ReNeuron *CustomEx*™

Developing a panel of optimized extracellular vesicles for enhanced delivery of therapeutics

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Overview

- ***CustomEx*™** is ReNeuron's novel EV platform.
- It consists of **EVs derived from stem cell producer lines representing a variety of tissue types**.
- Producer cell banks are **clonal and conditionally immortalised, enabling scalable and stable production of cells and functionally bioactive EVs**.
- **Unique EV types show distinct preferences for uptake** into selected target cell populations.
- EVs generated using this platform can be **engineered to express biologically active payloads.**
- ***CustomEx*™** offers potential advantages over a 'one-size-fits-all' approach.

Methodology

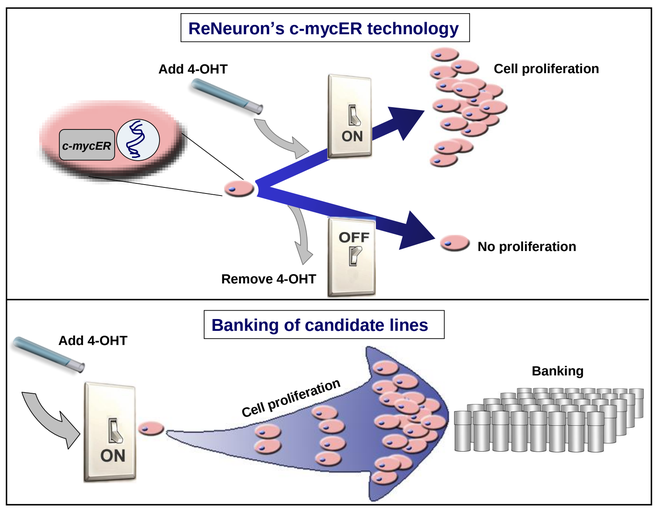


Figure 1. *CustomEx*™ EV producer cell line culture.

ReNeuron's producer cell lines are derived from primary cell material which has been transduced with a c-MycER fusion protein construct and undergone clonal selection[1]. This construct renders the cells responsive to the tamoxifen analogue 4-OHT. When cultured in the presence of 4-OHT, the proliferative rate and phenotype features are stabilized, enabling consistent, scalable and tunable cell production, compatible with GMP requirements.

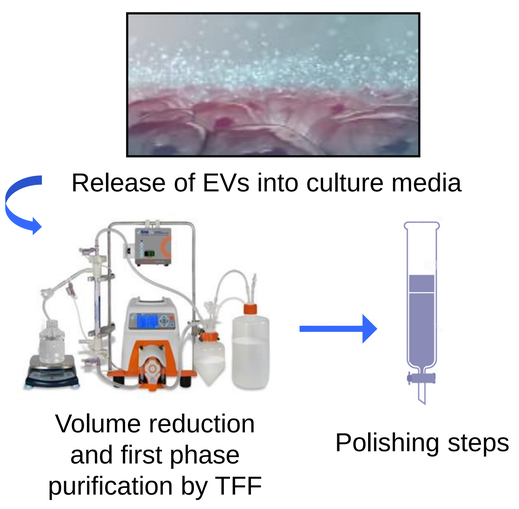
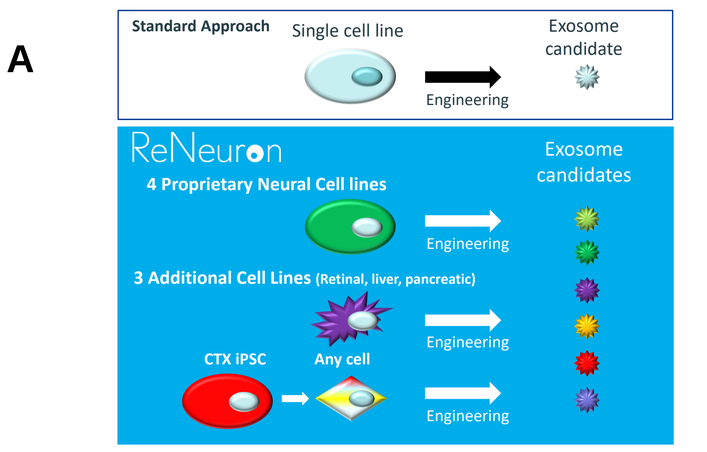
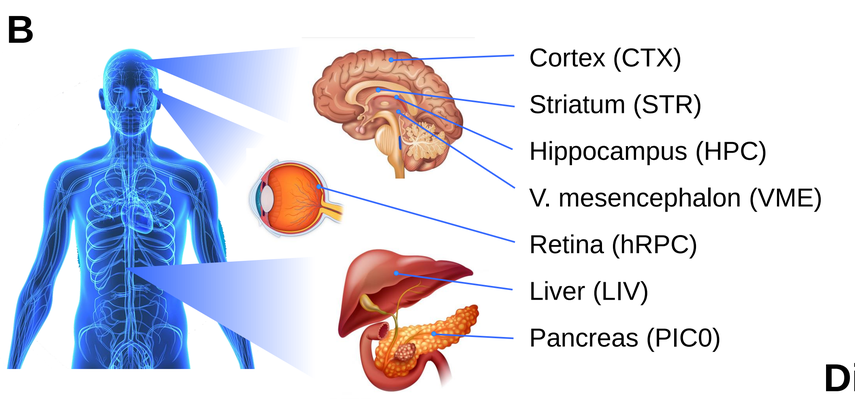


Figure 2. EV isolation strategy.

EVs are released into the culture media spontaneously by producer cells. Subsequently, EVs are purified from conditioned culture media using volume reduction and first phase purification by TFF followed by chromatographic polishing steps.

Data





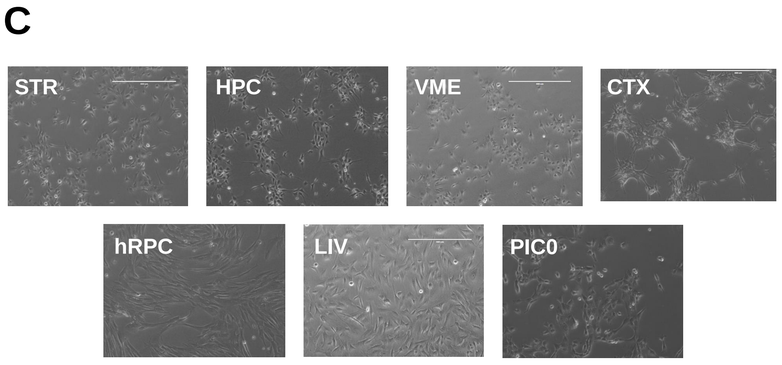


Figure 3. Producer cell origin and morphology

(A) ReNeuron's approach utilizing a repertoire of EV producer cell lines offers an alternative to the 'one-size-fits-all' strategy which relies on a single EV source type. (B) ReNeuron is developing EV producer stem cells originating from the striatum, hippocampus, cortex and ventral mesencephalon. Outside the brain parenchyma, sites of producer cell origin include the retina, the liver and the pancreas. (C) Conditionally immortalized cells are grown in proprietary serum-free media. Phase-contrast micrographs highlight unique morphological features reflecting the tissue origin and cell type.

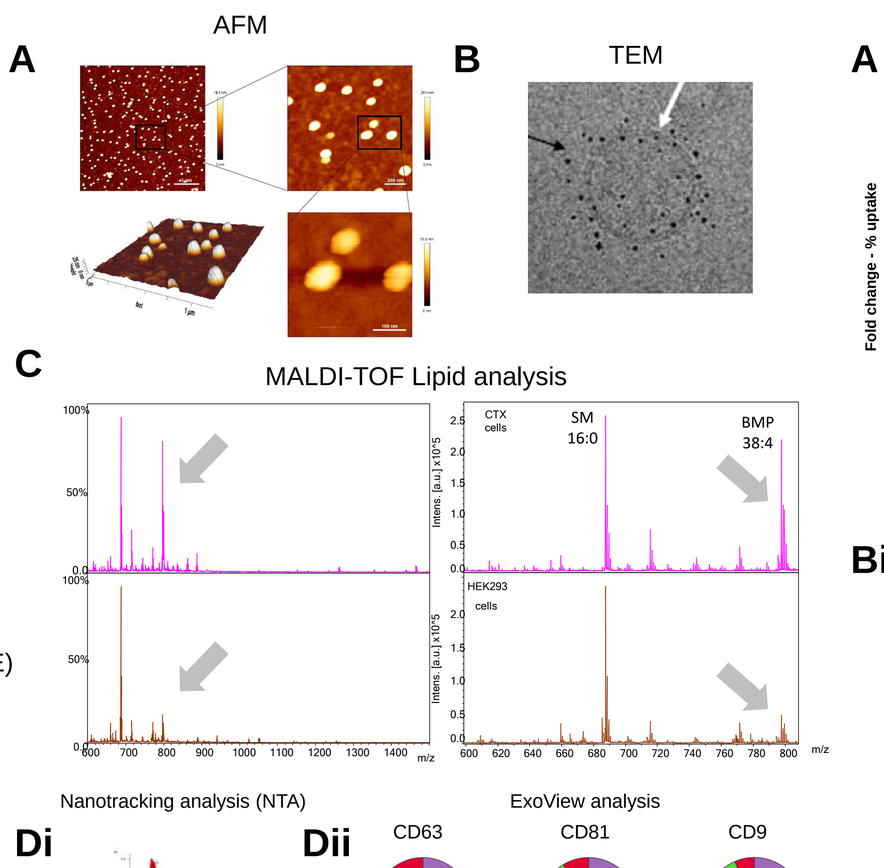


Figure 4. EV characterization and analytics

(A) Analysis of CTX EVs using atomic force microscopy (AFM) at low resolution (top left), mid resolution (top right) and high resolution (bottom right) allows assessment of EV diameter, height (bottom left) and preparation purity. (B) Transition electron microscopy (TEM) of CTX EVs stained with anti-CD63 and anti-CD81 antibodies conjugated to 6nm (white arrow) and 10nm (black arrow) gold particles. (C) Comparative MALDI-TOF mass spectroscopy of EV lipid membrane composition. CTX EVs contain higher levels of Bis(monoacylglycero)phosphate, and heavy gangliosides which is typical for CNS derived EVs.[2] (D) Selected *CustomEx*™ EVs were analyzed by i) NTA and ii) surface marker analysis using an ExoView platform. Pie charts represent degree of CD63, CD81 and CD9 co-expression on EVs captured by anti-CD63, CD81 and CD9 capture antibodies.

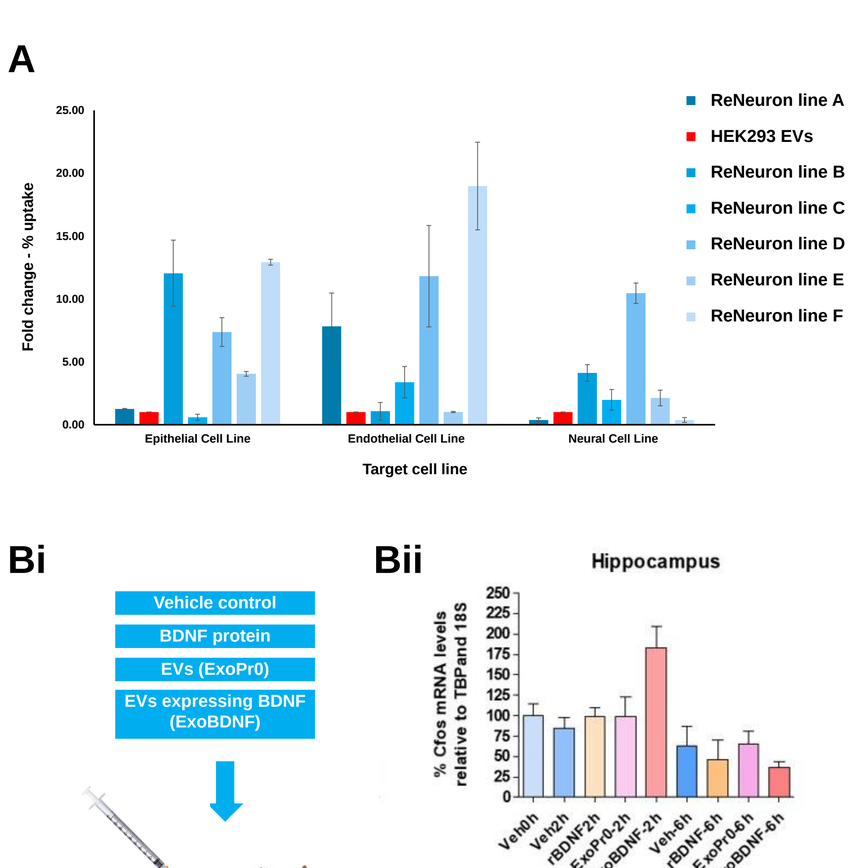


Figure 5. Analysis of EV uptake and *in vivo* payload delivery

(A) A panel of *CustomEx*™ EVs and HEK293 EVs were labelled using a fluorescent maleimide conjugate and purified. The fluorescently labelled exosomes were incubated with 3 target cell lines of epithelial, endothelial and neural origin. Following incubation, target cells were harvested, washed and exosome uptake was measured by flow cytometry. (B) CTX EVs were engineered to express brain-derived neurotrophic factor (BDNF) specially on the EV surface using proprietary ReNeuron's proprietary scaffold. i) Healthy murine hosts were treated with either vehicle control (Vehicle), recombinant human BDNF (rBDNF), native CTX EVs (ExoPr0), or CTX EVs expressing BDNF (ExoBDNF); administered intrathecally (lumbar puncture). ii) After 0, 2 and 6hr, upregulation of the BDNF target gene c-Fos was measured by qRT-PCR in harvested brain regions.

References and Acknowledgements

1. Pollock *et al*, Exp Neurol. 2006 May;199(1):143-55. doi: 10.1016/j.expneurol.2005.12.011
2. Magistretti *et al*, Front Neurol. 2019:6;10:859. doi: 10.3389/fneur.2019.00859

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