Thermally triggered theranostics for pancreatic cancer.

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Thermally Triggered Theranostics for Pancreatic Cancer

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Introduction

Introduction

- Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent epithelial, exocrine pancreatic cancer, which shows for more than 80% of the malignant neoplasms of the pancreas. The extracellular section of PDAC is normally a dense stromal, called desmoplastic, which has been demonstrated to affect drug delivery and reaction to gemcitabine in preclinical models. Thus, it is highly demanded to increase the efficacy of this treatment as well as exploring alternative therapies.

- Iron oxide-gold as hybrid nanoparticles (HNPs) have recently been the focus of a number of investigations and are becoming increasingly applicable in biomedicine (Fig. 1).

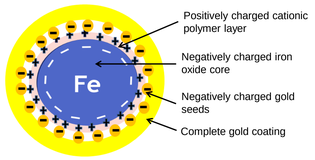
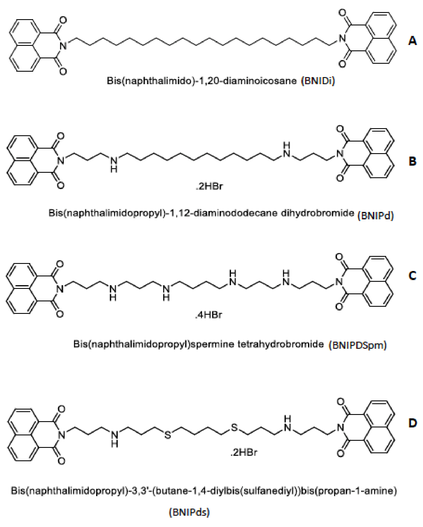


Figure 1. *Schematic diagram of gold-iron oxide core-shell nanoparticle presenting electrostatic charges within each layer.*

- By using both iron oxide and gold within one drug delivery vehicle, a multifaceted and stable system can be fabricated. This takes the advantage of the surface chemistry and SPR of the gold whilst holding the magnetic character of the iron oxide, offering imaging, heating and drug carrier potentials.

- Bisnaphthalimide derivatives have exhibited exciting potential as chemotherapeutic agents, which is due to their interactions with DNA by intercalation (Fig. 2)



	Drug	No. charges
A	BNIDI	0
B	BNIPd	2
C	BNIPDSpm	4
D	BNIPds	2 & SH

Figure 2. *Basic bisnaphthalimide chemical structure.*

- Arginine-glycine-aspartic acid (RGD) peptides have high affinity to integrin $\alpha_v\beta_3$, which plays a significant role in tumour angiogenesis. This peptide is a receptor for the extracellular matrix proteins with the exposed RGD tripeptide sequence and expressed on many different types of cancer as for pancreatic cancer.

Results

- Successful fabrication of the iron oxide core and subsequent coating steps to achieve the HNPs was evident from the TEM (Fig. 3A), UV-Vis (Fig. 3B) and zeta potential measurement (Fig. 3C).

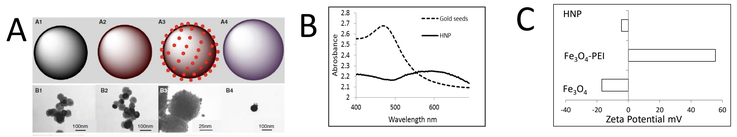


Figure 3*. Physicochemical characteriation of the nanoparticle synthesis steps using A) transmission electron microscopy, B) UV-Vis spectroscopy and C) zeta potential measurement.*

- The HNPs ability to act as localised nanoheaters after laser irradiation (Fig. 4A) and MRI contrast agents (Fig. 4B) was assessed whilst suspended in an agar phantom. The data suggested that the HNPs possessed similar contrast-ability to commercially available Feridex® and were capable of temperature increases over 30 degrees.

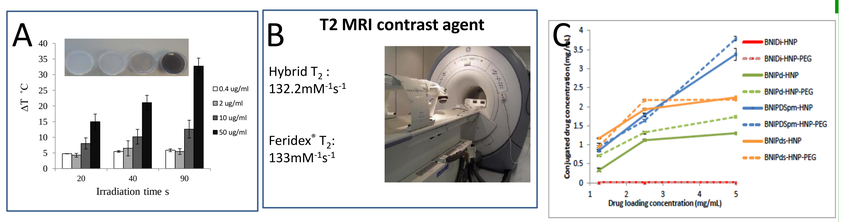


Figure 4. *Physical properties of HNPs including A) ability to act as a nano heater after laser irradiation (1064 nm, Nd: YAG), B) ability to act as a T2 contrast agent and C) ability to electrostatically bind charged drug molecules onto the surface.*

- The ability of drug molecules to electrostatically attach onto the HNP surface as investigated and measured by HPLC (Fig. 4C). The data suggested that increasing the number of amine groups in the polyamine chain of the bisnaphthalimido drugs resulted in greater conjugation and stability (Fig. 5 A1&A2). The presence of a thiol resulted in dative covalent binding onto the gold surface and these molecules were used as a control.

- Drug release profiles were determined over 14 days to evaluate the strength of binding of the bisnaphthimido drugs of varied charges (Fig. 5 B1-3). The data showed that increasing the positive charge in the polyamine backbone due to the presence of primary amine groups resulted in increased binding. The drug with no charge did not bind and was 100% released immediately. With 4 charges in the backbone the drug was steadily released over the experimental period.

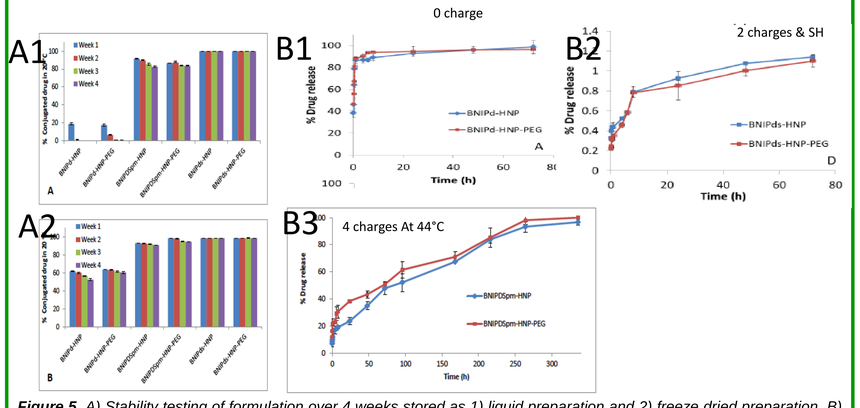


Figure 5. *A) Stability testing of formulation over 4 weeks stored as 1) liquid preparation and 2) freeze dried preparation. B) Drug release profile of birnaphthalimido drugs from HNP surface in cell culture media of 1) 0, 2) 2 and 3) 4 amine charges in the polyamine backbone.*

Results

- The formulations cytoxicity was determined using MTT assay and trypan blue exclusion on human pancreatic adenocarcinoma (BxPC-3) cells (Fig. 6A). The data showed a significant reduction in IC50 after drug conjugation onto HNPs compared with free drug. RGD was added onto the formulation to confer a targeting effect, however, this did not have any significant effect on the IC_{50} values observed (Fig. 6B) Cellular uptake studies also showed that increased drug content was achievable after conjugation (Fig. 6D), after conjugation of the RGD s significant increase in drug was achieved in the PEGylated formulations.

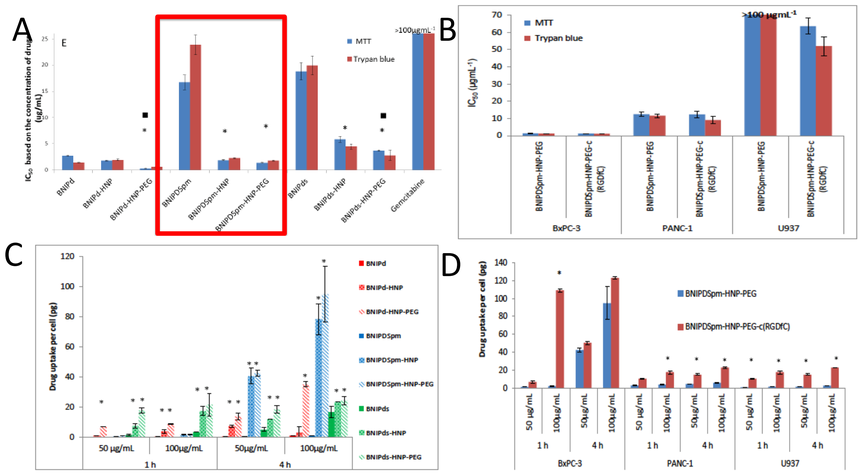


Figure 6. *Cytotoxicity testing of novel formulations on BxPC-3 cells measured by MTT assay and trypan blue exclusion both A) without and B) with attachement of a cyclic RGD targeting peptide. Cellular uptake of drug molecules measured by high performance liquid chromatography both C) without and D) with the presence of a cyclic RGD targeting peptide.*

- Cellular morphology after exposure to the free drugs (4 charges) and HNP formulations (4 charges) was assessed after 4 h using AFM. The images show that after this sort time the cells exposed to the free drug (Fig. 7B) or uncoated HNPs (Fig. 7C) do not exhibit any morphological changes and look healthy, whereas the cells exposed to the novel formulations look to be undegoing cellular breakdown as a result of the increased cytotoxicity (Fig. 7D).

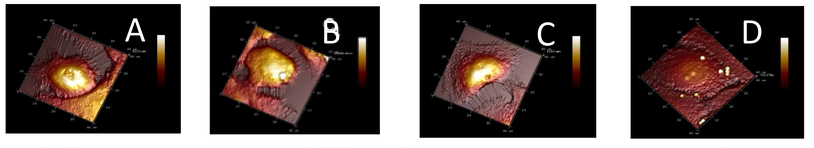


Figure 7*. AFM topographical analysis of fixed BxPC-3 cells after exposure to A) cell culture media (control), B) free drug (4 charges), C) uncoated HNPs and D) HNP-drug (4 charges) for 4h.*

- Laser irradiation of HNPs (50 µgmL^{-1}) *in vitro* over 60 sec showed a temperature rise of 9 degrees (Fig. 8A). 4 h after irradiation the cells were fixed and imaged for topography showing no structural damage (Fig. 8B). This suggests these particles could be exploited in thermally triggered drug release. The threshold energy required drug *in vitro* is being investigated.

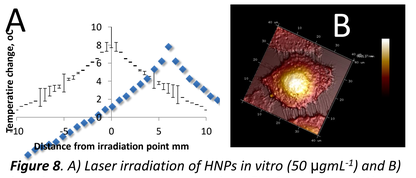


Figure 8*. A) Laser irradiation of HNPs in vitro (50 µgmL^{-1}) and B) imaging of cellular morphology using AFM 4 h post irradiation.*

Conclusion

This work highlights the multifunctional nature of hybrid nanoparticles for use in image guided triggered drug delivery. Further work is on-going to evaluate the in viivo potential of these systems in pancreatic cancer xenograft models.