Developing a sprayable drug delivery system using repurposed pectin and high drug-loaded nanoparticles for Glioblastoma Multiforme

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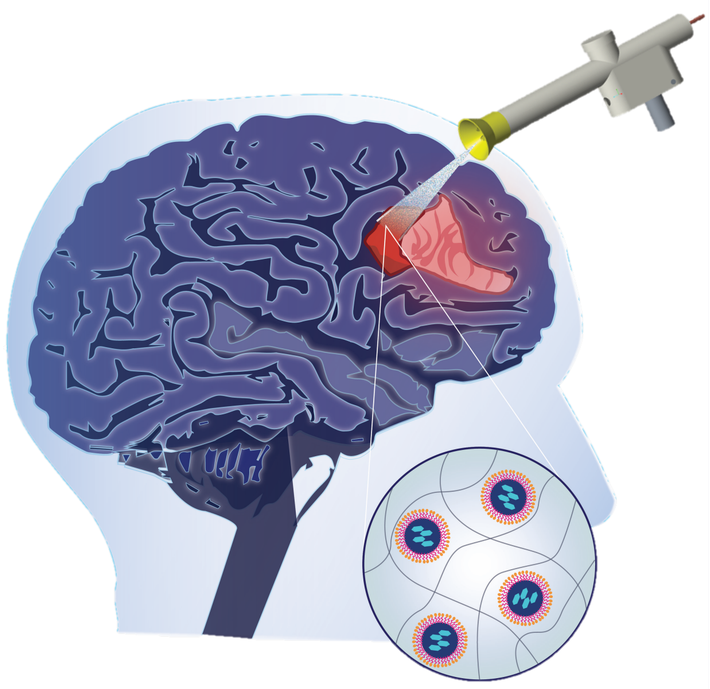
Background & Aims

- Glioblastoma multiforme (GBM) is a WHO grade IV malignant brain tumour with a median of 14 month survival time and a 100% mortality rate.
- Current standard-of-care therapy for GBM is surgery with concurrent chemoradiotherapy 4 weeks later; however reoccurrence is certain, usually within 2 cm of the surgical resection margin.
- Local delivery of a <u>multitude of chemotherapeutics immediately after surgery</u> may enhance residual disease exposure to therapeutic doses and potentially kill any remaining cells within the brain parenchyma.
- *The aim of this work is to develop a novel drug delivery system which sprays etoposide (ETO) and olaparib (OLA) nanoparticles held within a bio-adhesive gel (pectin), accurately onto the surgical resection margin, ensuring brain parenchyma penetration and controlled drug release.*



Bioadhesive gel

In vitro biocompatibility assays show pectin is non-toxic to human astrocytes and human blood

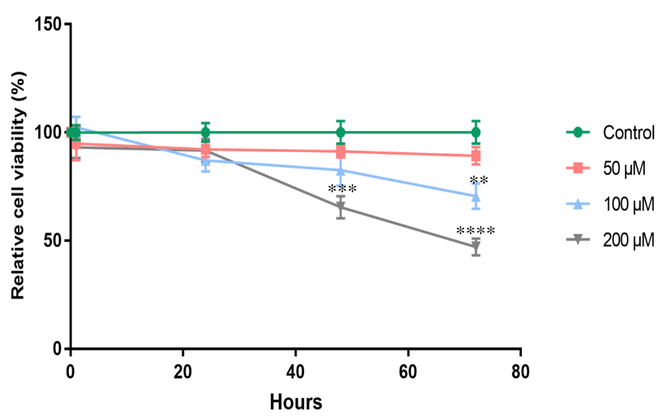


Figure 1: Human primary astrocytes showed no toxicity at 50 µM at all time points, however had significant toxicity when incubated with 100 µM Pectin at 72 hrs and 200 µM at 48+72 hrs, when analysed against the control using a one-way ANOVA with Dunnet's post-hoc test.

Table One: RBC haemolysis after 1 hr incubation with 50 – 200 µM of Pectin: Data shown as arithmetic mean ± SEM, n=6. Significant haemolysis defined as over 5%.

Samples		Optical density ± SEM	Haemolysis (%) ± SEM
PBS (negative)		0.083 ± 0.002	1.6 ± 0.1
Triton x-100 (positive)		2.290 ± 0.206	100.0 ± 9.1
Pectin	50 µM	0.098 ± 0.009	0.4 ± 0.4
	125 µM	0.115 ± 0.012	0.9 ± 0.4
	200 µM	0.123 ± 0.017	0.6 ± 0.4

In vivo studies show no adverse effects from Pectin

200 µM Cy5 labelled Pectin was orthotopically injected into mouse brains and left for up to two weeks, showing no neurological deficit.

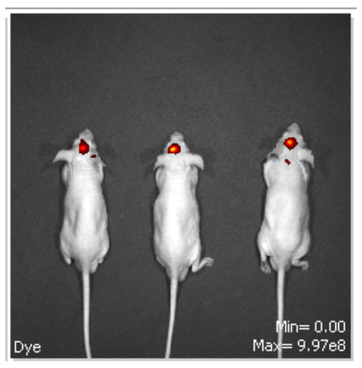


Figure 2. IVIS images confirmed pectin location

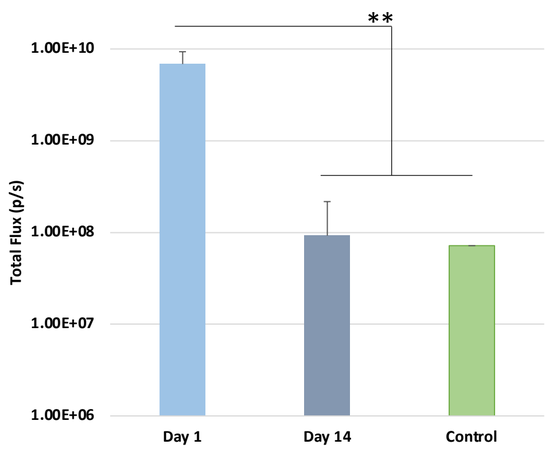
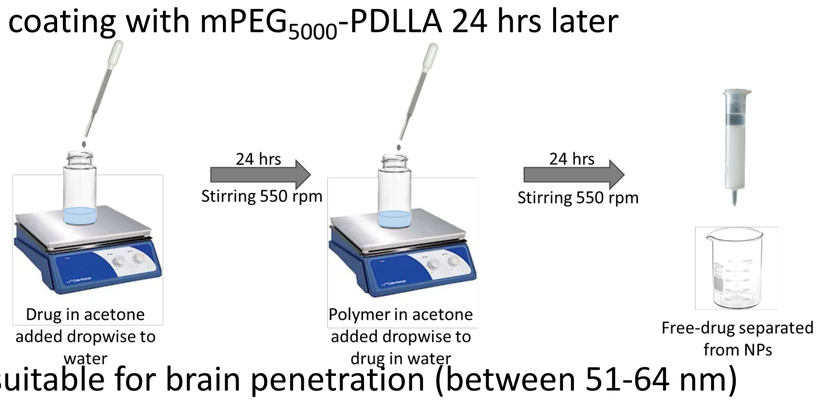


Figure 3. Loss of Cy5 signal in the brain after 2 weeks *in vivo* (one-way ANOVA with Dunnet's post-hoc test).

Polymer coated nanoparticles

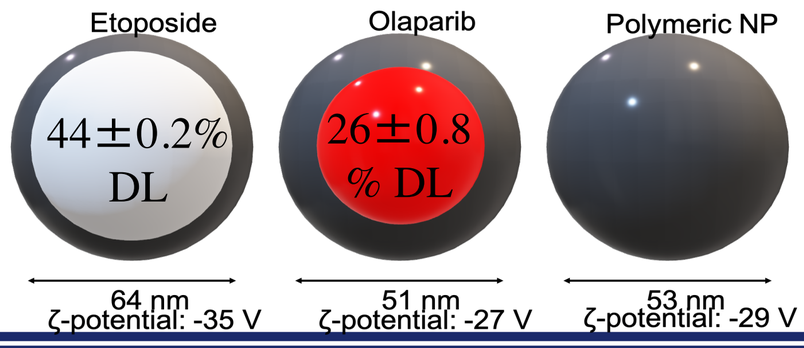
Polymer coating etoposide and olaparib nanocrystals gives high drug loading and suitable NP properties for brain delivery

Figure 4. Schematic to show how drug 0.25 mg/mL nanocrystals are initially made before coating with $mPEG_{5000}$-PDLLA 24 hrs later



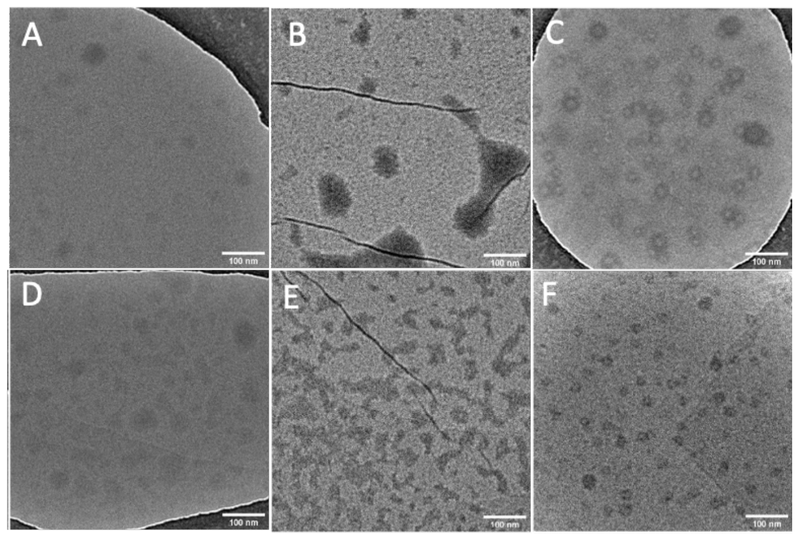
- NP sizes suitable for brain penetration (between 51-64 nm)
- Negative ζ-potential means less protein aggregation, thus potentially more diffusion
- Both systems had high drug loading (DL), found using HPLC against a calibration curve (mean ±SD):

$$\frac{Amount\ of\ drug\ encapsulated\ (mg)}{Amount\ of\ drug + polymer\ added\ into\ the\ system\ (mg)} x\ 100$$



TEM images show physical structure of polymer coated nanoparticles is different to drug or polymer alone

Figure 5. TEM images of (A) polymer alone, (B) OLA nanocrystals, (C) polymer coated OLA NPs, (D) polymer alone, (E) ETO nanocrystals, (F) Polymer coated ETO NPs.



Changes in physical structure (core shell versus plain black spheres) suggest successful encapsulation of drug within the nanoparticle, with only a few NPs showing original polymer-only characteristics.

OrbiSIMS imaging shows penetration of chemotherapeutics into brain tissue following being sprayed

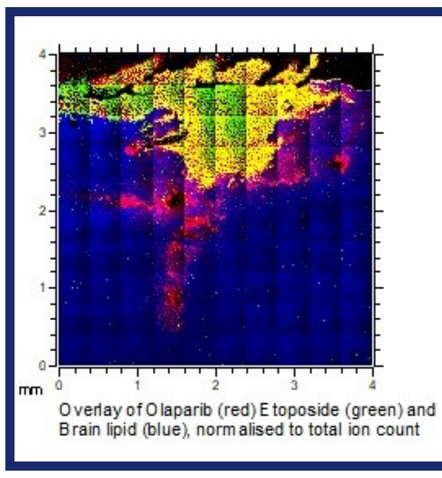


Figure 6. ETO and OLA penetration into a rat brain following being sprayed (Aptar Pharma hand pump device) within a pectin solution into a cavity.

Characteristic peaks:
Olaparib (red): $C_{15}H_{10}FN_2O^-$
Etoposide (green): $C_8H_9O_3^-$
Brain Lipid (blue): $C_{16}H_{31}O_2^-$

3 mm of penetration was found from a hand held spray device alone (without diffusion) suggesting a benefit to using a spray in the delivery system.

Conclusions and Future work

- ✓ Pectin was found to be non-toxic to human derived astrocytes below 100 µM for 48 hours
- ✓ A haemolysis assay showed no significant lysis of RBCs when incubated with gel for 1 hour
- ✓ *In vivo* results showed no initial signs of toxicity from the 200 µM gel for 2 weeks
- ✓ Polymer coated ETO and OLA show superior drug loading and show different physical structure to NPs alone
- ✓ OrbiSIMS methodology is optimised to show penetration of drugs in brain tissue
- ▪ Future work will trial safety and efficacy *in vivo*
- ▪ OrbiSIMS will be employed to assess penetration of drugs using the spray device versus injected control in *ex vivo* and *in vivo* brain tissue.

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