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Pump-Priming Grant Report

Extracellular vesicle uptake kinetics and tissue tropism in ex-situ machine-preserved livers: towards targeted therapeutics.

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Objective: *To evaluate EV biodistribution in ex situ perfused human liver*

Extracellular vesicles (EVs) are nanosized, membrane-bound particles released by all cells. By virtue of their varied bioactive cargo, EVs have been demonstrated to regulate immune responses, affect tissue regeneration, and influence cell fate. As a result, their potential as therapeutic entities has been explored in varied conditions including cancer, autoimmunity, and systemic inflammation, with recent progress to a number of clinical trials. Given their stability, their capacity for tissue-specific targeting, their ability to cross biological barriers, and an excellent safety profile, EVs are also being developed as vehicles for the delivery of therapeutics, with a particular focus on oligonucleotides (ON) payloads.

Nevertheless, despite tangible therapeutic outcomes associated with the delivery of EVs, there remains a gap in our understanding of the uptake kinetics, tissue tropism, and biodistribution of delivered EVs and of their payload. While limited small animal models demonstrate the reticuloendothelial system, and particularly the liver, to be responsible for the majority of the uptake of systemically delivered EVs, similar studies in human tissues are lacking. By the same token, murine demonstrations of the capacity of bioengineered EVs derived from different cellular sources to preferentially target specific liver cellular subsets also requires further elucidation in human, whole-organ models.

Ex-situ normothermic machine perfusion of the liver provides a unique preclinical setting to accurately investigate the uptake kinetics, dosing, and tropism of bioengineered EVs. To this end, in the first instance, fluorescently labelled EVs (CD63-GFP EVs) of different parental cellular sources will be delivered to livers undergoing normothermic machine perfusion (NMP). This will serve to inform further studies investigating cargo translocation.

Method

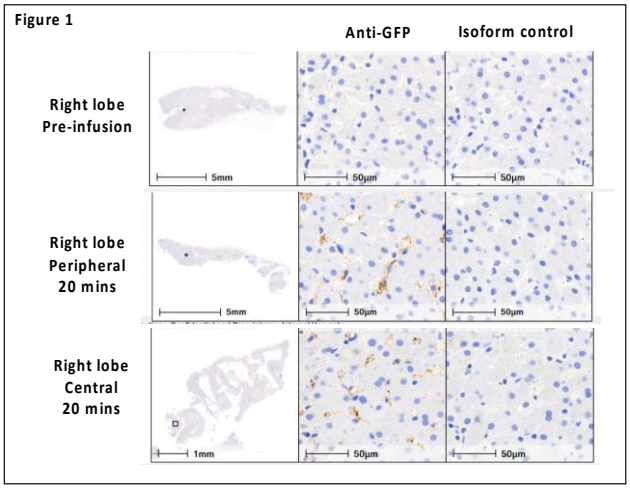
In brief, bioengineered EVs expressing CD63-GFP tag were derived from two different parent-cell colonies. Following multiple rounds of 4-6 weeks minimum harvest, pools of 10^{12} – 10^{14} GMP-standard EVs were derived and delivered via the portal vein in porcine and human livers undergoing NMP (as previously described). 20 minutes post-infusion, liver tissue samples were collected from central and peripheral aspects of multiple lobes (FFPE, snap frozen, and OCT). GFP signal was assessed by fluorescence slide scanner microscope and GFP IHC staining (Histologix).

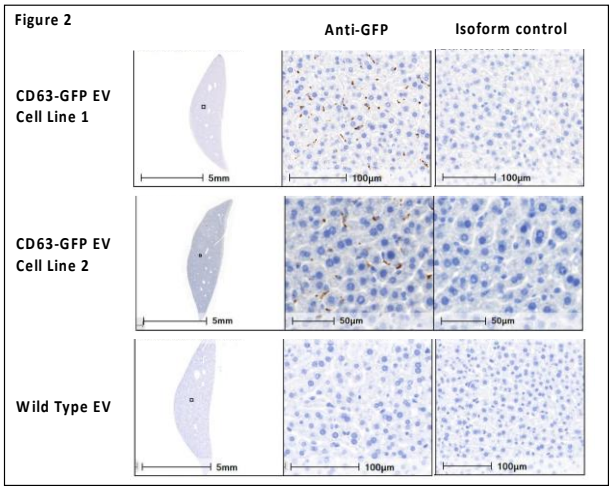
This work has been made possible by our unique collaboration with OrganOx, industry leaders in the field of ex-situ organ preservation, and EVOX, a company at the forefront of EV therapeutics.

Results

Given that this is a novel area of research without established protocols and investigating nano-particles which can be notoriously difficult to visualise, the bulk of the work conducted involved optimisation of experimental methods beyond the scope of this report. Adaptations and advances have been made in approaches to EV production, EV labelling, EV delivery, and IHC antibody selection among others.

In NMP human livers, uptake of CD63-GFP-labelled EV construct was confirmed 20 minutes post-infusion predominantly in sinusoids (Fig 1). Uptake was established in both the parental cell lines used for EV production. Analyses of variation of biodistribution are pending. Strong, specific, punctate signal was observed in tissues receiving EV-GFP constructs, while pre-infusion, isotype, and wild-type-EV negative controls all were negative for signal (Fig 2).





Summary and next steps

These preliminary results represent the first demonstration of bioengineered EV uptake within the human liver. As such, they form the basis for onward studies aimed towards a better understanding of dose-response relationships, of targeting to cell subsets, and of delivering EV-encapsulated payload. Having identified a number of payload candidates, going forward we aim to continue this work in collaboration with our partners in industry.

Outputs

This work has not been presented or published at this stage. These data serve as a) a proof of principle, and b) the first of a number of steps towards the ultimate goal of demonstrating payload delivery and efficacy. Work in this direction is ongoing.

We are very grateful to the OTF for supporting and making this preliminary work possible.