Development of nanoparticle loaded microneedles for drug delivery to a brain tumour resection site

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Abstract

Systemic drug delivery to the central nervous system (CNS) has been historically impeded by the presence of the blood brain barrier rendering many therapies inefficacious to any cancer cells residing within the brain. Therefore, local drug delivery systems are being developed to overcome this shortfall. Here we have manufactured polymeric microneedle (MN) patches, which can be anchored within a resection cavity site following surgical removal of a tumour such as isocitrate dehydrogenase wild type glioblastoma (GBM). These biodegradable MN patches have been loaded with polymer coated nanoparticles (NPs) containing cannabidiol (CBD) or olaparib (OLA) and applied to an *in vitro* brain simulant and *ex vivo* rat brain tissue to assess drug release and distance of penetration. MN patches loaded with methylene blue dye were placed into a cavity of 0.6% agarose to simulate brain tissue. The results showed that clear channels were generated by the MNs and the dye spread laterally throughout the agarose. When loaded with CBD-NPs, the agarose showed a CBD concentration of 12.5 µg/g at 0.5 cm from the MN insertion site. Furthermore, high performance liquid chromatography of *ex vivo* brain tissue following CBD-NP/MN patch insertion showed successful delivery of 59.6 µg/g into the brain tissue. Similarly, OLA-NP loaded MN patches showed delivery of 5.2 µg/g OLA into agarose gel at 0.5 cm distance from the insertion site. Orbitrap secondary ion mass spectrometry (OrbiSIMS) analysis confirmed the presence of OLA and the MN patch at up to 6 mm away from the insertion site

following its application to a rat brain hemisphere. This data has provided insight into the capabilities and versatility of MN patches for use in local brain drug delivery, giving promise for future research.

Keywords: Microneedles, nanoparticles, isocitrate dehydrogenase wild type glioblastoma

Introduction:

CNS tumours affected 330 000 people globally in 2016 alone, leading to 227 000 deaths (Patel et al. 2019). The incidence rate has also increased by 17.3% in comparison to 1990, making CNS cancers accountable for significant morbidity and mortality globally that is ever increasing (Bray et al. 2018). CNS tumours can place a significant burden on healthcare systems due to their potentially rapid development, associated neurological disability and very poor prognosis (Wei et al. 2014). The most common primary brain tumour type are gliomas, accounting for 80% of malignant brain tumours (Dolecek et al. 2012). Unfortunately, the median overall survival of isocitrate dehydrogenase (IDH) wild type glioblastoma (GBM) is only 14.6 months after diagnosis even with standard-of-care radical multimodal therapy (Stupp et al. 2009).

The lifespan of patients after diagnosis has not improved despite the considerable amounts of molecular and genomic research that has gone into advancing the treatment of GBM (Deorah et al. 2006), and despite a plethora of molecular targeted chemotherapy phase II clinical trials. This is in part also attributable to the lack of effective systemic chemotherapy owing to the presence of barriers preventing drugs from reaching the tumour sites. The blood-brain barrier (BBB) limits the penetrance of therapeutics into the brain, the blood-brain tumour barrier (BBTB) prevents entry into the tumour tissue, and a weak enhanced permeability and retention (EPR) effect leads to diminished accumulation of therapeutics within the tumour (Wei et al. 2014). These factors have led to a research focus into localised drug delivery to improve patient outcome after diagnosis (Bastiancich et al. 2016; Ung et al. 2015). Localised delivery involves the application of chemotherapeutics to the tumour resection cavity, aiming to prevent further growth of infiltrative cancer cells which could not be resected during

surgery (Manish Singh et al. 2014). The highly infiltrative nature of GBM cells means that they are able to disseminate within the CNS, leading to recurrences close to the surgical resection margin or in many cases within a few centimetres outside the resection cavity (Bastiancich et al. 2016). Therefore, it is imperative that local drug delivery technologies provide high and sustained concentrations of therapeutics at and beyond the resection site, are anchored within the resection cavity and are therefore in close contact with the brain parenchyma to unload chemotherapeutics for dissemination into the deeper brain regions.

Due to the inherent characteristics of some therapeutics, many small molecule drugs do not efficiently diffuse through brain tissue. Those with a low molecular weight and/or lipophilic agents are readily eliminated from the brain via systemic circulation (Seo et al, 2017), whilst other therapeutics which can form hydrogen bonds, ionic forces and hydrophobic interactions can be sequestered in brain tissue, hindering their diffusion(Wolak and Thorne 2013). Therefore, numerous nanocarriers have been investigated for local delivery to the CNS, including polymeric nanoparticles (NPs), liposomes and lipid nanocapsules (Wilhelm et al. 2016). The utilisation of nanotechnology for chemotherapeutic delivery increases solubility, protects drugs from degradation and increases their half-lives, therefore improving the efficacy of therapy (Alphandéry 2020). However, successful local delivery of polymeric NPs heavily relies on high levels of anticancer agents reaching the site of tumour cells (Wolinsky, Colson, and Grinstaff 2012). Tumour infiltration is detrimental to efficacious treatment, considering that the risk of reoccurrence is tightly correlated to the presence of left-over tumour cells surrounding the resection site (Sawabata et al. 2004). Recent work has demonstrated that NPs with 114 nm diameter can rapidly diffuse through brain parenchyma when coated by a dense poly(ethylene glycol) (PEG) layer (Nance et al. 2012), and that NPs with a 70 nm diameter diffused 100 times faster than similarly sized NPs without a PEG coating (Nance et al. 2014). Thus, it has been demonstrated that NPs have the ability of increasing the radius of effective treatment from the resection margin, in particular if applied within a local drug delivery technology permitting their anchoring within the cavity coupled with their sustained release over time.

Various localised delivery technologies have been investigated in recent years, with hydrogels and polymeric implants gaining much attention due to their versatility (Bota et al. 2007; Puente et al. 2018). These systems have the ability of being loaded with NPs allowing their sustained release over time. The only FDA approved drug delivery implant however, is the Gliadel® wafer (Ashby, Smith, and Stea 2016). It is a biodegradable co-polymer made of 1,3-bis-(p-carboxyphenoxy)propane (pCPP) and sebacic acid (SA) containing the chemotherapeutic BCNU (carmustine) (Juratli, Schackert, and Krex 2013). Nonetheless, there are several disadvantages posed by the Gliadel® wafer, namely poor drug diffusion coupled with rapid drug release, restriction to a mono-therapeutic system and the rigidity and size of wafers leading to their dislodgement within the resection cavity (Bastiancich et al. 2016b). An ideal localised drug delivery technology to overcome the disadvantages of the Gliadel® wafer are microneedles, which are efficient, biodegradable and minimally invasive recent inventions in the drug delivery field (Dugam et al. 2021).

Microneedles, which can be described as miniaturised needles with a shaft length of less than 1000 µm, have become a popular avenue in drug delivery research due to their ability to be inserted into the skin without causing the same painful response as a traditional hypodermic needle (Dugam et al. 2021). Specifically, dissolving microneedles are synthesised using a polymer selected to exhibit desirable characteristics, such as biocompatibility and timely dissolution, which is amalgamated with the drug / NP of choice. Once they are inserted into tissue, the polymer dissolves, simultaneously releasing the payload. Key advantages of this drug delivery system are the biodegradable nature of the microneedles, negating the need for surgical removal after administration, their precise anchoring at the resection site, and the uniform and close apposition to brain parenchyma to allow for NP diffusion into the diseased tissue (Dugam et al. 2021).

Whilst less research has been conducted into the ability of microneedles to be inserted into other tissue types, a recent article published by Lee *et al* demonstrated the possibility of microneedles being inserted in brain tumours, delivering theranostic NPs and photons as a treatment option (Lee *et al*. 2021). Moreover, given the multiple protrusions present in a microneedle array, microneedles may offer a solution to delivering a uniform quantity of drug with improved penetration, owing to piercing brain tissue, being moulded to the brain parenchyma, and remaining *in situ* until all the payload is released, offering significant advancements over the technology pertaining to the Gliadel® wafer. We therefore propose that microneedles are a promising technology for the local delivery of NPs to the brain resection cavity for the treatment of tumour cells disseminated in the neural parenchyma.

Due to the lack of advancements in GBM survival times with current therapies, new therapeutics are desperately needed to try increase patient lifespan. In this study, we developed a novel drug delivery system comprising PEG coated NPs with a high drug loading (cannabidiol (CBD) and olaparib (OLA)), formulated within a novel and biodegradable obelisk shaped microneedle design for prolonged and sustained delivery to the brain resection cavity. OLA, a poly(ADP-ribose) polymerase inhibitor and purported radiosensitiser, was chosen based on preclinical efficacy and safety profiles from recent GBM clinical trials, but which assessed systemic olaparib only (PARADIGM-2). CBD was selected due to its effect on limiting tumour growth and altering tumour microenvironment through its repression of P-selectin, apelin and interleukin-8 (Khodadadi et al., 2021). Moreover, it was also recently shown that CBD induced mitochondrial dysfunction leading to autophagic cell death in preclinical models of human glioma, through the activation of TRPV4 (Huang et al., 2021).

To the best of our knowledge, this is the first time that a microneedle array and a backing layer loaded with NPs (herein referred to as a MN patch) have been investigated for the local delivery of chemotherapeutics to the brain. We examined the drug distribution after release from the MN patch

in a brain simulant *in vitro* and in brain tissue *ex vivo*. We determined the capacity of the newly developed MN patch to release therapeutics utilising HPLC and Orbitrap-Secondary Ion Mass Spectrometry. Here, we test the hypothesis that a polymeric MN patch can be applied to a brain parenchyma and a pseudo-resection brain simulant model with close apposition, to aid brain penetration of drug compounds.

Materials and Methods:

Glycerol (CAS: 56-81-5), polyethylene glycol (PEG 400) (CAS: 25322-68-3), sodium carboxymethyl cellulose Mw 90,000 (CAS: 900-32-4) and agarose (CAS: 9012-36-6) were purchased from Sigma Aldrich and olaparib was purchased from Selleckchem. Polyvinylpyrollidone-co-vinyl acetate (PVPVA, Kollidon® VA 64 Mw 15-20 kDa) was provided by BASF (CAS: 25086-89-9). CBD was acquired from THC Pharm (Frankfurt, Germany). Silicone elastomer (Sylgard® 184) and Silicone elastomer curing agent (Sylgard® 184) were purchased from Wiesbaden, Germany.

Polymer synthesis

$mPEG_{5000}$-PLA_{100} and $tPEG_{1014}$-PLA_{100} were synthesised and characterised as per previous reported methods (Vasey et al, 2019, Phan et al, 2019) respectively, via PEG-initiated ring-opening polymerization of lactide block copolymer. Briefly, the desired amount of LA and $mPEG_{5000}$-initiator were weighed into a vial (pre-dried in an oven at 100 °C). Similarly, for the synthesis of $tPEG_{1014}$-PLA_{100}, LA and $tPEG_{1014}$-initiator were used. The [M]:[I] ratio was kept fixed at 100:1. $mPEG_{5000}$ and LA and $tPEG_{1014}$ and LA were dissolved in 10 mL of dichloromethane (Sigma-Aldrich) in a capped vial and the mixture was allowed to fully dissolve at room temperature. 1,8-Diazabicyclo[5.4.0]undec-7-ene (Sigma-Aldrich) was then added at 2% (*w/w*, compared to the monomer), to initiate the ring opening

polymerization. After 20 min of reaction time the polymer was purified via multi-precipitation steps using frozen hexane and dried in a vacuum oven with quantitative conversion of monomer into polymer (from 80 to 98%).

Olaparib NCPP formulation:

Nanocrystals coated with $mPEG_{5000}$-PLA_{100} (NCPPs) were prepared by a modified method from Styliari *et al.,*2020 and optimised in McCrorie *et al.,* 2020 for maximal drug loading (McCrorie et al., 2020, Styliari et al., 2020,). Briefly OLA was dissolved in 1 mL acetone:MeCN [1:1], before adding dropwise into 5 mL aqueous phase, stirring at 550 rpm. Drug-only nanocrystals were left stirring overnight to allow solvent evaporation, before $mPEG_{5000}$-PLA_{100} dissolved in 1 mL acetone was added dropwise into the drug nanocrystal aqueous phase whilst stirring. Samples were left stirring overnight to allow solvent evaporation before free drug was removed by centrifugation at 10000 x g for 5 minutes at room temperature (Eppendorf centrifuge 5430, Germany). NCPPs were then concentrated with gaseous N_2 to a predetermined volume and centrifuged again to remove any free drug.

Cannabidiol NCPP formulation:

Nanocrystals coated with $tPEG_{1014}$-PLA_{100}, previously designed for intrathecal delivery (manuscript under review), were similarly prepared by a modified method from Styliari et al., 2020 in a two-step nanoprecipitation technique. CBD was dissolved in 1 mL acetone and added to 5 mL water under constant stirring at 550 rpm. 4 hours later after the acetone evaporated, the PEG_{1014}-PLA_{100} dissolved in 1 mL acetone was added in a dropwise manner to the drug nanocrystal aqueous phase under constant stirring. Samples were subsequently left stirring overnight to allow for acetone evaporation. Free drug was removed by centrifugation at 4000 x g for 5 minutes at room temperature (Eppendorf centrifuge 5430, Germany). NCPPs were then concentrated with gaseous N_2 to a predetermined

volume. The drug loading in CBD NCPPs was determined by reverse-phase HPLC (as described below in: *Quantification of CBD and OLA release:*) after the reconstitution in solvent of lyophilised material.

NP characterisation:

The diameter (d.nm), polydispersity index (PDI) and zeta potential of NPs were determined using a Zetasizer Nano ZS (Malvern Instruments Ltd.). A NP dispersion was diluted in Milli-Q water at intensity in the range 10^4–10^6 counts/sec and measurements were performed at 25 °C on a 173° backscatter angle. The refractive index of materials was set to 1.46 for NCPPs and 1.33 for water dispersant. Results are reported as the mean of three independent measurements of three different batches (n = 3) ± standard deviation of the mean (SD).

Manufacture of nanoparticle loaded microneedles:

Stainless steel 304 master moulds with a 12 mm x 12 mm master structure with a 10 x 10 array of obelisk-shaped microneedles with a height of 1000 µM, base of 300 µM and pitch of 800 µM were made using a Kern Evo CNC Micro Milling Machine at the Precision Manufacturing Centre, University of Nottingham.

Polydimethylsiloxane (PDMS) moulds were fabricated using a 10:1 of silicone elastomer to silicone curing agent (Sylgard® 184), which was thoroughly mixed prior to being degassed. 1.25 ml of the mixture was transferred to the stainless steel 304 mould and held under vacuum for 5 minutes. After release from the vacuum, moulds were cured at 90 °C for 30 minutes before placing in an ice bath and removing from the stainless steel 304 mould.

Microneedles were made using a two-step casting process. Initially, the needle layer was fabricated using 15.8 % w/v polyvinylpyrrolidone-co-vinyl acetate (PVPVA), high performance liquid chromatography (HPLC)-grade water and 1.96 % w/v PEG 400 with OLA or CBD NPs. After being

degassed, 150 µL was pipetted into a mould and centrifuged at 4000 rpm for 15 minutes (Heraeus Multifuge 3s, Kendro Laboratory, Germany), allowing the formation of needles. Once centrifuged, excess liquid was removed from the moulds. The filled moulds were placed in a desiccator and allowed to dry overnight. Backing layer was made by combining and stirring 5.2 % w/v CMC, 0.65% w/w glycerol and either HPLC-grade water or OLA/CBD NCPPs, until fully dissolved. 200 µL was pipetted on top of the needle layer and further centrifuged at 3500 rpm for 10 minutes. The moulds were placed in a desiccator and left to dry for 72 hours. After this time, the MN patch was removed from the moulds and left for a further drying period within a desiccator.

Fracture force of microneedles:

A texture analyser (TA-XT Plus Texture Analyser, Stable Microsystems, UK) was used to evaluate the fracture force of the microneedles, paralleling the force applied to the microneedles upon insertion into biological tissue. The MN patch were attached to a 20 mm cylindrical aluminium probe with double-sided tape, with the needles facing downwards, and attached to a 50 kg load cell. The settings were adjusted such that a compression test was completed with a pre-test and post-test speed of 10 mm/second. The trigger force was set to auto at 0.02 N with no break mode. Results are reported as the mean for each formulation *(n* =4) ± standard deviation (SD).

Environmental scanning electron microscopy:

Microneedles were imaged using environmental scanning electron microscopy (ESEM) (FEI Quanta 650) to assess the structural uniformity after demoulding. A MN patch was attached to a vertical stub using carbon adhesive tape. Images were acquired using the low vacuum mode of the ESEM, with water vapour as the imaging gas and a large field detector. Operating voltage (kV) and gas pressure are displayed on the data bar for each image. Samples were remounted flat and imaged again at 0° tilt and 40° tilt using the same imaging conditions.

Insertion of microneedles into a brain simulant and ex vivo brain tissue:

In accordance with previously published literature, 500 ml of a 0.6% w/v agarose gel was formed to simulate brain tissue (Chen et al., 2004; Pomfret et al., 2013). Agarose powder was reconstituted with deionised water and left to set overnight. Microneedle arrays loaded with 180 µg methylene blue dye (hydrophilic), or OLA/CBD NCPPs respectively were inserted into the gel in order to assess whether they remained *in situ* and released the drug payload. Visual inspection was employed to assess distribution of the methylene blue dye throughout the agarose gel.

Drug-loaded microneedle arrays were inserted into 4 *ex vivo* female rat brains (age p55) by applying subtle thumb-pressure to the array for 10 seconds. Brains were sectioned into two hemispheres, with one patch applied to each (n=8 in total). Blank PVPVA microneedle arrays were used as controls.

Quantification of CBD and OLA release:

The concentrations of CBD in brain tissue and agarose and of OLA in agarose were determined by reverse-phase HPLC (Waters Alliance 2695 separations module) coupled to a photodiode array ultraviolet (UV) detector (Waters 996). Analytical conditions for CBD were previously published (Zgair et al., 2015) and applied with slight modifications. Briefly, the mobile phase consisted of acetonitrile:water (62:38, *v/v*), isocratic flow rate 1 mL/min and oven temperature of 55 °C. The stationary phase was comprised of an ACE C18-PFP column (150 x 4.6 mm, 3 µM) coupled with an ACE C18-PFP 3 µM guard cartridge (Hichrom Ltd, UK). Dichlorodiphenyltrichloroethane (DDT) was used as an internal standard. The absorbance of analytes was monitored at 220 nm.

Analytical methods for OLA were previously published (McCrorie et al., 2020) and applied for the quantification of OLA in agarose. Briefly, the mobile phase consisted of 10 mM pH 4 ammonium acetate:acetonitrile (55:45, *v/v*), isocratic flow rate of 1 mL/min and oven temperature of 40 °C. The

stationary phase was comprised of ACE 5 C18-PFP column (250 x 4.6 mm, 3 µM coupled with an ACE 5 C18 10 x 3 mm guard cartridge (Hichrom Ltd, UK). Erlotinib was used as an internal standard and absorbance was monitored at 254 nm.

Sample preparation for HPLC analysis:

Extraction of CBD and OLA from agarose for quantification by HPLC

1 mL ethanol (EtOH) was added to the agarose samples which were then sonicated for 30 mins. 700 µL EtOH was aspirated from the agarose samples and replaced by 1 mL of fresh EtOH. Samples were sonicated for another 30 mins, after which a further 700 µL of EtOH was aspirated and pooled with the initial samples. The EtOH was then fully evaporated over N_2 gas at 37 ^{o}C. Samples were reconstituted in 90 µL ACN and 10 µL internal standard. Sample injection volume was 20 µL for OLA quantification and 40 µL for CBD quantification. Calibration curve standards for CBD and OLA ranged between 0.1 to 100 µL/mL.

Extraction of CBD from brain tissue

Samples were prepared for HPLC analysis using a combination of protein precipitation and liquid-liquid extraction. Brain tissue was homogenised after dilution in water using a Stuart homogeniser (model: SHM1 1116) for 5 mins at 18 000 rpm. Tissue proteins were precipitated after the addition of 450 µL ACN and 450 µL deionised water followed by thorough vortexing for 5 mins (Ultraturax, UK). CBD was extracted by the liquid-liquid extraction method after the addition of 3 mL hexane. Samples were vortexed again for 5 mins followed by centrifugation for 10 mins (brand, 4 000 g). The upper hexane layer was separated and evaporated over N_2 gas at 37 ^{o}C. Mobile phase (62% ACN, 38% water) was used for reconstitution and 40 µL of sample was injected into the HPLC. All samples were run for 30 mins and analysed at 220 nm.

OrbiSIMS analysis:

The Orbitrap Secondary Ion Mass Spectroscopy (OrbiSIMS) instrument utilises a Q Exactive HF for Orbitrap MS. Mass calibration of the Q Exactive instrument was performed once a day using silver cluster ions. The MS spectrum was obtained in mode 4 (Passarelli *et al,* 2017) of the instrument using a 20 keV argon cluster beam (specifically, Ar_{3000}^{+} with a target current of 0.2 nA). Reference spectra for the control tissue, drug samples and 1 mg/mL drug spiked brain tissue were accumulated over 250 scans as the ion beam rastered in a random pattern (250 × 250 µm) in negative mode at the 240,000 at m/z 200 mass resolution setting. Charge compensation was applied throughout the analysis using a low energy floodgun (20 eV). Data was collected between 75 and 1125 Da for each spectrum from spots freeze-dried onto a glass microscope slide.

For drug penetration analysis, a MN patch containing 49.44 µg of OLA-NCPPs was gently pressed onto a hemisphere of fresh *ex vivo* rat brain and left at room temperature for 30 minutes. The brain hemisphere was then snap-frozen in liquid nitrogen and sectioned into 10 µm sagittal slices using a Leica CM3050S cryostat (Leica, Germany) at −22 °C. These slices were adhered to gelatin coated glass microscope slides, before being freeze-dried overnight. Once dry, samples were analysed for OLA presence utilising mode 4 of the OrbiSIMS as per optimisation. Three analysis spots were taken in total; one at the superior most point of the hemisphere where the MN patch was placed, in the middle, and distally, at the bottom of the section.

Ex vivo animal studies

Tissue collection for *ex vivo* studies was carried out under a non-licenced procedure in accordance to the 3Rs (replacement, reduction, refinement). The animals were housed and cared for according to the requirements of the UK Animals (Scientific Procedures) Act 1986 (ASPA) Code of Practice.

Statistical analysis

All data were expressed as mean ± SD or SEM. All statistical analyses were performed by GraphPad Prism 7 (GraphPad Software, UK. If three or more groups were analysed a one-way ANOVA was carried out with a post-hoc Tukey's test or Sidak's multiple comparisons test. If two groups were analysed a two-tailed t-test was performed. Differences considered statistically significant at *$p < 0.05$.

Results and Discussion

Generation of NPs

Firstly, two different NP systems were manufactured to enhance the drug loading potential in the MN patches; OLA NCPPs generated in prior work (McCrorie *et al,* 2020) and a modified method to generate CBD NCPPs. These NCPPs utilise PEGylated polymers to coat drug nanocrystals in order to aid drug diffusion (Nance *et al, 2012*); furthermore, prior work with this method generated high drug loading (Styliari *et al*, 2020 and McCrorie *et al*, *2020*), which is hoped to enhance future *in vivo* efficacy.

CBD NCPPs formulated by a double nanoprecipitation technique were physically characterised by DLS (Figure 1A). The size of the NCPPs was 121.8 ± 1.1 with a PDI of 0.079 ± 0.014 and zeta-potential of –39.9 mV ± 1.22 (n=3, measured in triplicates) (Table 1), which are larger and with a greater negative charge than those developed by Nance *et al*.(Nance *et al.* 2012). After removal of free drug by centrifugation and subsequent freeze-drying, the drug loading (DL%) was demonstrated to be 28.52 ± 2.45 and encapsulation efficiency (EE%) was 46.04 ± 0.03 as analysed by HPLC.

OLA NCPPs were made and characterised as previously described (McCrorie *et al,* 2020). Briefly, the OLA loaded NCPPs were 50.0 ± 0.734 nm (PDI: 0.174 ± 0.011) with a zeta potential of -20.1 ± 2.79

(mean ± SD, *n*=6). They have been shown to be stable in PBS, artificial cerebrospinal fluid and DMEM at 37 °C for up to 72 hours, and at 4 °C for up to 6 weeks in prior published work (McCrorie *et al,* 2020).

Microneedle characterisation:

The strength of the microneedles was assessed using a texture analyser to understand whether the microneedles could withstand being handled and inserted into brain tissue without detriment to the structure.

Figure 2A shows that the PVPVA microneedles loaded with OLA NPs exhibited a mean fracture force of 0.73 ± 0.03 N/needle whilst those loaded with CBD NPs had a slightly lower fracture force of 0.68 ± 0.03 N/needle (mean ± SD, *n*=4). In both instances, PVPVA microneedles loaded with drug demonstrate a statistically significantly higher fracture force than PVPVA microneedles without drug ($p < 0.0001$ and 0.0003 respectively), which may suggest that the addition of drug increases rigidity due to the NPs being crystalline and hence mechanically stronger than the PVPVA.

Given the novelty of this research, there is no data known to the authors regarding the strength required for successful insertion into brain tissue specifically; however previous research has studied microneedle insertion into human skin. In this instance, it has been shown that the fracture force should be no less than 0.098 N/needle to allow for successful insertion (Lee et al., 2015; Yu et al., 2017). Given the nature of the brain tissue being 'ultrasoft', typically with stiffness values in the region of 1 kPa, it is fair to assume a considerably less fracture force would be required for successful insertion compared to skin, which is a considerably stiffer tissue (Budday et al. 2015, Budday et al. 2020, Graham et al. 2019).

Micrographs obtained via ESEM demonstrate the successful manufacture of the obelisk PVPVA microneedles loaded with OLA/CBD NPs using a two-step casting method. As can be seen in Figure 2B-C, the microneedles are uniform in shape and alignment and remain intact after being demoulded,

suggesting that the total amount of drug loaded in the system will be available for release from the MN array. As shown previously, this supports the choice of PVPVA being the predominant material in the array, alongside its biocompatibility, plasticity and controlled release properties (Mellert et al. 3004, Patel et al. 2015).

A dye-release study was completed to gain further understanding into how NPs would be released and distribute into the surrounding brain tissue. Microneedle arrays were inserted into a pseudo-resection of 0.6% w/v agarose gel in order to mimic a tumour resection site. Figure 2D demonstrates that the microneedles were able to stay in place after insertion for up to 60 minutes, after which excess dye was removed. Although the distribution of methylene blue dye could not be accurately measured, there was clear evidence of channels formed by the microneedle insertion and dye spreading laterally. This is promising with respect to drug delivery suggesting that with time drug may be evenly distributed around the resection site, lessening the risk of tumour recurrence.

Given the complicated anatomical nature of brain tissue, MN patch loaded with CBD NPs were inserted onto *ex vivo* brain tissue to ensure adhesion. Figure 3A-B, clearly shows the MN patch adhered to the tissue and moulded to the shape of the tissue, demonstrating their flexibility and suitability for purpose. Moreover, after 30 minutes, the MN patch was unable to be removed from the site of application, given the inserted microneedles and backing layer had started dissolving to release drug (inset image, Figure 3B). Currently, Gliadel® wafers are commonly inserted into brain tumour resection sites; however, these can be dislodged and are susceptible to gravity. Here, it has been demonstrated that microneedles, which actively anchor into brain tissue may be able to overcome these issues, offering more uniform drug delivery with persistent apposition of microneedle patch to brain parenchyma harbouring residual infiltrative glioblastoma cells.

Overall, the successful formation and insertion of microneedles loaded with OLA/CBD NPs into both *in vitro* and *ex vivo* models of the brain prompted the need for quantification of drug release.

Drug release in agarose:

Microneedles loaded with OLA and CBD NCPPs successfully released drug into brain simulant (agarose gel) *in vitro* and brain tissue *ex vivo* as quantified by HPLC. Figure 4A-C represents the release profile of CBD after the application of microneedle array onto agarose gel, with samples analysed at three depths of 0.5, 1 and 1.5 cm away from the patch. Agarose was sampled at various stages after the application of the patch (30, 60 and 180 mins for CBD NCPPS and 30, 60, 180 and 320 mins for OLA NCPPs). CBD NCPPs were successfully released from the microarray patch into the brain simulant *in vitro* within 30 mins of application (Figure 4A), resulting in 9.4 μg/g CBD concentration at 0.5 cm depth. Within 180 mins the CBD concentration reached 12.5 μg/g at this depth. On the other hand, negligible CBD concentrations were detected by HPLC deeper into agarose (1 and 1.5 cm away from the patch; Figure 4B-C). This release pattern could be explained by an immediate burst release of the CBD NCPPs present in the needles during the first sampling point at 30 mins, with the drug present in the backing layer of the microarray patch taking longer to release into the brain stimulant to the same sampling area at the 180 min time-point.

Successful release into brain simulant *in vitro* was also demonstrated with OLA NCPPs loaded MN patches, as determined by HPLC analysis. Figure 4E-F represents the release profile of OLA following the application of MN patch onto agarose gel. The concentration of OLA at 0.5 cm agarose depth within 30 mins of application was determined to be 3.6 μg/g, reaching a concentration of 5.2 μg/g within 320 mins of application. Similarly to the CBD NCPPs release profile, the concentration of OLA further away from the microneedle patch (1 and 1.5 cm, Figure 4E&F) was negligible as detected by

HPLC (< 0.7 µg/g). Previous work in our group has shown an average IC_{50} of 14.8 µM across 4 patient-derived and commercial cell lines (paediatric and adult GBM [data not shown]); at 0.5 cm the concentration was 12.0 µM (at 320 minutes), which has shown that IC_{50} concentration levels have almost been achieved, which could be enhanced by adding greater amounts of NPs to the MN patch. Furthermore, a study by Azoulay. *et al., 2020* showed that 65% of GBM tumours reoccur within this 0.5 cm window from the resection site, and therefore a MN patch could be efficacious in this population of patients (Azoulay et al., 2020) if the MN patch has the same effects *in vivo*. However, diffusion of the drug from the MN patch *in vivo* will also be affected by elements not present in the agarose brain simulant, such as extracellular matrix pore size, charge and flow of cerebrospinal fluid and blood following surgical trauma, and hence, the 0.5 cm penetration distance observed in the simulant may not be totally representative of *in vivo* drug penetration.

Drug release in ex vivo brain tissue:

CBD NCPPs loaded in microneedles were successfully applied to *ex vivo* rat brain to determine the release of CBD into freshly excised tissue. Figure 5 displays the brains used and how they were sectioned into hemispheres prior to patch application. Furthermore, the mouldability of the microneedle patches is apparent in Figure 5B, after their application to brain tissue at room temperature.

Successful release into brain was demonstrated with CBD NCPPs loaded in microneedles as determined by HPLC analysis of the whole brain after patch application (Figure 5C). The amount of CBD quantified in brain was 59.6 µg/g within 30 mins, from the initial CBD drug loading of 370 µg within the MN patch.

OrbiSIMS analysis

OLA NCPPs-loaded MNs were also applied to *ex vivo* rat brain hemispheres. Following 30 minutes of MN patch application at room temperature, the hemispheres were snap frozen and sagittal sections taken. These sections were freeze-dried to remove excess moisture and then analysed using OrbiSIMS. OrbiSIMS is a surface analysis technique which ionises a material surface using a primary ion beam and separates the ions utilising either a time-of-flight or an Orbitrap mass spectrometer. With the latter, the instrumentation can identify ions with <2 ppm accuracy.

Four sagittal sections were analysed at the point of MN patch insertion, in the middle of the section (approx. 3 mm) and at the bottom of the section (approx. 6 mm). As shown in Figure 6C, the OrbiSIMS data suggests that the OLA molecular ion ($C_{24}H_{23}FN_4O_3^+$; m/z 434.1756) is observed in the top and bottom analysis point of the section and two ions for the backing layer polymer, sodium carboxymethyl cellulose, ($C_6H_{11}O_6Na^+$; m/z 202.0451, and $C_5H_9O_5Na^+$; m/z 172.0344) were seen in all three analysis points.

The drug diffusion pathway through the brain may be accountable for the lack of the OLA molecular ion in the middle analysis point, whereby the drug has bypassed the 20 µm area analysed in this single spot analysis of the brain tissue. A recent paper from our group has shown OLA distribution in brain tissue following administration to a rat brain. The paper shows that OLA does not distribute uniformly through the tissue; the drug seems to follow the 'path of least resistance', whereby it follows a clear channel through the tissue, noted by a lack of brain lipids within the channel. Although OLA was present throughout the brain tissue, there was a greater abundance within this lipid free channel. This data shows that OLA does not reach all brain tissue in a uniform manner and therefore could account for the lack of molecular ion seen in the middle spot analysis of this animal (McCrorie *et al*, 2022). Furthermore, the sodiated molecular ion ($C_{24}H_{23}FN_4O_3\ Na^+$) was found in the top and middle analysis point in a second animal, further suggesting a brain-dependant drug diffusion pathway (supplementary figure S1). Another possibility is that it could be a result of the concentration below

the limit of detection. However, we have shown that OLA is detectable in rat brain tissue down to at least 25 μg/g when analysing in negative mode (McCrorie *et al*, 2022). Nevertheless, OrbiSIMS has shown that OLA and the MN patch are detectable throughout brain tissue following the application of a MN patch for 30 minutes at room temperature, giving promise for its application for local drug delivery in a neuro-oncological setting.

Conclusions

Whilst reliance on drug diffusion upon release from a local delivery system has previously demonstrated clinical utility and adoption for glioblastoma, there are limitations to drug diffusion extent. We provide proof-of-concept insight into the use of polymeric MN patch as a local drug delivery system to the brain, whereby persistent and close apposition to a brain resection cavity and insertion of MN patches into brain parenchyma, offered up to 0.6 cm diffusion of CBD and OLA drug compounds. Scientific evidence presented here, warrants evaluation of safety and efficacy in preclinical allograft or patient-derived xenograft tumour resection models of glioblastoma using CBD and OLA. Furthermore, the MN patch delivery system is amenable for incorporating a broad range of next-generation molecular targeted therapeutics predicated on patient-tailored genome biology.

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Figure Legends

Figure 1. Nanoparticle characterization data showing A) DLS traces of CBD NCPPs formulated with $tPEG_{1014}$-PLA_{100}, CBD nanocrystals without a polymeric coating and tPEG only micelles. CBD NCPPs were significantly larger in particle size than the blank tPEG control micelles (*n*=3, mean ± SD, *** p<0.001) (tPEG control particle size was 85.44 ± 1.16 nm and CBD NCPPs were 121.8 ± 1.1 nm; there were no significant differences in PDI). B) DLS data of OLA-NCPPs following preparation (mean ± SD, *n*=3), with a particle size of 50.0 ± 0.734 nm and PDI of 0.174 ± 0.011.

Figure 2. (A) Fracture force of a range of PVPVA microneedle arrays loaded with OLA and CBD. Data expressed as mean ± SD (*n*=4), analysed using one-way ANOVA, P<0.0001, GraphPad Prism 9.2.0. (B) ESEM micrograph of PVPVA microneedles loaded with OLA. (C) ESEM micrograph of PVPVA microneedles loaded with CBD. (D) Methylene blue loaded PVPVA microneedles inserted into 0.6% w/v agarose to simulate distribution of drug released from microneedles in brain tissue. Arrows indicate channels formed by MN insertion. Scale bars: B left image = 1 mm B right image =500 µm; C left image = 2 mm, C right image = 1 mm; D left image = 30 mm, D right image = 30 mm

Figure 3. Images to show how MN patches were placed onto rat brain hemispheres (A) from above and (B) from the side, with the inset image showing that the removal of the MN patch is not possible due to the adherence to the tissue and the dissolving of the MN patch.

Figure 4. Release profile of CBD and OLA NCPPs in agarose over time following the application of CBD loaded MN patches. Agarose sampled at 3 different depths measured from the application of the CBD MN patch. (A). 0.5 cm., B). 1.0 cm and C). 1.5 cm) and after the application of the OLA MN patch (D). 0.5 cm., E). 1.0 cm and F). 1.5 cm).(n=3 and n=2 in duplicates, respectively, means +/- SEM)

Figure 5. Release of CBD NPs in rat brain *ex vivo* following the application of CBD loaded MN patches. Brains were sectioned in two hemispheres (depicted by the red dashed line in A) and 8 MN patches were applied to the left and right hemispheres of four rat brains (B). (C) Amount of CBD in brain was quantified after 30 mins of application at room temperature (*n*=8, means +/- SEM).

Figure 6. (A) Schematic to show the location of analysis spots for OrbiSIMS analysis following the application of a MN patch for 30 minutes at room temperature. (B) Selected ions from the MN patch backing layer in the control animal, treated with a blank MN patch (no NPs) and (C) the same peaks seen in an animal treated with an OLA-NCCP loaded MN patch, displaying the olaparib molecular ion.