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Chromacity Spark 1040 nm femtosecond laser system used as irradiation source for heating of hybrid gold-iron oxide nanoparticles in biological media

TECHNICAL REPORT

Purpose

Hybrid gold-iron oxide nanoparticles have been shown to hold great potential for heat triggered drug delivery [1,2]. The magnetic core of the particles enables magnetic resonance imaging whilst the gold surface coating can act as a localised heat source after laser irradiation by exploitation of the surface plasmon resonance. Previously, we have reported heating properties in agar [3], inside cells [4] and in tumour bearing xenograft cadavers [4] after irradiation with a 1064 nm laser source. The purpose of this work was to test whether the Chromacity Spark 1040 nm femtosecond laser source was A) useful for laser irradiation of these nanoparticles and B) more effective than our previous laser source.

Methodology

Agar phantoms

Hybrid nanoparticles were suspended in 2 % agar phantoms at 5 $\mu g ml^{-1}$ and 50 $\mu g ml^{-1}$. The gels were irradiated for 60 s using the Chromacity Spark 1040 nm femtosecond system and changes in heat monitored using an Optris PI640 thermal imaging camera (Optris, Germany). The subsequent 60 s following irradiation was also monitored to understand the cooling profile of the particles. Heat dissipation was deduced from the infrared images up to 12 mm from the point of irradiation. All reported thermal change is in respect to a control agar phantom containing no nanoparticles.

In vitro

Human pancreatic adenocarcinoma (BxPC-3) cells were seeded into 6-well plates (50,000 cells/well) containing quartz coverslips (Alfa Aesar, USA) and incubated at 37 ˚C with 5 % CO_2. Hybrid nanoparticles (5 $\mu g ml^{-1}$ & 50 $\mu g ml^{-1}$) were incubated with the cells for 24 h. Cells were washed with phosphate buffered saline (PBS) four times and the coverslips removed from the plate. The cells were irradiated using the Chromacity Spark 1040 nm femtosecond laser source for 60 s or 4 repetitions of 60 s with a 60 s cooling period between. After irradiation the coverslips were returned to the 6 well plate, fresh media added and incubated for 24 h. After this time the cytotoxicity was measured using trypan blue exclusion as previously described. Cytotoxicity was measured in relation to control cells with no nanoparticles.

In vivo

Female Nu/Nu mice, five weeks of age (n=3), (Charles River, UK) were kept in pathogen-free conditions (weight of mice was 20–25 g). Human pancreatic cancer cell line BxPC-3 was cultured to 90 % confluence in RPMI 1640 supplemented with 10 % fetal bovine serum and 1 % penicillin streptomycin. The cells were washed twice with cold PBS and harvested with trypsin for 10 min at 37 ˚C. The cells were washed three times with PBS and resuspended in 50:50 media:PBS. The tumour cell suspension (3.0 × 106 cells in 100 µl of 50:50 PBS:media) was injected subcutaneously (s.c.) in the right flank of each mouse. When the tumour became palpable (approximately after one week), measurements in two dimensions with Vernier callipers were carried out twice a week and the volume of the tumours calculated according to Equation 1.

$$V = \frac{4}{3}\pi\left[\frac{(D1+D2)}{4}\right]^3 \qquad (1)$$

Once tumour volume reached a maximum of 0.9 cm^3 the mice were sacrificed and used for imaging immediately post mortem. Hybrid nanoparticle solution (0.2 $mgkg^{-1}$ or 0.02 $mgkg^{-1}$ 100 µl) was injected intra-tumorally (I.T.) using a 26 gauge needle (Vet-Tech, UK). The tumour was irradiated using the Chromacity Spark 1040 nm femtosecond laser source for 60 s and temperature change & heat dissipation measured using an Optris PI640 thermal imaging camera (Optris, Germany). The ΔT data was calculated in relation to a tumour bearing mouse control containing no nanoparticles.

Results

The laser irradiation studies in agar phantom showed heating increase of up to 48 ˚C for the 50 $µgml^{-1}$ and 27 ˚C for the 5 $µgml^{-1}$ samples (Figure 1A). The maximum heating was achieved in both cases with a plateau in the graph being observed thereafter. The cooling profiles for both concentrations looked similar, whereby, heat was rapidly lost, followed by a slower return to ambient temperature. The heat dissipation studies (Figure 1B) showed that in both cases the heat spread from laser source was approximately 12 mm in each direction.

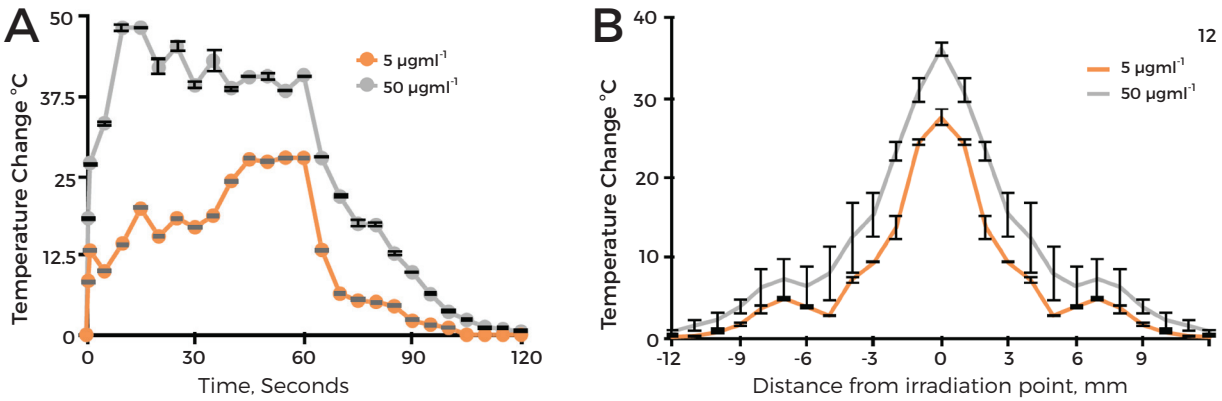

Figure 1. Infrared monitoring of hybrid nanoparticles in 2% agar after 60 s irradiation with the Chromacity Spark 1040 nm femtosecond laser source showing **A)** Thermal change over 120 s and **B)** Heat dissipation from laser source at 60 s irradiation (n=3,±SD).

The cell viability was measured in BxPC-3 cells containing either 50 $µgml^{-1}$ or 5 $µgml^{-1}$ hybrid nanoparticles, 24 h post irradiation (Figure 2). Here it was observed that those cells containing the lower particle concentration, did not experience any adverse effect on their viability, even after the multiple irradiation cycles. However, those cells which contained the higher concentration of particles (50 $µgml^{-1}$) were no longer viable after both 60 s irradiation and multiple irradiations. This is probably due to the temperature increase inside the cells exceeding the threshold temperature (50 ˚C) after which cells can no longer survive.

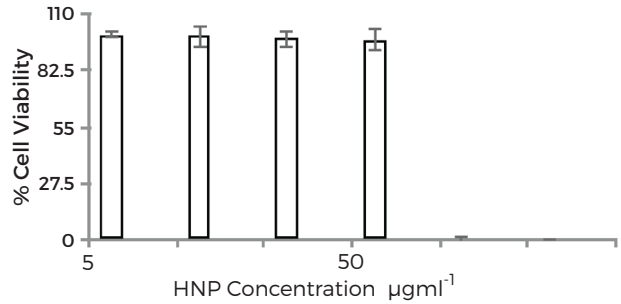

Figure 2. Cell viability of BxPC-3 cells containing 50 $µgml^{-1}$ and 5 $µgml^{-1}$ hybrid nanoparticles 24 h post irradiation (n=3,±SD).

Thermal rise and heat dissipation was measured in a xenograft bearing cadaver. The hybrid particles were injected intra-tumorally at the equivalent concentrations dependant on mouse weight. Here the data showed that a greater increase in heat was observed compared to the agar phantoms with up to 80 ˚C heating increase at 50 $µgml^{-1}$ and a 26 ˚C increase at 5 $µgml^{-1}$ (Figure 3A). The reason for the difference to the agar data is not known. However, pancreatic cancer forms very dense solid tumours, and perhaps this has an impact on localised heating. Figure 3B shows the heat dissipation in the tumour tissue. Here we observed that at 5 mm radius from the irradiation point, the majority of heat had been lost with only small thermal changes beyond this point.

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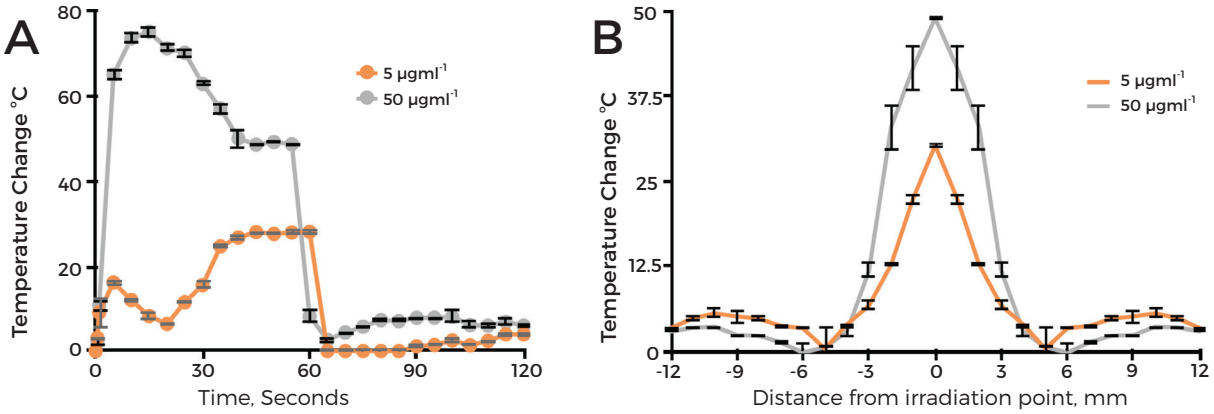

Figure 3. Infrared monitoring of hybrid nanoparticles in tumour bearing nude mouse xenograft cadavers after 60 s irradiation with Chromacity laser source showing **A**) Thermal change over 120 s and **B**) Heat dissipation from laser source at 60 s irradiation (n=3,±SD).

Benefits

Comparing these studies with the previous reports on hybrid nanoparticle heating shows that the Chromacity Spark 1040 nm femtosecond system has major advantages compared to the previously used 1064 nm source. Consistently, we observed that the Chromacity system provides greater thermal rise both in agar and in tumours. Previously, at 5 μgml^{-1} no notable heating was observed compared to control samples. However, in this study in xenografts, those injected with 5 μgml^{-1} experienced 27 °C temperature increases. Additionally, there was an 8-fold increase in temperature at 50 μgml^{-1} compared with the 1064 nm irradiation study. These exciting findings show that for biomedical application more than a 10-fold reduction in nanoparticle concentration could be administered in order to experience similar heating effects. This would not only render formulations more cost effective but also reduce any question over the toxic burden of nano-carrier or long-term accumulation. This system would enable either localised tumour ablation or thermally triggered drug delivery which is highly localised to the laser irradiation point thus minimum impact on surrounding tissue would be experienced.

Reference

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Chromacity

Livingstone House,
Research Avenue North
Riccarton, Edinburgh
EH14 4AP

Phone: +44 (0) 131 449 4308
Email: sales@chromacitylasers.com
Web: www.chromacitylasers.com
Twitter: @chromacityltd