EXTRACELLULAR VESICLE ENGINEERING: OPTIMISING NATURE'S NON-IMMUNOGENIC, DRUG DELIVERY PLATFORM

In this article, Anna Cifuentes-Rius, PhD, Research & Innovation Manager at Exopharm, discusses the exciting potential of extracellular vesicles to advance drug delivery, in particular their natural ability to carry API payloads across difficult-to-traverse biological barriers.

With the promise to simultaneously boost both the safety and efficacy of their medicinal cargo, nanoparticle drug delivery technologies that can transport a protected therapeutic payload to target cells are of increasing interest to pharmaceutical, biopharmaceutical and vaccine development. As such, lipid nanoparticles (LNPs) have gained recognition as a useful delivery platform.[1] Tailored LNP formulations were an enabling technology for the novel mRNA vaccines that were swiftly rolled out to help turn the tide of the covid-19 pandemic. LNPs and liposomes have also been deployed to improve the safety and pharmacokinetic profile of small molecule drugs, such as Doxil (doxorubicin – Sequus Pharmaceuticals, CA, US), and to enable *in vivo* delivery of siRNA, such as Onpattro (patisiran – Alnylam Pharmaceuticals, MA, US).

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Despite these notable successes, LNP vectors have not proven to be the therapeutic delivery panacea. Many tissues are still beyond the reach of synthetic drug delivery vectors, and concerns remain over safe and effective redosing. These significant challenges can lead to suboptimal delivery and therapeutic efficacy, as well as potentially harmful side effects. There remains a clear and pressing need for delivery systems that silently shepherd a therapeutic cargo to its intended target tissue.

Although LNP research continues to advance, the discovery and accelerated development of extracellular vesicles (EVs) suggests that these nanoparticles may be naturally suited to drug delivery.[2] EVs are nano-sized lipid vesicles secreted from living cells and present in cell culture media and other biological fluids. EVs have evolved as the body's own system for safely delivering cargo – transferring RNAs, proteins and other bioactive small molecules, for example – from cell to cell, often in a targeted fashion. By harnessing their natural capabilities and modifying them as required using EV engineering techniques, EVs offer significant promise as a valuable addition to the current suite of nanoparticle-mediated drug delivery modalities. As such, EVs are attracting increased attention from the pharmaceutical industry.[3]

EXTRACELLULAR VESICLES – A BRIEF HISTORY

EVs were first described in the early 1980s but were dismissed initially as the garbage bag of the cell.[4] The ejection of these lipid bilayer-wrapped nanoscopic particles by cells was thought to be a cellular waste disposal system, and EVs therefore received little further attention at the time.

The reappraisal of EVs' natural biological role was sparked by the 1996 discovery that EV release and uptake was a key mode by which immune cells co-ordinate their activity against pathogens. EVs' true nature as a delivery system, carrying bioactive molecules between cells was underscored in 2007 by the landmark discovery that EVs can carry an RNA cargo that can directly influence protein expression in recipient cells.

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As the subsequent decade and a half of increasingly intense research activity has established, EVs represent a highly conserved mechanism by which cells of all types communicate with each other. Rather than jettisoned waste, EVs contain a curated cargo of biomolecules, released to deliver a particular message to other cells throughout the body.

Following this extremely exciting discovery, EVs rapidly gained attention in the sphere of stem cell regenerative medicine when it was shown that native EVs released by stem cells were the main active component of the therapy – the EV-rich supernatant collected from cultured stem cells was shown to have the same therapeutic benefit as infusions of the cells themselves. Native EVs released by stem cells and blood platelets have progressed through early phase human clinical trials and are currently under investigation for a range of applications, from wound healing to cosmetics.

More recently, the EV field has been increasingly focused towards drug delivery. A range of techniques have been developed to load EVs successfully with therapeutic cargoes, including small molecule drugs, proteins and RNAs. Overall, the more that the properties of EVs and their natural behaviours in the body have been explored, the more that EVs appear to lend themselves ideally to drug delivery. These characteristics equip EVs with advanced properties that are, in most cases, superior to their synthetic counterparts.

IMMUNOGENICITY: DRUG DELIVERY UNDER THE RADAR

Evidence suggests that EVs have an intrinsic circulatory stability, as befits their role in cell-to-cell communication.[5] EVs naturally have a negatively charged surface and can carry surface signalling proteins, such as CD47, that stave off mononuclear phagocytic system clearance. Their natural lipid bilayer structure does not trigger hypersensitivity or elicit neutralising antibodies. Critically, this immuno-quiet nature should enable ongoing and repeated dosing of EV-loaded therapeutics, in contrast to synthetic systems, such as PEGylated LNPs, where antibody recognition can compromise subsequent dosing.

In terms of toxicity and immunogenicity, decades of data from blood transfusions attest to the safety with which allogeneic EV-rich biofluids can be infused into patients. More specifically, EVs from allogeneic cell sources have been shown not to trigger a significant immunological response.

Pioneering early-stage EV clinical trials have also shown that EVs can be safely administered to humans. While EV technology is evolving rapidly, the low immunogenicity observed thus far indicates their potential in delivering therapeutics when an immune response needs to be avoided, such as with autoimmune diseases.

LEAPING BARRIERS: ENHANCED TARGETING CAPABILITIES

The body can be considered as a nested set of biological barriers, from systemic to intracellular, that keep different biosystems compartmentalised across various length scales. These barriers can be highly restrictive in what may cross, hampering drug delivery to specific cells and tissues.

Arguably, the biggest factors driving the search for new drug delivery modalities are, firstly, the significant resistance met when crossing these barriers and, secondly, the limited tissue-targeting capabilities of systemically delivered therapeutics, either alone or via viral or non-viral vehicles. For example, targeting any cell type besides hepatocytes remains a significant challenge yet to be solved for intravenously injected LNP drug formulations manufactured at clinical scale.

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EVs naturally enjoy privileged status in the body and are actively transported across many barriers. As such, EVs can natively target and deliver a cargo to highly restricted areas of the body, depending upon the type and activation status of the parental cell. For example, EVs released by certain tumour cells show a strong tropism for other tumour cells. Other cell types shown to be targeted by specific EV populations range from epithelial cells to lung fibroblasts to pancreatic cells.

Another illustrative example would be oral drug delivery, where surviving the harsh conditions of the gastrointestinal tract before entering the body through the gut lining represents the first barrier to be crossed. EVs in milk naturally possess oral uptake characteristics and can carry a bioactive cargo from the gut into systemic circulation. During early life, the EVs present in breast milk are a key mode of biomolecular transfer from mother to baby. EVs in cow's milk also possess the necessary protein coating to enter the body through the gut. In fact, bovine milk-derived EVs are currently being developed as oral drug delivery vectors.

Once in circulation – whether by oral, intravenous or other mode of entry – the brain is a key target for many therapeutics currently under investigation, from those targeting neurodegenerative conditions, such as Alzheimer's disease, to those for central nervous system cancers, such as glioblastoma. The blood-brain barrier (BBB) represents one of the most challenging obstacles to therapeutic delivery, restricting the passage of almost 98% of small molecule drugs. EVs have been shown to be able to deliver a functional cargo to the brain. Intranasal delivery of EV therapeutics is one potential route of entry that may be able to bypass the BBB. Intravenous EV infusion has also been shown to be able to deliver a therapeutic across the BBB and into the brain. For example, in a monkey model of cortical brain injury, intravenous administration of EVs derived from stem cells led to significantly enhanced recovery of motor function.[6]

To increase cell selectivity and tissue tropism, interest in designing and engineering EVs to express select cell-targeting moieties on their surface is increasing in laboratories around the world. For example, one development in this field has been the creation of an array of fusion proteins consisting of an EV membrane protein combined with a specific

cell-surface receptor targeting component. Cell types that can be selectively targeted by drug-loaded EVs in this fashion include cardiomyocytes, neurons and tumour cells. EVs derived from readily cultured HEK293 cells are proving to be a platform well suited to the development of engineered EVs for targeted drug delivery. For synergic benefits, it is possible to co-deliver combinations of therapeutic types to target tissues within the same particle.

Further barriers present themselves once a drug delivery capsule reaches its intended cell target. EVs appear to enjoy a natural advantage when crossing the cell membrane barrier. Several studies have shown how EVs can be taken up and deliver their cargo – both small molecule drugs and RNAs – into cells faster and more efficiently than, for example, LNPs.[7,8] Although, at present, EVs may be more challenging than LNPs to load with a high concentration of RNA cargo, EVs' more efficient transfer of RNA into the target cell cytosol can more than outweigh this current shortcoming. Moreover, techniques to measure actual EV loading are still being refined and, currently, may underestimate the true loading efficiency.

Furthermore, EV RNA-loading research and development is progressing rapidly. For example, it was recently shown that cells load specific RNAs into EVs preferentially based on the presence of a particular sequence recognised by the cell. This finding potentially offers an improved way to tap into cells' own RNA-loading machinery to produce EVs that are highly and selectively enriched in a therapeutic RNA.

INNATE POTENTIAL

Understanding of EV biology has come a long way since their initial description as cellular garbage. EVs are now recognised to roam naturally throughout the body, delivering a bioactive cargo from cell to cell in a targeted fashion. As such, EVs clearly warrant thorough investigation as a drug delivery platform. EV properties are particularly attractive in the context of the known limitations of other delivery technologies.

One bottleneck to large-scale, clinical-grade production of EVs has been the isolation of EVs from cell culture media.[9] Research-scale EV purification has typically focused on ultracentrifugation, which is labour-intensive and not directly scalable, however, alternative processes are being found. EV isolation based on the combination of bespoke resins with highly scalable industry-standard ion-exchange equipment and protocols promises to alleviate this bottleneck, and has proven fit for purpose to purify EVs for human clinical trials.[10]

Inspired by their ideal behaviour as drug delivery nano-vehicles, companies specialising in EV-based therapeutics development have established a growing expertise in large-scale production and harvesting of EVs, loading EVs with a specific therapeutic cargo and decorating the EV surface with selective targeting moieties. These small, cell-derived vesicles appear to offer big potential for targeted drug delivery.

ABOUT THE COMPANY

Exopharm (ASX:EX1) is a clinical-stage biopharmaceutical company that uses EVs to deliver a new class of transformative medicines. Using Exopharm's technologies, the company provides advanced customisation of EVs to improve delivery of active ingredients, including DNA, RNA, small molecules and proteins, to selected cell types and organs. Moreover, Exopharm's LEAP manufacturing technology provides access to large quantities of high-purity EVs for research and clinical uses. Exopharm uses variations and combinations of these technologies to pursue its own product pipeline as well as to enable its biopharma partners to improve delivery of their drug candidates.

ABOUT THE AUTHOR

Anna Cifuentes-Rius, PhD, is responsible for bridging Exopharm's innovation and commercial teams, helping shape Exopharm's research and business development strategy. Before joining Exopharm, Dr Cifuentes-Rius was an academic scientist leading a research team towards the development of targeted nanoparticles for drug delivery. She has worked at world-leading universities, such as the Monash Institute of Pharmaceutical Sciences (Parkville, Australia) and Massachusetts Institute of Technology (MA, US). Dr Cifuentes-Rius's innovations have resulted in 30 publications, one patent and over AUS$1 million (£564,000) in secured funding. She has been awarded several awards in recognition of her emerging leadership, such as the 2020 MIPS Early Career Researcher Award.

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