Megavoltage Radiotherapy with Photodynamic Treatment to Enhance Tumour Cell Kill

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PURPOSE / OBJECTIVE(s)

When treating malignant disease with therapeutic radiotherapy, the dose able to be delivered to the tumour can be the limited by the surrounding normal tissue. An innovative approach being investigated is to use nanoparticle aimed at maximising the effect of radiation on the tumour whilst limiting the damage to normal tissue. Ultimately our objective is to enhance the therapeutic window by increasing the tumour control probability whilst reducing normal tissue toxicity.

MATERIAL & METHODS

The nanoparticle (NP) material is prepared using a precipitation approach, whereby all of the structure. The organic solvent is then removed and the constituents are dissolved in an organic solvent. The NPs are then added dropwise to Phosphate Buffered Saline (PBS), resulting in the precipitation of NPs in which the particle size controlled. Using a standard MTS protocol, the media is diluted to achieve the required concentration of 0.25 mg/ml for the nanoparticles used.

Cell Culture

Human prostate cancer cells (DU145) were cultured and maintained in Minimal Essential Media (MEM) with Alpha modifications with 10% foetal bovine serum (FBS) and 1% antibiotics (Penicillin-Streptomycin). The cells initially were cultured and grown to 80% confluence in a 25 cm2 flask and then were sub-cultured. Incubation conditions during the experiments was 37˚C with 5% CO2 in a humidified environment.

Cytotoxicity assay

DU145 cells were seeded in 96 well plates (1,000 cells/well) and incubated for 24 hr. The cells were combined with 0.25 mg/ml NPs + photofrin (Por) . At 24 hr after the treatment of NPs, cells were exposed to a range of doses between 0 and 6 Gy with 6-MV X-ray under the laboratory conditions. After the irradiation, the culture medium was changed and the cells were then incubated. Subsequently, the medium was removed and 100 ml of a new culture medium and 20 ml of MTS reagent were added into each well. After 45 min incubation, the absorbance (optical density) of the solutions were measured using a CLARIOstar microplate reader (BMG LABTECH Inc, Ortenberg, Germany) at a wavelength of 490 nm. The percentage of surviving cell relative to the control group were calculated.

RESULTS

Figure 1 - Cell Toxicity vs Concentration

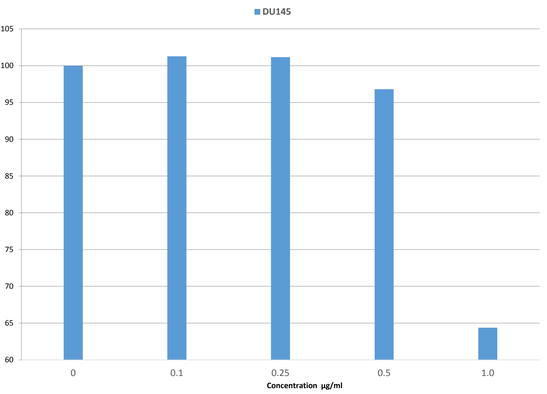


Figure 1 – Toxicity as a function of Nanoparticle concentration.

The developed nanoparticles can be toxic to cells at a given concentration. Figure 1 shows the maximum concentration of nanoparticles that can be used without toxicity to the normal cells. From the preliminary results it has been determined that if the concentration of nanoparticles was kept below 30µg/ml there was no toxicity to the cells.

The decision was to use a concentration of 25µg/ml. for this research.

Figure 2 - % Cell Viability vs Dose

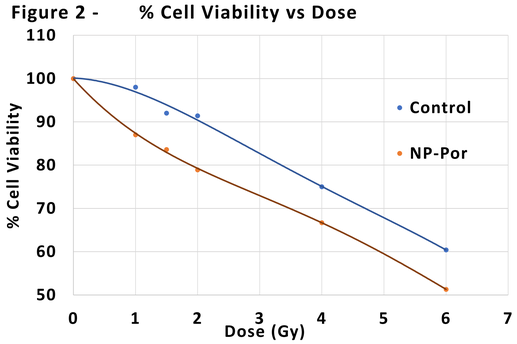
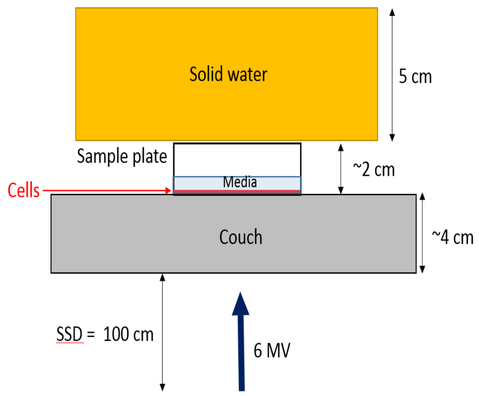


Figure 2 – Percentage Cell Viability vs Dose.

The graph demonstrates that irradiation of NP + Por compared with control results in an approximate 15% increase in tumour cell death. With standard fractionated radiotherapy between 1.8 to 2 Gy, the graph indicates enhanced tumour cell kill which could be clinically relevant.

Figure 3 - Experimental Geometry



Linac: Elekta Synergy , 6MV
Field Size 20cm x 20cm
Gantry Angle to T180°
SSD:104cm Source target Distance
Dose Rate 380MU/min
Dose: 0, 1, 1.5, 2, 4, 6

SUMMARY / CONCLUSION

Previous work on NP has focused on low (KV range) energy where the photoelectric effect dominates. With radiotherapy treatment, high energy (MV) x-rays are dominated by Compton interactions and these previously used NP are of limited use. It has also been shown that metallic nanoparticles can florescence under exposure to low energy ionising radiation.

This research aims at utilise the fluorescent property of the nanoparticles when exposed to MV photons. This generates red light which reacts with a conjugated photodynamic therapy agent such a photophrin. This results in the generation of reactive oxygen species (ROS) which enhances tumour cell killing. This is very preliminary work and to date has been focused on proof of concept. The results of this research demonstrate that the addition NP-Por increases cell kill by approximately 15%.

The ongoing research is to determine the following:

1) the minimal NP concentration to yield optimal dose enhancement.
2) the response of the cells to:-
    1) radiation alone,
    2) NP + radiation,
    3) NP + Por + radiation and
    4) NP + tumour cell marker + Por + radiation
4) the optimal Particle size, geometry, and coating
5) the impact on cell with fractionated treatment.

This preliminary data is encouraging, given the dose enhancement of between 15%-20%. With validation this may result in an increase in tumour cell kill with reduced toxicity to the surrounding normal tissues.

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