Diels-Alder mediated release of combination therapies from hybrid nanoparticles

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Abstract

Pancreatic cancer or pancreatic ductal adenocarcinoma (PDAC) is the deadliest type of cancerous malignancy with a 5-year survival rate of only 7%.

The historic first line treatment is a drug called gemcitabine, which displays effectiveness in only 22% of patients over 12 months.[1]

Combination therapies such as GemCap, has been shown to improve patient outcomes, by operating under different mechanisms reducing the likelihood drug resistance.

Hybrid nanoparticles (HNPs) comprised of an iron oxide core and outer gold coat have shown great potential for heat triggered drug release. This work seeks to utilize this knowledge to develop a combination therapy using both gemcitabine and capecitabine.

Introduction

This novel formulation consists of a HNP core surface functionalised with a cytotoxic drug such as gemcitabine and capecitabine via a thermally labile Diels-Alder linker.
The proposed novel formulation initially involves the design and synthesis of a hybrid gold coated iron oxide nanoparticle. [2]

- The linker's function is to anchor the chosen cytotoxic drug to the HNP surface.
- The linker is specifically a Diels-Alder cycloadduct which provides several advantages over a conventional polymer linker for example.
- One advantage us that the Diels-Alder cycloadduct can be formed without side products, spontaneously, without the need for catalysts which are important for biomedical applications.
- The cycloadduct is temperature sensitive and undergoes the reverse reaction when heated, again without the formation of side products, and this temperature induced reversal can occur at 44 ˚C. [3]
- This ties in well when combined with the HNP, in which the heat generating surface plasmon properties can be utilised to facilitate retro Diels-Alder mediated release of attached cytotoxic agents such as gemcitabine and capecitabine.

HNP Method

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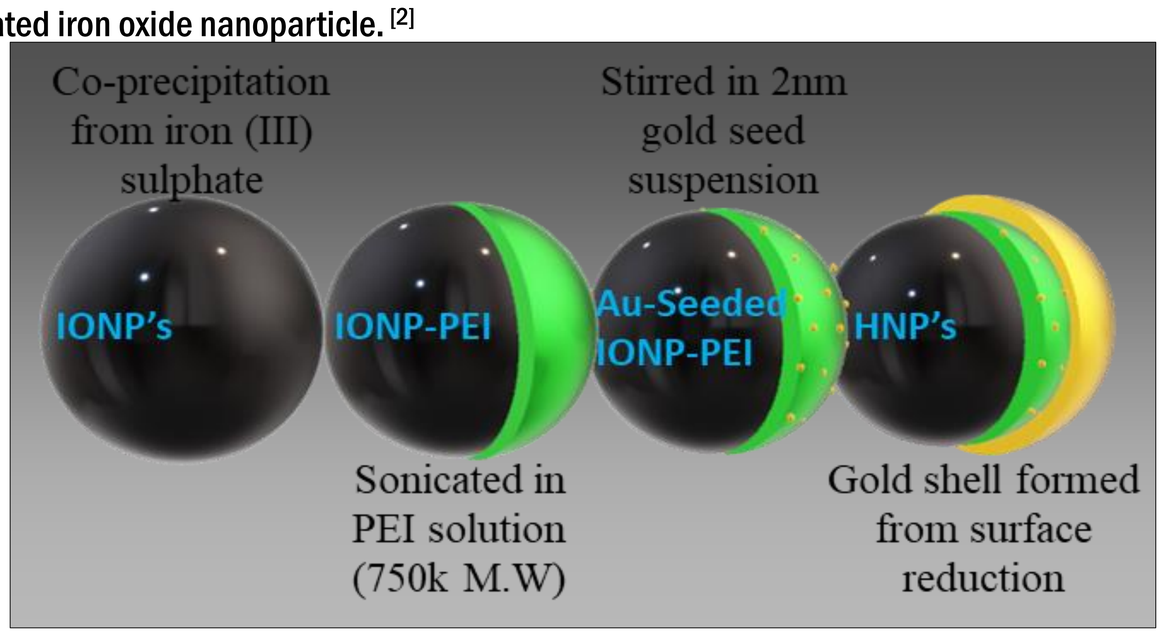
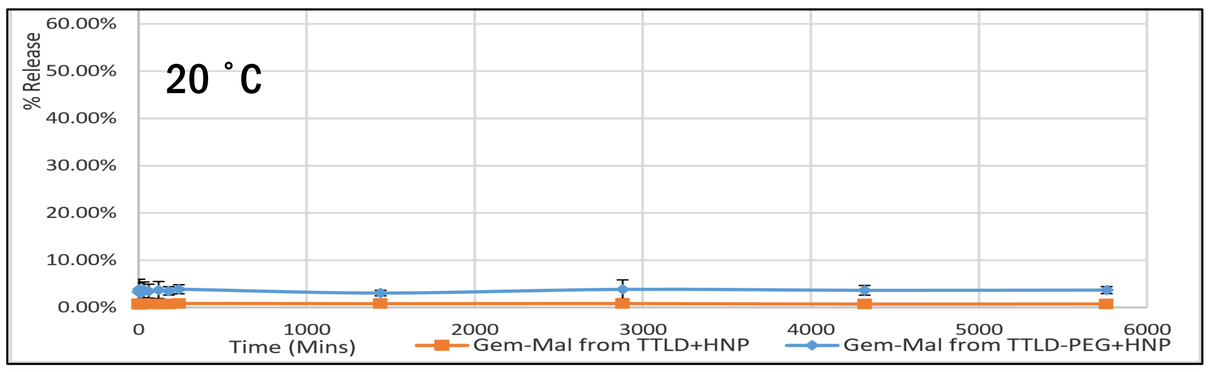
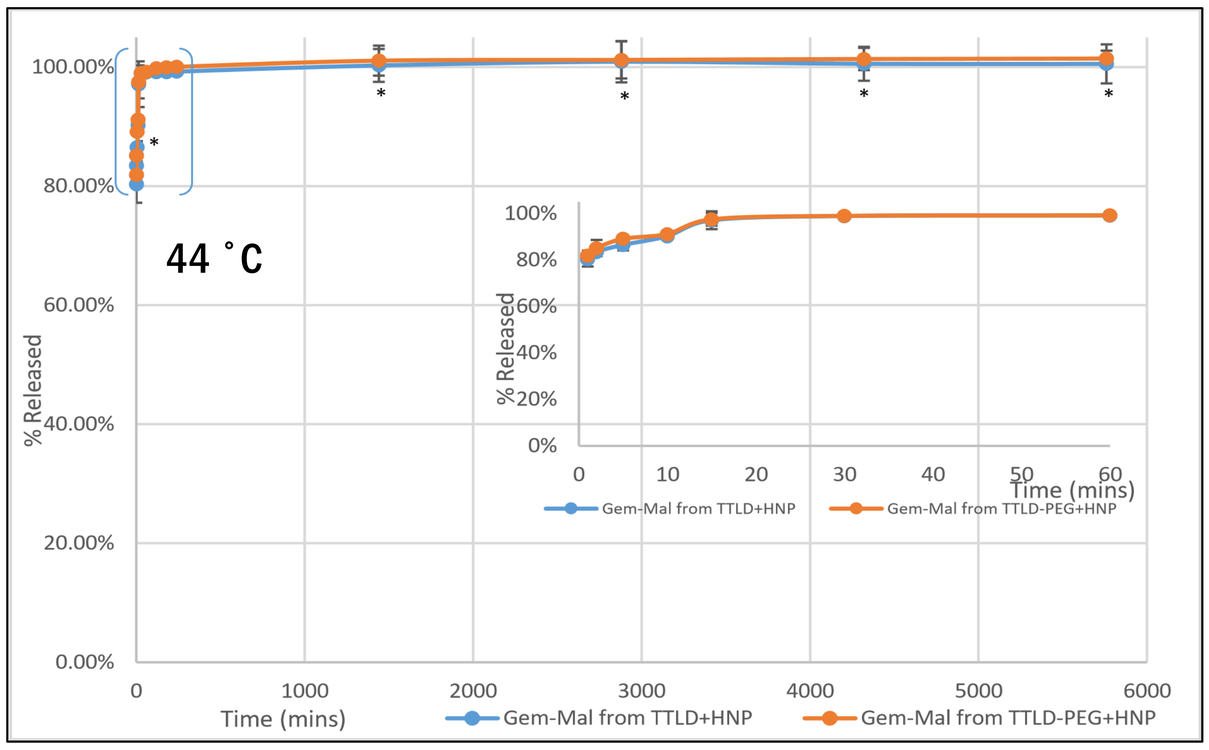


Zeta potential measurements of the IONP's, PEI-IONP's and HNP's were 14.8, 23.7 and 16.2mV respectively, which are indicative of each successful stage of the overall HNP synthesis.

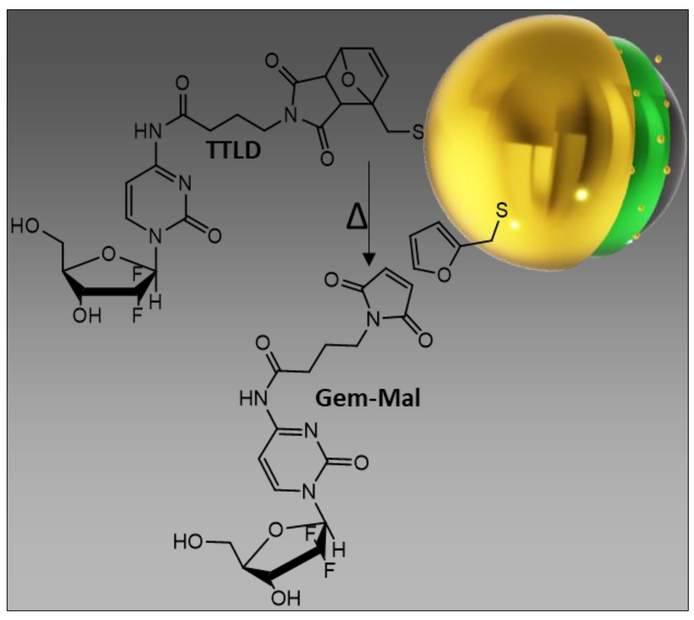
Drug Release Studies

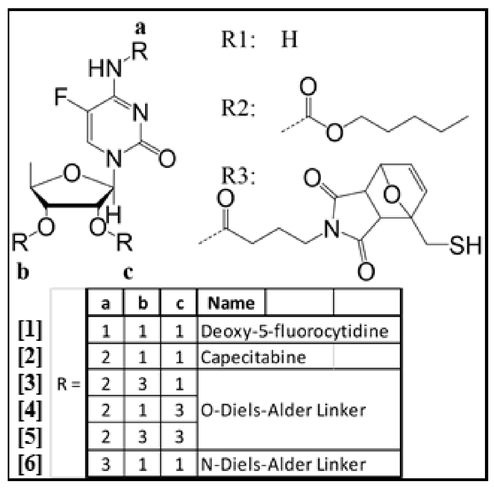




HNP – Linker – Drug Formulation Diagram

The gemcitabine-linker-HNP formulation has already been shown to have improved cytotoxicity compared to gemcitabine alone.[2] Historically Gem-Mal's cytotoxicity was determined by MTT and trypan blue assay to be 4.6 times lower than gemcitabine. The 11-fold enhanced uptake aided by the HNP's, coupled with rDA mediated release at 44 ˚C, led to an 26% increase in cytotoxicity compared to gemcitabine, confirming its temperature driven activity[3]





		a	b	c	Name
[1]	R =	1	1	1	Deoxy-5-fluorocytidine
[2]		2	1	1	Capecitabine
[3]		2	3	1	
[4]		2	1	3	O-Diels-Alder Linker
[5]		2	3	3	
[6]		3	1	1	N-Diels-Alder Linker

Future Work

Following on from preliminary studies with gemcitabine, the current task is to facilitate the attachment of capecitabine to the Diels-Alder cycloadduct at various potential points such as; the pyrimidine amine group, the 3-OH, and/or the 5-OH ribose hydroxyl groups.
All four capecitabine-linkers (Figure 1 (3-6)) will be evaluated for their release rates at elevated temperatures to determine the optimal linker anchor points. The initial use of protecting groups such as tert-butyloxycarbonyl (Boc) may be used to facilitate linker attachment desired locations. The capecitabine-linkers will be attached to HNP's via a gold-thiol bond.[3]
Successful capecitabine-linkers synthesis and drug loading onto HNP's will be confirmed by NMR and HPLC. The dual drug loaded HNP's cytotoxicity will be determined by trypan blue exclusion and MTT assay.