

SPECIAL ISSUE ARTICLE

The emerging role of exosomes as novel therapeutics: Biology, technologies, clinical applications, and the next

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Abstract

The extracellular vesicles (EVs) research area has grown rapidly because of their pivotal roles in intercellular communications and maintaining homeostasis of individual organism. As a subtype of EVs, exosomes are made *via* unique biogenesis pathway and exhibit disparate functional and phenotypic characteristics. Functionally, exosomes transfer biological messages from donor cell to recipient cell, which makes exosomes as a novel therapeutic platform delivering therapeutic materials to the target tissue/cell. Currently, both academia and industry try to develop exosome platform-based therapeutics for disease management, some of which are already in clinical trials. In this review, we will discuss focusing on therapeutic values of exosomes, recent advances in therapeutic exosome platform development, and late development of exosome therapeutics in diverse therapeutic areas.

KEYWORDS

clinical trial, exosomes, inflammatory diseases, therapeutic platform

1 | INTRODUCTION

For maintaining homeostasis of organisms, cell-to-cell communication controls multiple biological processes such as cellular proliferation, differentiation, apoptosis, biological mediator secretion, and so on. Cellular communication is mediated largely by either direct cell-to-cell contact or soluble factors including cytokines/chemokines, hormones, neurotransmitters, lipid mediators, and extracellular vesicles (EVs).¹⁻³ Of these, EVs are emerging as novel intercellular communication mediators in both normal and pathophysiological conditions.

EVs can be classified into three major subtypes including microvesicles, exosomes, and apoptotic bodies which show different characteristics in size (microvesicles:50-1,000 nm, exosomes:30-200 nm, and apoptotic bodies:1-5µm), content, morphology, and biogenesis

mechanism.^{1,4} Especially, both microvesicles and exosomes act as safe containers of diverse biological molecules for intercellular communication and play a crucial role in cell-cell communication *via* delivery of functional proteins, nucleic acids, and lipids. In this review, we will provide overview focusing on exosome biology, therapeutic values of exosomes, recent advances in exosome therapeutics development.

1.1 | Exosome biogenesis

The biogenesis of exosomes has been proposed *via* either endosomal sorting complex required for transport (ESCRT)-dependent or ESCRT-independent pathway. ESCRT consists of about 20 different kinds of proteins that can be grouped into four complexes (ie,

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ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III), and each of complexes has its own unique role during exosome biogenesis.⁵ In ESCRT-dependent pathway, ESCRT-0 initiates the pathway by engaging ubiquitinated cargo proteins which are sequestered in the pre-MVB endosomes (intra-vesicle containing endosome). Both ESCRT-I and ESCRT-II bind ubiquitinated proteins to sort the proteins into the bud of endosome. ESCRT-II coordinates assembly of ESCRT-III, vesicle scission complex, and subsequently, ESCRT-III cleaves fully established vesicles from the membrane.⁶⁻⁹ At the end of the pathway, these ESCRT complexes are disassembled and recycled for next round of exosome biogenesis. In the ESCRT-independent pathway, membrane lipid components including sphingomyelinases and ceramide induce spontaneous budding of intra-luminal vesicles (ILVs) into MVBs.^{10,11} The interactome study of tetraspanin-enriched microdomains (TEMs) by mass spectrometry revealed that tetraspanins are involved in exosome cargo sorting processes in human lymphoblast derived exosomes.¹² Finally, in both exosome biogenesis pathways, via actin and microtubule filament-mediated transport system exosomes in MVBs are secreted out extracellularly.^{13,14} During this trafficking process, molecules including cortactin and Rab GTPases play a pivotal role in MVB and plasma membrane docking for exosome secretion.^{15,16}

Exosomes secreted via biogenesis pathways show heterogeneity in terms of size and composition of biological cargos even generated from same producing cells.^{17,18} On the contrary, exosome biomarkers which are universally contained in the exosomes have been identified¹⁰: Proteins including heat-shock proteins (eg, HSP70 and HSP 90), MVB biogenesis proteins (eg, Alix and TSG101), and tetraspanins (eg, CD9, CD63, and CD81) are commonly loaded in/onto exosome and used as exosome biomarkers.^{10,19} Although exact biological function is not fully uncovered, it has been known that various forms of nucleic acids including fragmented DNA, mRNA, miRNA, piRNA, snoRNA, snRNA, rRNA, tRNA, Y-RNA, and scRNA can be loaded into exosomes.²⁰ Exosomes also contain lipids including cholesterol, phosphatidylserine (PS), sphingomyelin, phosphatidic acid, sphingolipid, and ceramide, of which cholesterol, PS, and sphingolipid are mostly found in the exosomal membrane.²¹

1.2 | Clinical applications of exosomes

As of August 12, 2020, a key word search in the US-NIH clinical trial database with "exosomes" gives 182 clinical studies search result.²² In most of these studies, exosomes are being used as diagnosis biomarkers from body fluids such as blood and urine based on the findings that in vivo circulating exosomes carry biological materials (eg, proteins and nucleic acid) representing current physiological conditions. For example, miRNA expressions such as miR17-92, miR-15, and miR-1246 are highly associated with multiple types of cancer, which are readily detected by analyzing circulating exosomes from the patients. Therefore, miRNA analysis of circulating exosomes can be used as non-invasive method for cancer diagnostics.^{23,24}

Now, the therapeutic potential of exosomes is also being investigated.^{17,25} As it has been well known that exosomes inherit many physiological characteristics of originated cells, exosomes can be used as cell-free therapeutics with comparable potency but better safety profile compared to original cell therapy. Moreover, therapeutic potential of exosome as therapeutic vehicles is also being investigated by loading active pharmaceutical ingredients (API) into exosomes to deliver APIs safely and specifically to the target tissues/cells. Now, the exosome therapeutics field has been hot up in the past few years, and as described below, a number of companies are actively developing exosome-based therapeutics in various disease areas (Table 1).

So far, there are couple of companies in clinical stage of exosome therapeutics development: Codiak Biosciences is one of the major biotech start-up companies developing exosome therapeutics and is dedicated to develop therapeutic exosomes to deliver siRNA API targeting KRAS (G12D) mutation in pancreatic cancer.²⁶ Recently, they got investigational new drug (IND) approval for Phase Ia/b study in pancreatic cancer patients from US FDA (NCT03608631). The company has also teamed up with Sarepta Therapeutics, the leader in precision genetic medicine for rare disease, to develop engineered exosome therapeutics to deliver RNA for neuromuscular diseases. Avalon Globocare is developing engineered exosome therapeutics, and with its lead pipeline, AVA-201 (miR-185 loaded exosomes produced from mesenchymal stem cells) in Leucoplakia, the company plans to have its first clinical study in United States in

TABLE 1 Biotech companies preparing for exosome human clinical trials

Product	Development stages	
Type of exosome	Pre-clinical	Phase I and IIa
Naïve exosome	ExoCoBio	Aegle Therapeutics
	Capricor	Exopharm
	AgeX Therapeutics	United Therapeutics
	ReNeuron	
	Avalon	Codiak (Not yet recruiting)
	Evox	
Engineered exosome	ILIAS	
	Carmine Therapeutics	
	Aruna Bio	
	Anjarium	
	Adipomics	
	Brainstorm Cell Therapeutics	
	Exocure Biosciences	
TriArm Therapeutics		

Note: Naïve exosomes are naturally produced by cells. Engineered exosomes use exosomes loaded up with other material such as proteins, nucleic acids, or other biomolecules.

the year 2020.²⁷ In the same year, Aegle Therapeutics also plans to have a Phase I/IIa clinical study in dystrophic epidermolysis bullosa patients with their naïve regenerative exosomes, AGLE-102, isolated from bone marrow mesenchymal stem cells (NCT04173650). Exopharm has started its PLEXOVAL Phase I study using exosomes isolated from human platelets for wound healing (NCT number, undisclosed). United Therapeutics has IND approval for a Phase I trial (NCT03857841) of UNEX-42, EVs secreted from human bone marrow-derived mesenchymal stem cells, against bronchopulmonary dysplasia, a condition common in preterm infants that receive assisted ventilation and supplemental oxygen. There are multiple other exosome therapeutics companies in pre-clinical stage of their pipelines: Evox Therapeutics has developed technologies which allow API molecules (eg, proteins or nucleic acids) to be actively loaded into the exosomes as membrane anchored molecules. The company's lead pipeline targets a lysosomal storage disorder called Niemann-Pick Disease type C. Recently, Evox Therapeutics has licensed-out its exosome-based RNA interference/antisense RNA delivery technology to US big pharma Eli Lilly for neurological disease. ILIAS Biologics is also developing exosome therapeutics. The company has unique patented technology which allows active loading of large size proteins into luminal side of exosomes. With its current lead therapeutic candidate targeting inflammatory diseases, ILIAS Biologics plans to submit the IND application to the US FDA in the year 2021. ILIAS Biologics has other therapeutics pipelines in various disease indications which are mostly at the pre-clinical stage of development. Compared to cell therapies, exosomes might be able to overcome safety issues surrounding persistent cell proliferation, while having the same therapeutic efficacy. Capricor Therapeutics, a company originally developing stem cell therapies using cardiospheres heart stem cells, is now evaluating therapeutic value of exosomes derived from cardiospheres to treat cardiac and inflammatory conditions.²⁸ ReNeuron, a CTX stem cells therapeutics company, also is investigating the possibility to use CTX stem cells derived exosomes as drug- and gene-delivery vehicles in stroke to promote post-stroke rehabilitation.²⁹

Currently, there are about 20 biotech companies around the world developing exosome therapeutics with various therapeutic exosome platform technologies.^{30,31} Some of these companies already moved on to more promising stage of clinical development with their own exosome engineering technologies improving efficacy and safety profiles. Next, we will overview currently available exosome therapeutic platforms and their potential use in multiple therapeutics areas.

1.3 | Therapeutic exosome platforms and their potential use in clinic

Exosomes are increasingly being recognized as novel modulators for different therapeutic purposes, including vaccination, biological targeting agents, and drug delivery tools.³²⁻³⁴ Thus, several applications for exosome-mediated therapy have been proposed. Herein,

we will focus on the application of exosomes as drug delivery systems. Therapeutic exosome platforms can be largely sub-grouped into either naïve exosomes or artificial exosomes: Naive exosomes are therapeutics exosomes derived from non-engineered producing cells. Artificial exosomes are therapeutics exosomes either derived from engineered producing cells or made via in vitro engineering post-isolation. More details of each therapeutic exosome platform will be discussed as following.

1.3.1 | Naïve/natural exosomes

As natural cell-derived exosomes, known as naïve exosomes, by carrying not fully identified functional molecules inherit functional characteristics from their parent cell (Figure 1A), they have been effectively employed to provide an alternative way of developing strategies to cure or modify disease processes.³⁵⁻³⁸ Recent studies have suggested that mesenchymal stem cell (MSC)-derived exosomes might represent as novel therapeutics with advantages over parent MSCs such as lower immunogenicity and tumorigenicity.^{37,38} Phase I clinical trials evaluating therapeutic potential of MSC-derived naïve exosomes have been either completed or are underway in multiple disease areas (NCT04173650 and NCT03857841).²² Although too early for clinical use, these clinical studies together with pre-clinical observation suggest that MSC-derived naïve exosomes may hold promise for a cell-free therapeutic strategy.

1.3.2 | Artificial exosomes with engineering technologies

To improve API loading efficacy and in vivo target specificity, multiple exosome engineering technologies are being used. Especially, related to API loading into exosomes, currently there are two major technical approaches are being used including passive or active API loading into exosomes.

The passive cargo-loading methods involve an incubation procedure that recruit bioactive agents into exosomes through sonication, electroporation, freeze-thaw cycles, and extrusion (Figure 1B).³⁹⁻⁴⁴ However, a major drawback of this method is that during the membrane disruption process, there is high potential of damaging exosome structure with compromised targeting properties.⁴⁵⁻⁴⁷ The active cargo-loading methods provide cells with the means to incorporate API proteins or nucleic acids into exosomes during their biogenesis process. The cargo can be endogenously loaded by genetically modifying the parental cells to overexpress desired API proteins or nucleic acids of interest (with or without additional modification to promote cargo packaging), which is then naturally incorporated into the exosomes. One of active cargo-loading technologies is anchoring cargo on exosome inner surfaces via transgene expression in parent cells (Figure 1C): System Biosciences, an exosome biotech company, developed specific peptide sequences which target interior exosomal membrane proteins, allowing the fusion protein to

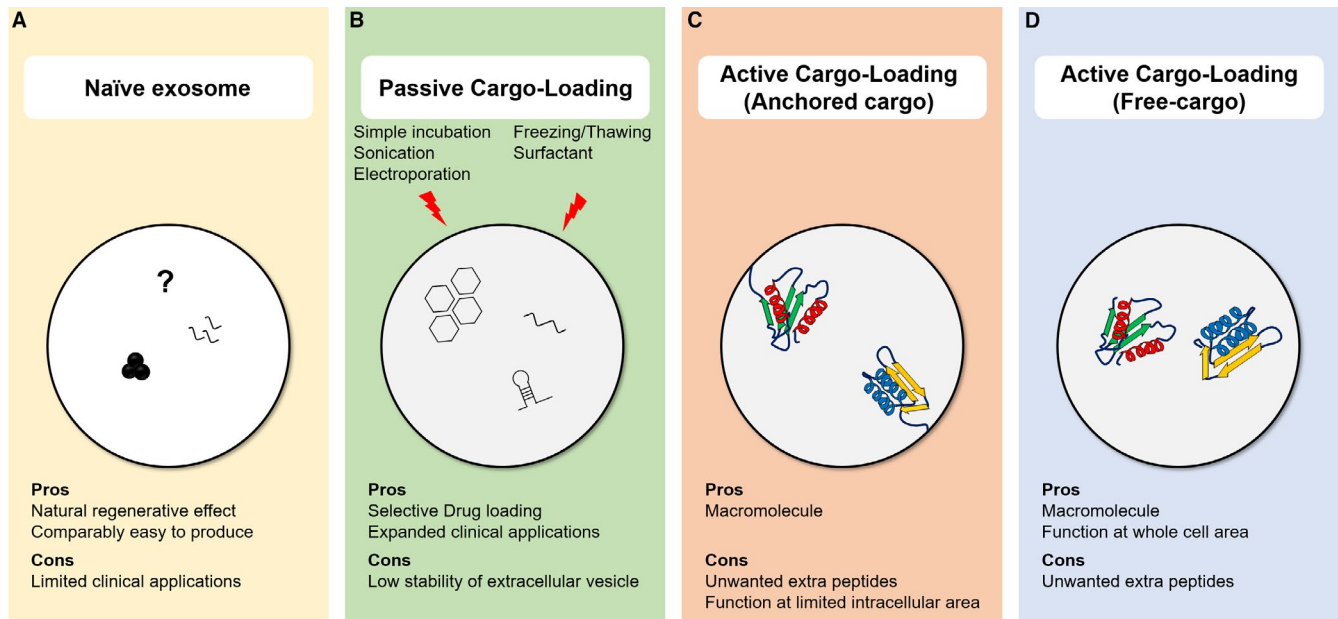


FIGURE 1 Exosome engineering methods for encapsulation of therapeutic agents. The techniques of loading cargo into exosomes can be divided into four approaches. A, Naïve exosomes are relatively simple technique, but the bioactive molecules contained in them are unknown. B, Passive methods are based on the use of sonication, Freezing/Thawing cycles, or electroporation to destabilize exosome membrane integrity, allowing drugs to be introduced into exosomes. C and D, Active methods spontaneously load interested molecules by hijacking the exosome biogenesis pathway. These techniques are divided into two approaches depending on whether the loaded cargo can reside as free form in the exosome

be packaged into exosomes for secretion.⁴⁸ Evox Therapeutics also developed another API loading techniques by simply making fusion proteins between protein API and exosomal membrane proteins such as CD63, CD81, and syntenin.⁴⁹ However, the caveat this approach is that the API remains attached to the exosomal membrane in the target cells, which may significantly limit their biological function in the target cells. Alternatively, a novel technology called “EXosomes for Protein Loading via Optically Reversible protein-protein interaction (EXPLOR)” has been developed by ILIAS Biologics to overcome this limitation.⁵⁰ EXPLOR technology makes it possible for getting high API loading yield into the exosomes with controllable way that in the target cells protein APIs can be delivered as free form to show unlimited biological function (Figure 1D). The next question is how we can achieve target-specific delivery of loaded API without premature immune-mediated clearance. By applying various exosome tissue targeting technology to their own API loading platforms, exosome therapeutics companies try to provide solutions for this question: System Biosciences employed exosome surface engineering post exosome isolation which decorates the outer membrane of exosomes with streptavidin to bind to biotinylated targeting moiety such as target-specific single-chain variable fragment (ScFv). This technology enables exosome-based targeted delivery of API to HIV-infected target cells in vivo. Codiak Biosciences uses Prostaglandin F2 Receptor Inhibitor (PTGFRN) and Brain Abundant Membrane Attached Signal Protein 1 (BASP-1) proteins as scaffolds for anchoring of direct targeting ligands and therapeutic molecules to the surface and the lumen of exosomes, respectively. However, these engineered exosomes need to be considered for potential drawback,

including induction of immunogenicity by exogenous modification, unlike naïve exosomes. Although several studies showed that the engineered exosomes can deliver therapeutic RNAi safely into target tissues/cells with minimal immunogenicity even after repeated doses,^{26,41,51} still it is not known whether the cargo has no immunogenic activity or not.

Next, we will overview more details about this EXPLOR technology and discuss the clinical potential of “Exo-target,” therapeutic exosomes with EXPLOR technology, in multiple disease areas.

1.3.3 | Artificial exosomes with EXPLOR technology (Exo-target)

EXPLOR technology utilizes photo-reactive binding modules, cryptochrome2 (CRY2) and N-terminal of CRY-interacting basic-helix-loop helix 1 (CIBN) isolated from *Arabidopsis thaliana*. In Exo-target producing donor cells, CRY2 protein is fused to a cargo protein API, and CIBN is conjugated with a representative marker of exosomes, CD9 protein. Blue-light illumination induces the reversible protein-protein interaction (PPI) between CIBN and CRY2 fusion proteins.^{52,53} With continuous blue-light irradiation, the cargo proteins are guided to the inner surface of the cell membrane or the surface of early endosomes. Mature MVBs then readily secrete cargo protein-carrying exosomes, Exo-target, from the cells by membrane fusion with the plasma membrane. After exocytosis, Exo-target can be easily isolated and purified from producing cell culture supernatant with blue-light removal which allows the exosome loaded cargo

protein APIs present as free form in the luminal side of Exo-target. Purified Exo-target can be used for cargo protein APIs delivery into the cytosolic compartment of target cells *via* membrane fusion or endocytosis processes (Figure 2).

Recently, Exo-srI κ B, Exo-target loaded with super-repressor I κ B (srI κ B) by EXPLOR, was proposed as a potential medicine in inflammatory disease such as sepsis and preterm birth (PTB).^{54,55} The srI κ B indicates a degradation-resistant I κ B mutant in which both Ser32 and Ser36 are replaced to Ala. It blocks nuclear translocation of NF- κ B even in the presence of pro-inflammatory stimuli and thus inhibits the expression of specific NF- κ B target genes. As 293T cell-derived exosomes are one of the most widely used as tools for drug delivery,⁵⁶⁻⁵⁸ Exo-srI κ B was generated from HEK293T cells. The role and potential of Exo-srI κ B in sepsis and PTB are as follows.

Sepsis is a syndrome associated with severe infection and is one of the leading causes of deaths world-wide. Yet there are no specific therapies available and mortality continues to be high especially in low-income countries. Based on findings from pre-clinical studies, there is growing interest on therapeutic intervention for nuclear factor (NF)- κ B pathway to limit inflammatory injury during sepsis.^{59,60} To date, numerous small molecule inhibitors for NF- κ B pathway have been developed, but none of them have been clinically approved.⁶¹ Recently, ILIAS Biologics generated Exo-srI κ B and clearly verified its therapeutic potential in sepsis pre-clinical models.^{50,54} The data from Exo-srI κ B *in vivo* pre-clinical studies showed the srI κ B pharmacology, driving significantly greater anti-inflammatory activity in septic animal models: Exo-srI κ B were administered intraperitoneally (i.p.) by either single or multiple dose (four times every 6 hours, 10⁹ particles/dose) in lipopolysaccharide (LPS)-induced or cecum ligation and puncture (CLP)-induced septic mouse model, respectively. In these septic mouse models, Exo-srI κ B treatment ameliorates systemic inflammation associated mouse mortality and morbidity compared to naïve exosome treatment (*ie*, Exo-naïve) generated from same parent cells.⁵⁴ Neutrophils and macrophages are major components of the innate immune system and play critical roles in orchestrating host immune response during sepsis.⁶² At the early phase of sepsis, they secrete an increased level of pro-inflammatory factors, which exacerbate the inflammatory response and subsequently contribute

to disease mortality and morbidity.⁶³ In septic animals, administered Exo-srI κ B via intravenous (iv) injection are targeted to these innate immune cells within 30 min and inhibit overwhelming pro-inflammatory response (*ie*, TNF- α , IL-1 β , and IL-6), which subsequently ameliorates tissue/organ damages including kidney and liver. Taken together, these findings suggest therapeutic potential of Exo-srI κ B in treating hyper-immunity of sepsis which has significant medical unmet need (Figure 3A).

Although the causes of PTB are numerous, it is well known that inflammation represents a highly significant risk factor in PTB.⁶⁴⁻⁶⁶ Current PTB prevention strategies are that minimizing contractions of maternal uterine tissues to prolong labor or administering corticosteroids to provide enough time for fetal lung maturation. Multiple drugs (*eg*, tocolytics and progesterone) have been developed; however, none have significantly reduced the risk of PTB in nulliparous subjects over the past 30 years.⁶⁷ The potential therapeutics for PTB include cytokine-suppressive anti-inflammatory drugs,⁶⁸⁻⁷⁰ cell-penetrating peptides and small molecule inhibitors,⁷¹⁻⁷³ non-specific inhibitors of NF- κ B pathway,⁷⁴⁻⁷⁷ and flavonoids.^{78,79} However, very few of these drugs have been in clinical trials and none are in-use clinically due to key issues such as placental permeability, *in vivo* half-life and stability, effectiveness in reducing the fetal inflammatory response, and their toxicity.

Recently, University of Texas Medical Branch and ILIAS Biologics reported fetal-maternal communication during pregnancy is mediated by exosomes.⁵⁵ They used mT/mG, a cell membrane targeted and two-color fluorescent Cre reporter mouse. Prior to Cre-mediated excision, membrane-localized tdTomato (mT) expression is widespread in cells/tissues. Cre recombinase expressing cells express membrane-localized EGFP (mG) fluorescence replacing the mT fluorescence to identify placental permeability of exosomes, Exo-Cre (Cre-loaded exosomes by EXPLOR) were injected intraperitoneally into pregnant mT/mG mice. The maternally administered Exo-Cre was transferred fetal tissues across placental barriers, inducing expression of mG instead of mT. These results propose that exosomes are able to have a therapeutic effect on PTB by passing through the placenta. NF- κ B is a well-known mechanistic mediator in infection-associated PTB.⁸⁰⁻⁸⁴ In infection-induced PTB pre-clinical

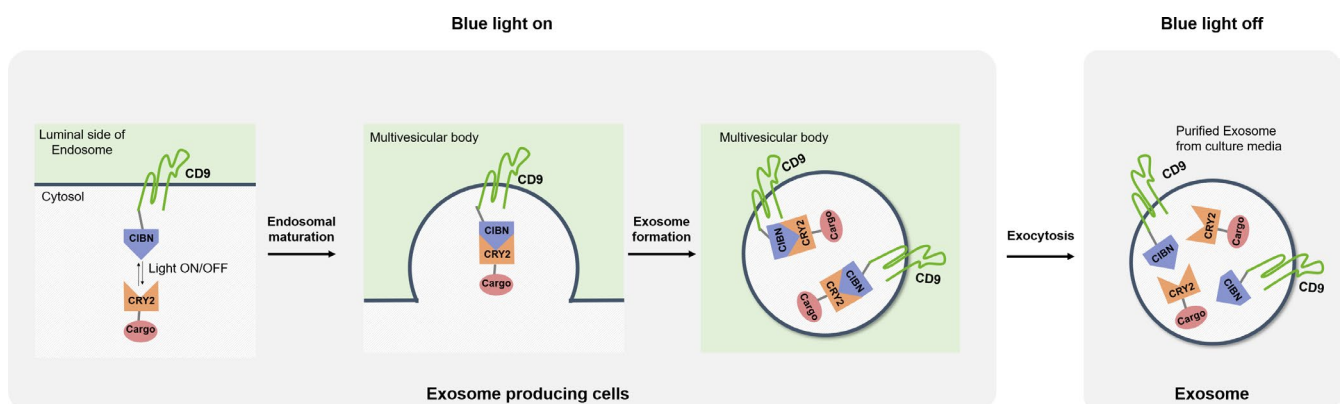


FIGURE 2 Scheme of EXPLOR technology. The cargo proteins are actively loaded into exosomes under light irradiation. The interaction is reversible; thus, the cargo proteins can be detached from exosome membrane as free forms in the lumen of exosomes

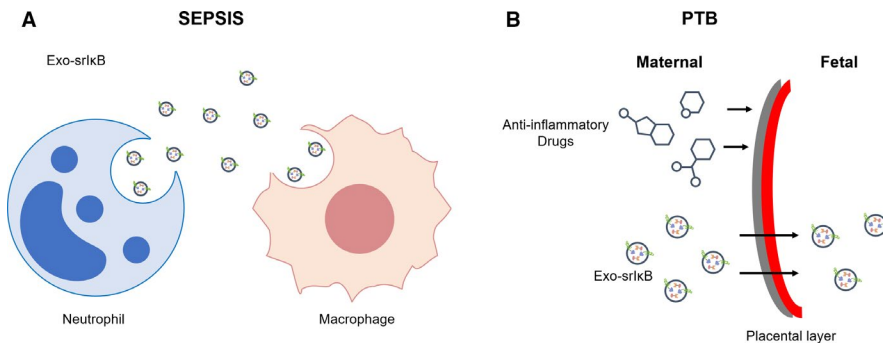


FIGURE 3 The role and therapeutic potential of Exo-srlkB in inflammatory disease. A, Exo-srlkB is successfully delivered to target neutrophil and macrophage in sepsis model. B, Unlike small molecules, Exo-srlkB can inhibit/delay preterm birth by penetrating the placental layer and delivering drug substances to fetus

model, Exo-srlkB treatment delayed PTB by 24-hours, improved pup viability, and significantly reduced histologic chorioamnionitis in fetal, not maternal (unpublished data). Taken together, these findings suggest Exo-srlkB may provide a potential intervention for reducing the host inflammatory response associated with PTB (Figure 3B).

1.4 | The challenges for bringing exosomes into the clinic

Up to now, there are enough scientific evidence accumulated to support therapeutic value of exosomes in various human diseases. For successful exosome therapeutics development, the CMC (*ie*, chemistry, manufacturing, and control) development for good manufacturing practice (GMP) grade therapeutic exosome production needs to be established. The CMC development for exosome therapeutics covers multiple area including establishing master cell bank (MCB), process development for large quantity of exosome production/isolation and quality control (QC)/analytical method development for therapeutic exosome production.⁸⁵⁻⁸⁸ As exosome is a novel therapeutic platform, in the field so far there is no gold standard process established for the CMC of exosome therapeutics. Currently, most of exosome therapeutics companies are working on developing CMC process suitable for their own exosome therapeutic platforms.⁸⁹ Here, we will briefly overview the biggest hurdles we are facing to solve for successful exosome therapeutics development and current status of CMC development in the field.

As exosomes are products secreted from cells, cell selection is crucial first step for generating exosomes without changing the composition and function of the exosomes. In order to produce exosomes in small or large scale, several types of cells including human cardiac progenitor cells, bone marrow mesenchymal stem cells, adipose tissue-derived stem cells, monocyte-derived dendritic cells, and HEK293 cells have been cultured in fixed or moving culture systems such as the flask and bioreactor.⁸⁹⁻⁹² Further studies are focusing on developing exosome producing stable cell-lines which can produce API loaded exosomes with better yield and less variability. In parallel, scalable culture system in optimal serum-free culture condition using well-established bioreactors is desired to manufacture clinical grade exosomes.

Next, establishing a robust exosome purification process is also crucial to obtain high yield and purity therapeutic exosomes.⁹³

During exosome therapeutics production, impurities such as host cell proteins, DNA, cellular debris, and non-exosomal EVs (*eg*, apoptotic bodies and microvesicles) should be controlled and removed as thoroughly as possible.^{94,95} To increase production yield, diverse methods for exosome separation and concentration from the cell culture medium have been applied: ultracentrifugation using centrifugal force and weight, size-based separation methods using column filled with porous polymeric beads and tangential flow filtration using ultrafiltration membranes, precipitation-based separation methods using polymer and protein organic solvent, immunoaffinity-based separation methods using specific bindings between exosome membrane-bound antigens and immobilized antibodies, ion-exchanged chromatography based on electrostatic interaction, and microfluidics-based separation using microfluidic control technology.⁹⁶⁻¹⁰¹ Still, there are significant needs for technical improvement for these methods to improve exosome separation capability, scalability, yield, and cost for commercialization.

QC/analytical method development for exosome therapeutic product is essential for successful exosome therapeutics development. Certainly, current limitation in exosome analytical technologies is one of biggest huddles for large-scale exosome manufacturing.^{87,88,102} Currently, exosomes are being defined and identified according to the proteomic identification criteria including transmembrane proteins (*eg*, tetraspanins) and cytosolic proteins (*eg*, TSG101, Rab proteins, or annexins), established by the International Society of Extracellular Vesicles (ISEV).¹⁰³ Although electron microscopy and particle size distribution analysis methods are generally accepted in the field, more advanced and accurate methods to evaluate morphology and size of exosomes are also required. Additional more reliable *in vitro* or *in vivo* exosome QC and characterization methods are needed, which can possibility be achieved with advanced technologies using chromatography, nanoFACS, microfluidics analysis, etc.^{87,102}

None the less, by overcoming these technical difficulties of manufacturing, several leading exosome biotech companies successfully already entered or are entering into clinical trials, albeit in an early stage or MSC-derived naïve exosomes. Exopharm (Australia)'s purification technology called Ligand-based Exosome Affinity Purification (LEAP) was applied to Plexaris, which is being developed as a wound treatment at Phase I clinical stage in Australia. The company demonstrates that LEAP technology enables production and purification of clinical grade and scale exosome, and

takes exosome-based therapeutics to clinical trials successfully.¹⁰⁴ Kalluri's group in University of Texas MD Anderson Cancer Center reported a large-scale production of GMP-compliant exosomes from bone marrow-derived MSCs loaded with siRNA to target Kras G12D available for clinical use for the first time in the world.²⁶ Aegle Therapeutics developed a new technology for the isolation and purification of extracellular vesicle including exosomes with high purity and therapeutic grade. The company demonstrates the method offers a simple large-scale production and purification of extracellular vesicles without complicated equipment at GMP condition. However, the details of the techniques listed above about how their exosomes are produced and isolated have not been released. ILIAS Biologics¹⁰⁵ employs engineered suspension cells to produce therapeutic exosomes by the EXPLORs technology in single-use bioreactors. Master and work cell banks for non-clinical and clinical production were successfully established from stabilized cells, based on productivity, viability, and characteristics. The company optimized cell culture scale and manufacturing process to produce large quantities of a variety of homogeneous engineered exosomes in the high yield and purity. To isolate highly pure exosomes, culture media harvested from the bioreactors is processed through centrifugation, filtration, chromatography, and concentration. Purity, contents, and potency in the final products are analyzed by size-exclusion chromatography, immunoblotting, nanoparticle tracking analysis, protein quantification, and cell-based activity assay. With active pre-clinical pharmacological and toxicological studies for first-in-human study, ILIAS Biologics is currently attempting to develop robust GMP production process that allows exosome therapeutics to translate into clinic.

2 | SUMMARY AND PERSPECTIVE

In both normal and pathophysiological conditions, exosomes play pivotal roles as messenger delivering biological messages between tissues/cells. Due to their biological function and characteristics, exosomes can be utilized as novel biological platform for disease diagnose and therapeutics. With non-invasive liquid biopsies, circulating exosomes can be analyzed to determine the presence of cancer biomarkers including oncogenic proteins and nucleic acids (eg, miRNAs, mRNAs, and DNAs). Therapeutically, due to their unique characteristics as EVs transmigration tissue barriers (eg, blood-brain barrier and placenta), high yield capacity for intercellular cargo delivery, high biocompatibility and low immunogenicity, exosomes are actively being studied as therapeutic vehicles by loading many different forms of therapeutic APIs into exosomes via multiple exosome engineering technologies. With demonstrated therapeutics potentials in pre-clinical studies, there are exosome biotech companies entering into clinical development including ILIAS Biologics. Although there are still significant technological hurdles present for large-scale manufacturing, these clinical stage leading companies are paving the way for successful exosome therapeutic development by overcoming scientific and technological challenges, which

can give clear guidance for other following exosome therapeutics companies. Finally, with scientific and technological innovation in exosome biology, we expect to have successful novel therapeutic platform development to treat the various diseases having significant medical unmet needs in the near future.

CONFLICT OF INTEREST

C.C is an inventor of a patent related to this work filed by ILIAS Biologics Inc (no. KR 10-1877010 and US 10,702,581), the founder and shareholder of ILIAS Biologics Inc YS, YK, JY, and CHP are minor shareholders of ILIAS Biologics Inc SS-M. is a paid consultant for ILIAS Therapeutics. All other authors declare that they have no conflict of interests.

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