°C, the control phantoms (figure 2(d)) displayed an increased, homogeneous bubble density similar to the one observed in the middle zone of the irradiated phantoms. No bubbles were detected before or after irradiation in the control phantom without nanodroplets (data not shown), confirming that the radiation-responsive behavior can be attributed to the presence of the superheated nanodroplets.

Bubble signals were counted as described in section 2.6 and displayed in figure 3. Afterwards, phantoms were grouped per condition, irradiated (n=3, pre and post) and control (n=3, pre and post), and potential differences between the three ROIs (spatial differences in bubble density) were assessed with Student’s t-tests for each condition. Only the irradiated group, post-irradiation, exhibited a statistically significant difference in bubble density between the three zones (p<0.05). This confirmed the homogeneous dispersion of the droplets in the phantom, as the bubble density in non-irradiated samples was spatially uniform. Then, we evaluated the increase in bubble density between pre and post images for irradiated and control groups with a one-tailed paired t-test. For both irradiated and control groups, we observed a significant increase (p<0.05) in

![Figure 2: Top: Ultrasound images of a gelatin phantom with dispersed nanodroplets before (a) and after (c) exposure to 62 MeV protons (10 Gy dose delivered in the forward position, 20 Gy in the reverse position). (e) Bubble number profile across the phantom, averaged over eleven imaging frames. Bottom: Corresponding images, before (b) and after (d) immersion at 25°C and profiles (f), for a control phantom with dispersed nanodroplets, displaying only spontaneous vaporization over time (no irradiation Figure (f) misleading. What you measure in (f) is the bubbles after irradiation but without irradiating the phantom right? I mean, pre irradiation and post irradiation mean here only a certain point in time. This would gain being clarified directly on the graph).](image1)

![Figure 3: Delineation of regions of interest in the two irradiated regions and in the zone distal to both Bragg peaks, on a post-irradiation ultrasound image. The round markers indicate bubble counts. The vaporization profiles across the image lateral axis are computed over the red region of interest.](image2)
bubble density, indicating that all phantoms exhibit a certain degree of spontaneous vaporization over time. Finally, we investigated whether the bubble density increase was more pronounced in the irradiated group. The mean and standard deviation of the difference in bubble count between images acquired before and after irradiation are displayed in figure 4 for each ROI of the control and irradiated groups. Statistically significant (p<0.01) differences in bubble density were observed between irradiated and control phantoms for the left and right ROIs, corresponding to the 10 Gy and 20 Gy zones respectively. No significant difference between irradiated and control groups were found for the zone distal to the Bragg peaks, confirming that the observed response is due to proton irradiation.

3.3 Proton range verification

Lateral bubble intensity profiles (figure 2(e-f)) were derived from the ultrasound images (figure 2(a-d) as explained in section 2.6. Figure 5 shows the resulting intensity profiles for phantoms irradiated with 62 MeV and 46.8 MeV protons, as well as the corresponding absolute range measurements. For both beam energies, the bubble density profiles did not follow the characteristic Bragg profile, but instead appeared as switch-like functions, with higher bubble densities for 20 Gy irradiations compared to 10 Gy (figure 2(e-f)). Additionally, for each phantom, the bubble density abruptly dropped a few millimeters proximal to the dose maximum position.

Signal shifts, calculated as the difference between the R_{50} value obtained from the absolute range measurements and the position corresponding to a 50% drop in bubble density (circle in figure 5), are listed in table 3. One of the phantoms irradiated with 46.8 MeV was discarded, due to bad image quality disabling profile extraction. On average, a signal shift of 2.60 ± 0.26 mm was obtained for irradiation with 62 MeV protons and a shift of 3 mm was observed for 46.8 MeV protons. Due to the limited resolution of ultrasound images (pixel size is 0.25 mm in the lateral direction, bubble landmarks can span several pixels) and the low number of phantoms, we cannot establish whether there is a difference between the range shifts observed with 62 MeV and 46.8 MeV protons. However, we confirm that the radiation response is related to the proton range and conclude that the distance between the end of the dense bubble region and the proton range can be measured with submillimeter repeatability.

Table 3: Signal shifts measured for five irradiated phantoms

<table>
<thead>
<tr>
<th>Phantom</th>
<th>Beam Energy</th>
<th>Signal shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% Gelatin</td>
<td>62 MeV</td>
<td>2.7 mm</td>
</tr>
<tr>
<td>25 µM NDs</td>
<td>62 MeV</td>
<td>2.8 mm</td>
</tr>
<tr>
<td>6% Gelatin</td>
<td>46.8 MeV</td>
<td>3 mm</td>
</tr>
<tr>
<td>50 µM NDs</td>
<td>46.8 MeV</td>
<td>3 mm</td>
</tr>
</tbody>
</table>

3.4 Nanodroplet sensitivity to proton dose and fluence

After achieving a statistically significant radiation response for large proton doses (10 and 20 Gy), we assessed whether the same results could also be obtained for clinically-relevant doses. Since less vaporization events were expected, the droplet concentration in the phantoms was doubled. An ultrasound image acquired after delivery of a 2 Gy dose in the Bragg peak with 62 MeV protons is displayed in figure 6. Again, a distinct zone of high bubble density was observed proximal to the Bragg Peak, confirming the capability of the superheated nanodroplets to detect clinically-relevant doses.
The relationship between the different irradiated doses and the resulting bubble counts is depicted in figure 7. As before, isometric ROIs were defined in zones irradiated with 2, 10 and 20 Gy as well as the corresponding zones distal to the Bragg peak. The latter served as internal reference and the resulting bubble counts were subtracted from the values obtained from the irradiated zones to account for spontaneous droplet vaporization. The 0 Gy data points were analogously obtained from the control phantoms. A linear regression line was fitted through the 0, 10 and 20 Gy data points, which were all acquired for the same droplet concentration. Bubble counts for the 2 Gy irradiation were rescaled by a factor of 0.5 to account for the doubled droplet concentration. However, since we would ignore potential concentration dependent effects, these data points were not used for curve fitting. Nevertheless, the 2 Gy data points were located well within the calculated confidence interval of the fit.

It must be noted that we used ROIs encompassing substantial parts of the irradiated zone, within which the proton dose was not uniform. While this is justified since bubble density profiles were approximately flat over these regions, this also infers that no real dose sensitivity was observed, as a detector sensitive to the proton dose would have a response following the Bragg profile. Hence, the trend displayed in figure 7 should be interpreted as a linear relationship to the proton fluences corresponding with the different radiation doses, since the former are indeed nearly constant over the ROI range.

4. Discussion

In this study, we evaluated the radiation sensitivity of nanometer-sized superheated droplets in proton beams to assess their potential as proton dosimetry and range verification tools. The theoretical threshold LET value for droplet vaporization was determined to be 370 keV/µm. Hence, neither sensitivity to the primary proton beam (exhibiting a maximum LET of 70-90 keV/µm at the distal end of the Bragg peak) nor to secondary alpha particles (LET ranging from 130 to 190 keV/µm) (Grassberger and Paganetti 2011) was expected. This was confirmed by the nearly flat bubble intensity profiles observed, with no enhancement in the Bragg peak location, which would have been observed for both protons and alpha particles. Instead, we hypothesize that bubble vaporization was caused by nuclear recoils, whose LET can range from several hundreds to a thousand keV/µm (Grassberger and Paganetti 2011), created from interactions with either the primary proton beam or secondary neutrons. However, the contribution of the latter was assumed negligible as we did not observe a significant increase of bubbles in the region distal to the Bragg peak.
To confirm that bubble vaporization was induced by recoil ions, we extracted nuclear reaction cross sections of C, N, O and F (atoms present in the gelatin matrix and nanodroplets) from the TENDL-2014 database, which relies on the advanced nuclear reaction simulation software TALYS (Koning et al 2014), and displayed them together with a bubble intensity profile in figure 8(a). The average proton energy at each depth was determined based on the PSTAR (Berger et al 2005) residual CSDA range in water and used to evaluate the reaction cross section at these positions. For each atom, the reaction cross section drops – similarly to the bubble profile – proximal to the Bragg peak. This is due to the Coulomb barrier of the nucleus, which has to be overcome for a non-elastic nuclear reaction to take place (Newhauser and Zhang 2015). For oxygen, this threshold energy is 8 MeV, which corresponds to a residual range in water of 0.83 mm. In section 3.3, the distance between the 50% drop in bubble density and the proton range measured in water was estimated to be 2.60 mm. We measured a difference of 1.8% in density between pure water and a 6% gelatin matrix, leading to a CSDA range decrease of 0.59 mm (Berger et al 2005) that is accounted for in figure 8. However, a small discrepancy between the steep drop in oxygen recoil production and the experimentally determined vaporization profile remains. This indicates that the presence of recoil nuclei is insufficient for droplet vaporization. Indeed, superheated drop detectors are LET-dependent, and the maximal amount of energy transferred to recoil nuclei decreases with the energy of incident protons (Seltzer 1993). Therefore, we also evaluated the track-averaged LET of oxygen recoils produced along the phantom depth. The average energy transferred to heavy recoils (A>4) from proton-oxygen nuclear interactions was extracted from the ICRU report n°63 (ICRU 2000). For these energies, the range of an oxygen ion in water with a density of 1.018 g/cm³ was determined with SRIM (Ziegler 2013) and used to calculate the track-averaged LET. The result is depicted in figure 8(b), together with the theoretical threshold of 370 keV/µm, and a bubble intensity profile. The depth at which the track-averaged LET of oxygen recoils drops below the LET threshold coincides with the start of the drop in bubble intensity. The measurement uncertainties are displayed on figure 8(a) and (b) as shaded areas. Uncertainties on the absolute range position arise from the large step size (1 mm) employed to measure the proton dose deposition profile, and propagate on the estimated position of the non-elastic cross sections and track-averaged LET of recoil ions, which depend on the residual proton energy. The 95% confidence interval on the position of the drop in bubble density is represented as a grey area. Furthermore, longitudinal range straggling of 62 MeV protons in water was simulated using TRIM (Ziegler 2013) and determined to be 508 µm. As the initial energy dispersion of the proton beam is unknown, we did not account for range straggling in figure 8. Despite the aforementioned uncertainties arising from the limited resolution of the experimental measurements, results shown suggest that the features of the observed bubble density profiles can be related to the non-elastic reaction cross-section and energy deposition characteristics of oxygen recoils. This supports the hypothesis that the radiation-induced nucleation theory is applicable to droplets of nanometer sizes.

The transition from droplets of several microns, commonly used in superheated drop detectors, to nanometer-sized droplets has two important implications. Firstly, the assumption that the recoil ions responsible for droplet vaporization are formed within the superheated liquid (D’Errico 2001) no longer holds, as the nanodroplet diameters are several times

![Figure 8: (a) Overlay of the droplet vaporization profile in the phantom with the non-elastic nuclear interaction cross-sections for relevant atoms and the proton depth-dose profile. (b) Overlay of the droplet vaporization profile in the phantom with the average track-averaged LET of oxygen recoils and the proton depth-dose profile. The shaded area surrounding the Bragg Peak represents measurement uncertainties on the proton range, which propagate to uncertainties on the residual proton energy in the phantom (in red) and on the non-elastic cross-section and track-averaged LET estimates (in blue). Uncertainties on the position of the 50% drop in bubble density are represented by the grey area.](image-url)
smaller than the mean range of recoil ions. Hence, both recoils produced inside the droplets and in the surrounding gelatin matrix can induce droplet vaporization. For this reason, we considered oxygen as the dominant recoil ion, given its relative abundance in the phantom matrix. Secondly, while negligible in micro-emulsions, the contribution of the Laplace pressure to the pressure inside the droplet (pL) becomes important for small droplet radii as described in equation 9.

\[ \Delta P_{\text{Lap}} = \frac{2\gamma}{R} \]  

Here, \( \gamma \) denotes the surface tension at the droplet interface and therefore depends on the polymerized PCDA layer. A positive Laplace pressure will decrease the degree of superheat of the droplets, and hence raise the LET threshold for droplet vaporization. Since we did not experimentally measure the surface tension, nor could adapt values described in the literature, we did not account for the Laplace pressure. Theoretical considerations and experimental observations support the assumption of a negligible surface tension. Indeed, the nanodroplets employed in this study have an outstanding in-vial stability (Toumia et al 2019), while models predict a fast dissolution of nanodroplets with a positive surface tension (Mountford and Borden 2016). Additionally, it has been described that perfluorocarbons can decrease the overall surface tension of droplets (Unger and Matsunaga 2010). Moreover, due to the large polydispersity of the droplets used, it is reasonable to assume that part of the population (i.e., the large droplets) will be relatively unaffected by the Laplace pressure, while only the smallest droplets might experience a decreased superheat.

Next to proton range verification, we also evaluated whether the bubble density profiles were correlated with the radiation dose. No direct sensitivity to the primary proton beam was established due to the limited degree of superheat of the nanodroplets. Nevertheless, a linear relationship between bubble counts and irradiation dose was obtained for high proton doses of 10 and 20 Gy. We explain this relationship by the fact that the amount of nuclear reactions and thus recoil ion production is dependent on the proton fluence. As a rule of thumb, primary proton fluence decreases by 1% for every centimeter of tissue traversed (Durante and Paganetti 2016) due to nuclear reactions. Hence, for the limited size of the ROIs (7 mm) along the proton beam, the fluence can be assumed constant. To double the dose from 10 to 20 Gy, also the fluence at a certain location was increased twofold, which is captured by the nearly perfect linear relationship in figure 7. This linear response tends to hold for clinically-relevant doses (2 Gy), although future work will be required to assess the validity of this relationship at smaller fluences.

Consequently, the experimental findings presented in this contribution show that superheated nanodroplets can provide indirect information on the proton range and fluence, by generating ultrasound contrast upon interaction with high-LET nuclear recoils. The latter provides one of the major advantages of the presented approach over current state-of-the-art range verification tools such as PGI and PET imaging, which are also based on nuclear reactions. Since this technique is not relying on specific reaction channels, like prompt gamma emitting channels or channels in which positron emitting isotopes are generated, it has the potential to detect a larger amount of nuclear events. Moreover, the indirect detection of nuclear recoils via individually detectable microbubbles, provides a strong, inherent signal enhancement.

To accurately relate the observed signals to the primary proton beam, Monte Carlo (MC) simulations are required. Apart from providing a means for in vivo range verification, these could potentially be employed for in vivo proton dosimetry by taking advantage of the fluence dependency. To assess this hypothesis and better understand the LET vaporization thresholds, we are currently implementing comprehensive MC simulations describing individual nanodroplets as sensitive detectors. However, MC simulations of nuclear interactions suffer from uncertainties arising from the limited amount of experimental data available to describe interaction cross-sections for biologically-relevant targets (Paganetti 2012).

Therefore, increasing the degree of superheat to sensitize nanodroplets to the primary proton beam might be beneficial, as the vaporization profiles could be directly related to the proton range and dose distribution. Additionally, nuclear recoils only represent a very small percentage of all interactions, leading to a low fraction of nanodroplets undergoing vaporization. For in vivo applications, a high yield might be recommended to minimize the required droplet concentration and related potential side-effects. However, highly superheated droplets will be more prone to spontaneous vaporization. Hence, the appropriate choice of degree of superheat will depend on the achievable signal-to-noise ratio.

One could argue that the temperature of 25°C chosen for this study is unsuitable as it does not fully reflect the response of the nanodroplets at physiological temperature, due to the high dependence of the radiation response on the degree of superheat. At 37°C, the reduced degree of superheat for CaF2 droplets, \( s = 0.33 \), is theoretically still insufficient to expect a response to the primary proton beam. However, the proton range would have to be inferred from vaporization profiles related to secondary radiation products, comprised not only of heavy recoil ions, but potentially also of light secondaries such as alpha particles and deuterons, as the track-averaged LET threshold would drop to 145 keV/µm. Moreover, the composition of the nanodroplets superheated liquid core can be altered to appropriately tune the degree of superheat to the desired LET thresholds. Indeed, a variety of perfluorocarbons with different boiling points have been employed to formulate nanodroplets (Sheeran et al 2017).
The use of ultrasound imaging to noninvasively evaluate vaporization events has numerous advantages for clinical applications such as its inexpensiveness, small footprint, short examination times and real-time capabilities. In our study, we did not employ an ultrasound pulse sequence tailored for microbubble contrast agent imaging, and the limited image quality and resolution affected the accuracy of the estimation of the absolute position of the drop in bubble density. Additionally, the algorithm developed for bubble detection was not optimal for zones of high bubble density. To verify that the bubble counting algorithm did not introduce any bias in the results, we compared it with results obtained by considering the mean grey value as the relevant metric for vaporization (instead of the number of bubbles) and found no noticeable difference. In order to benefit from the improved ultrasound image resolution in the axial direction, the ultrasound probe should be positioned parallel to the proton beam. The droplet concentration in the phantom should also be varied to maximize contrast between irradiated and non-irradiated regions.

Proton irradiations were performed in an experimental research facility, with a passively-scattered monoenergetic beam whose characteristics differ from clinical proton beams. Particularly, the features of the vaporization profiles, with a steep drop in front of the proton range, might be altered in a spread-out Bragg Peak. Future studies will establish the response of the superheated nanodroplets in clinical proton beams, including scanned beams.

Additionally, we will follow-up the presented results with two different research tracks; firstly, we will refine our investigation on the droplets response to secondary recoil ions with additional experimental observations and extensive in silico analyses. Through comparison of simulation results and experimental findings, the LET threshold estimate given in this work will be verified. Moreover, the influence of a physiological temperature of 37°C on the nanodroplet radiation response will be evaluated. Secondly, the nanodroplet design will be optimized to allow sensitization to the primary proton beam. This would enable direct imaging of the in vivo proton range and would provide a promising candidate for in vivo proton dosimetry.

5. Conclusion

In this contribution, we investigated the potential use of nanodroplets for proton dosimetry and range verification. Downscaling radioresponsive micro-emulsions used in superheated drop detectors to injectable phase change contrast agents produced in vivo radiation sensors detectable with ultrasound imaging. Applying the thermal spike theory to our experimental conditions revealed that the decalfluorobutane liquid core vaporizes when exposed to high-LET secondary particles generated during nuclear reactions of the proton beam. Nanodroplet dispersions exposed to monoenergetic proton beams of 62 MeV and 46.8 MeV, at 25°C, exhibited spatially confined bubble vaporization regions proximal to the Bragg peak, confirming the potential of these nanodroplets as range verification devices. While ultrasound signals dropped before the actual proton range due to LET dependencies, the resulting signal shift was determined with submillimeter precision. Additionally, the bubble density was linearly related to the proton fluence. Lastly, the potential of the developed technique was proven at clinically-relevant doses of 2 Gy. Future work will aim at confirming these early findings and refining the range estimates accuracy. Proton irradiations at 37°C will be carried out to assess the relevance of these proof-of-concept data at physiological temperatures. Finally, nanodroplet design will be optimized to ensure sensitization to the primary proton beam, enabling direct in vivo proton range verification and potential in vivo proton dosimetry.

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