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REVIEW ARTICLE OPEN ACCESS

Extracellular Vesicle Lipids and Their Role in Delivery

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ABSTRACT

Small extracellular vesicles (sEVs) possess many advantageous characteristics which highlight their potential as nanocarriers for biomedical applications, including the ability to cross the blood brain barrier, improved biocompatibility and exhibit tissue tropism. Despite this potential, the clinical translation of sEVs has been hindered by a variety of factors and lipid nanoparticles (LNPs) remain as the gold standard for nanocarriers, indicating a knowledge gap which could unlock the potential of sEVs. A growing body of research suggests that the lipid profile, rather than the proteome, of sEVs may be contributing to these beneficial characteristics much more than previously thought. This review highlights and discusses the current state of the field in terms of lipid composition between sEVs originating from various cell sources and the roles which the different lipids play in the function of sEVs as natural nanocarriers within the body. We also discuss the potential of various EV-mimetics and synthetic EVs (synEVs) in terms of clinical translation which may provide a means to allow wider therapeutic adoption of EVs.

1 | Introduction

1.1 | Extracellular Vesicles

The communication between cells is integral to their function and organisation within a multicellular organism to create a cohesive cellular community and maintain tissue homeostasis (Gurunathan et al. 2019). This intercellular communication is achieved through multiple mechanisms including direct cell-to-cell contact, the secretion of specific molecules and via extracellular vesicles (EVs) (Gurunathan et al. 2019). EVs are particles released by all cells that contain a range of biological molecules including proteins and nucleic acids, encapsulated within a lipid bilayer membrane. There are two main subpopulations including exosomes and ectosomes. Ectosomes are generated via outward budding of the plasma membrane and include the subclasses oncosomes (1000–10,000 nm), apoptotic bodies (100–5000 nm)

and small ectosomes (<100 nm) (Dixon et al. 2023; Meehan et al. 2016; Van Niel et al. 2022; Zhang et al. 2018). Exosomes, however, are produced through repeated inward budding of the plasma membrane forming intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). These MVBs subsequently fuse with the plasma membrane to release the exosomes into the extracellular space (Van Niel et al. 2022) and they range in size from ~50 to 150 nm (Dixon et al. 2023). Due to the difficulty in differentiating between the EV subtypes the International Society for Extracellular Vesicles agreed the term small Extracellular Vesicle (sEV) was to be used for EVs less than 200 nm in size (Théry et al. 2018). Of the different classes of EVs the most extensively researched for therapeutic application has been exosomes (Shrivastava and Morris 2021). Therefore, to maintain the correct terminology the term sEV shall be used in place of exosomes to indicate “exosome-like” biological nanoparticles unless referring specifically to the exosomal pathway of EV formation.

Abbreviations: BBB, blood brain barrier; CE, cholesterol esters; Chol, cholesterol; CNS, central nervous system; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOPS, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine; EV, extracellular vesicle; GP, glycerophospholipid; LNP, lipid nanoparticle; LPC, lysophosphatidylcholine; MPS, monocyte-phagocyte system; MSC, mesenchymal stem cell; MVB, multivesicular bodies; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEG, poly(ethylene) glycol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIP, phosphatidylinositol-phosphorylated; PS, phosphatidylserine; SIP, sphingosine-1-phosphate; sEV, small extracellular vesicle; SM, sphingomyelin; SP, sphingolipid; synEV, synthetic extracellular vesicle; TME, tumour microenvironment.

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sEVs contain functional proteins (Simpson et al. 2009), lipids (Vidal et al. 1989), carbohydrates (Mohseni et al. 2025) and a wide array of cellular nucleic acids including messenger RNA (mRNA) (Valadi et al. 2007), micro-RNA (miRNA) (Hunter et al. 2008), and many other types. This carrying capacity, in conjunction with the innate ability of sEVs to deliver their cargo to other cells, is central to their function in intercellular communication as well as their major role in many physiological processes. As a result, they are implicated in a wide array of normal and pathological states involving intercellular communication, including mammalian reproduction/development, immune responses to infection, angiogenesis and many more (Kalluri and LeBleu 2020; Kaur et al. 2014). Pathological roles of sEVs can be seen in processes such as inflammation and cancer (Chow et al. 2014; Wu et al. 2016), inflammatory bowel disease, neurodegenerative disease, type 2 diabetes, obesity and rheumatoid arthritis (Console et al. 2019; Scrivo et al. 2011). Interestingly, the dual role of sEVs is highlighted in their putative connection with the pathogenesis of neurodegeneration. In this, sEVs could be involved in both the clearing of misfolded proteins, providing a neuroprotective function, or the aggregation of misfolded proteins, promoting protein aggregates and contributing to the progression of the disease (Kalluri and LeBleu 2020).

1.2 | Beneficial Characteristics of sEVs

As sEVs are extensively produced by most if not all cells they are widely spread throughout the body and have been isolated from multiple bodily fluids including blood, lymph, bile, urine, cerebrospinal fluid and milk (Zhu et al. 2020). This widespread biodistribution, together with their innate biocompatibility, mean sEVs are an ideal candidate for the delivery of a wide range of therapeutics. For example, one of the greatest barriers for the treatment of central nervous system (CNS) conditions is the inability to efficiently cross the blood brain barrier (BBB) (Xie et al. 2019). Indeed, most small-molecule drugs and almost all macromolecular drugs are unable to cross the BBB due to factors like molecular size and hydrophilicity (Xie et al. 2019; Baratta 2018; Bender 2018). However, sEVs exhibit this ability suggesting their promise in treating neurological conditions (Terstappen et al. 2021). This, in combination with their limited immunogenicity, further point to the therapeutic potential of sEVs as nanocarriers (Wiklander et al. 2015).

One of sEVs most unique abilities is their capability to exhibit tissue tropism (Wiklander et al. 2015). For example, sEVs isolated from ovarian cancer cells show a greater accumulation in ovarian tumours compared to epithelial cell-derived sEVs (Kim et al. 2017). This presents the potential to package therapeutics within a guided delivery vehicle and allow for a much higher level of tissue-specific accumulation. Overall, these unique characteristics of sEVs highlight their potential as nanocarriers for many drugs and therapeutics.

1.3 | Loading sEVs

Currently there are three distinct techniques exist which can be utilised to load cargo into sEVs. These are (1) the loading of isolated naïve sEVs *ex vivo*; (2) transfecting or infecting parental cells

directly with a therapeutic, which is subsequently packaged into sEVs and (3) genetic manipulation of cells to introduce DNA that encodes the desired therapeutic that can be packaged into sEVs. *Ex vivo* loading is achieved using various methods including physical loading via electroporation (Kim et al. 2017), sonication (Lamichhane et al. 2016) or incubation (Zhang et al. 2022), or chemical loading using reagents like Exo-Fect (Mcandrews et al. 2021). However, many of these techniques lead to dysfunction of the sEV membrane structure resulting in reduced cell uptake, particle aggregation, leakage of the cargo and reduced tropism (De Abreu et al. 2021; Johnsen et al. 2016). Physical methods like electroporation have poor encapsulation efficiencies of <5% (Munagala et al. 2021) and disrupt the membrane (Han et al. 2024), while transfection with agents like Exo-Fect are better at ~35% (Munagala et al. 2021) it can induce significant cytotoxicity (Han et al. 2024). Genetic manipulation, such as the EXOtic system (Kojima et al. 2018), allows for the directed packaging of therapeutic RNAs directly into sEVs, however loading efficiency is low.

While the success of some studies utilising therapeutic sEVs is promising, the current inability to manipulate them efficiently following their harvest hampers their efficient production, and their broader clinical application is limited by poor production and isolation yields, and the lack of a standardised method for sEV isolation and purification (Lu and Huang 2020). This has led to division on how to best utilise and mass produce sEVs for their therapeutic application (Shrivastava and Morris 2021).

1.4 | Lipid-Based Nanocarriers

Lipid nanoparticles (LNPs) currently represent the current gold standard for the delivery of genetic therapies and vaccines such as those deployed in response to the SARS-CoV-2 pandemic (Packer et al. 2021). This is in part due to their ease of production and their ability to achieve high encapsulation efficiencies of therapeutic cargo, regularly achieving encapsulation efficiencies of >80% (Cui et al. 2022). LNP characteristics like the size, lipid composition and molar ratio of nitrogen to phosphate (N/P) can all impact the biodistribution and therapeutic effects, indicating that optimisation and control of these conditions will allow for the greatest therapeutic effect (Maeki et al. 2022). LNPs however, are currently hindered by the fact that only 1% of their cargo is able to escape the endosome and be delivered to cells (Pei and Buyanova 2019), and they are unable to distribute to a wide range of tissues with the vast majority localising within the liver, spleen and kidney (Chen et al. 2016; Veiga et al. 2023). Another issue arises from the commonly used lipid, poly(ethylene) glycol (PEG), as this is used in a variety of commercial products and as a result, a large proportion of individuals have antibodies against it, which upon administration can elicit an immune response, leading to rapid clearance via the monocyte-phagocyte system (MPS) (Batrakova and Kim 2015), hindering the ability of the LNPs to deliver their cargo (Ju et al. 2022).

1.5 | The Role of Lipids in sEVs

The advantageous characteristics of sEVs, including the innate bioavailability, safety profile and tissue tropism have been

believed to mainly be due to the characteristic proteins on their surface, however recent research has highlighted the possibly dominant role the lipid composition plays in these beneficial characteristics. This was highlighted by Wang et al. (2023) who developed selective organ-targeting (SORT) LNPs which were able to be accurately targeted towards the lungs, spleen or liver solely through the manipulation of the lipid profile via addition of DOTAP, 18PA or DODAP, respectively. Recently Su et al. (2024) also showed that through the manipulation of the lipid profile of LNPs they were able to direct their nanoparticles to avoid the liver and spleen and target them towards the lungs. Additionally, Jia et al. provides a summary of some of the modulations made to the lipid composition of LNPs and its affect on the biodistribution (Jia et al. 2024). This is indicating that surface proteins may not be a required component to achieve some of these beneficial characteristics like biodistribution. Based on the potential of sEV research, Lu et al. (2018) formulated liposomes which attempted to mimic the lipidomic membrane composition of sEVs which therefore had no characteristic sEV proteins. This was done by using the most commonly found lipids in sEVs which included phosphatidylcholine (PC), spingomyelin (SM), cholesterol (Chol), phosphatidylserine (PS) and phosphatidylethanolamine (PE). However, instead of PC, PS and PE they used 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). These were then formulated at a molar ratio to mimic that which is found in sEVs to create EV-mimetic nanoparticles. However, in this article EV-mimicking nanoparticles which have been developed entirely synthetically will be referred to as synthetic EVs (synEVs), in order to differentiate them from EV-mimetics which utilise natural sEVs and cellular plasma membranes to construct their nanocarriers. These nanoparticles were as a result, pure lipid membranes mimicking that of sEVs, without the characteristic surface proteins and were still able to maintain a strong safety profile, maintain serum stability (indicating their bioavailability) and effectively deliver their cargo. More details will be provided on this work later in the review, however this highlights the possibly dominant role the specific lipid composition of sEVs could be playing in their beneficial characteristics as nanocarriers. These synEVs could then help bridge the gap between the beneficial characteristics of sEVs and the ease of production and therapeutic relevance of LNPs and a greater knowledge of the lipid profile of sEVs from different sources could help achieve this.

2 | Lipidomics of sEVs

As lipids are suspected to be playing a more dominant role in the beneficial characteristics of sEVs, then it would be expected that those derived from different cell lines would possess quite different compositions, and with careful analysis of the lipid profile it could identify trends which could be impacting factors like tissue tropism. Therefore, a more robust knowledge of the lipidomics of sEVs from various cell lines and under different conditions could elucidate the roles different lipids are playing. The lipid compositions of sEVs derived from 18 cell lines are presented in Table 1. The table presents the lipid compositions from left to right from oldest to most recently published. However, only five of these present data on the cholesterol molar percentage

(mol %). Cholesterol is normally enriched in sEVs compared to cells and contributes to almost half of their lipid composition (Skotland et al. 2019). Therefore, to better compare the results the cholesterol mol % was set at 43 and the remaining lipids were recalculated from the original data as was previously performed by Skotland et al. (2019).

2.1 | Lipid Classes

As seen in Table 1 the lipid composition can vary significantly in sEVs derived from different cell lines which may suggest different roles of lipids and a characteristic 'tissue profile' of lipids. The lipid species from Table 1 were then grouped into their classes in Table 2 to provide a general overview of the lipid composition into sterols, glycerophospholipids (GPs) and sphingolipids (SPs). Overall, of the studies which investigated sterols they maintained a fairly consistent mol % at ~43, while a larger degree of variation was observed in the levels of GPs and SPs. Although further research on the sterol composition of sEVs is required, as previously mentioned only 5 of the 18 studies published cholesterol content and one published a very low content at 15.3% in mast cells. Due to the large degree of variation from other reported cholesterol values and the fact that cholesterol is known to make up a large component of sEV membranes (Skotland et al. 2019) it was excluded from the rest of the discussion. Of the other four previously mentioned studies which published cholesterol data, the sEV GP mol % ranged from 34.9 in b-lymphocytes to 46.68 in reticulocytes. While in the studies which did not publish cholesterol data this ranged from 32.76 in mesenchymal stem cells (MSCs) to 52.5 in oli-neu cells. Sphingolipids contributed a smaller amount to the mol % of sEVs in the studies which published cholesterol data, ranging from 8.83 in reticulocytes to 23 in b-lymphocytes. While in those which did not, the sphingolipids mol % ranged from 7.42 in brain-derived sEVs, to 23.54 in metastatic colon cancer. The similarity in the ranges between the studies which published cholesterol data and those that did not indicates that the estimated mol % of cholesterol at 43 is accurate for the cell types analysed. The ranges observed also highlights that although sEVs have a similar overall lipid composition, they can have variations within this depending on the cell line which they derive from and this as a result could be impacting their characteristic properties. Interestingly, of the cells derived from the CNS (oli-neu and brain-derived) they appeared to produce sEVs with higher GPs at the expense of SPs. With oli-neu and brain-derived sEV's GPs making up roughly half of their mol % at 52.5 and 49.73, while the sphingolipid mol % was only at 8.2 and 7.42. However, the brain-derived sEVs were isolated by taking sections of tissue from post-mortem frontal cortices and through collagenase treatment followed by sucrose density fractionation, the sEVs from within the tissue were extracted. This therefore means there is no single cell source of these sEVs and they represent an 'average' of the sEVs found within the frontal cortex from all cell types. Although with this and the similarity in profile to the sEVs derived from oli-neu cells, it is suggestive that the CNS may have a characteristic sEV lipid profile. Further research into other CNS derived sEVs is required to confirm this but this lipid profile may impact delivery and communication of sEVs within the CNS and this could therefore be harnessed in the development of sEV mimetics which deliver to the brain and CNS.

TABLE 1 | Lipidomics of sEVs from different cell sources.

Lipid class	Reticulocytes (Vidal et al. 1989)	B-Lymphocytes (Wubboldts et al. 2003)	Mast (Laulagier et al. 2004)	Dendritic (Laulagier et al. 2004)	Oligoneuroblastoma (Trajtkovic et al. 2008)	Melanoma (Basic) (Parolini et al. 2009)	Melanoma (Acidic) (Parolini et al. 2009)	Platelets (Pienimäki et al. 2013)	HepG2/C3a (Chapuy-Regaud et al. 2017)	Adipocytes (Durcin et al. 2017)	HOSEPIC (Cheng et al. 2020)	SKOV-3 (Cheng et al. 2020)	Brain-derived (Sullivan et al. 2021)	Colon epithelial (Elmhallah et al. 2022)	Nonmetastatic colon cancer (Elmhallah et al. 2022)	Metastatic colon cancer (Elmhallah et al. 2022)	Mesenchymal stem cells (Amaro-Prellez et al. 2024)
Lipids	%	%	%	% ^b	% ^b	% ^b	% ^b	%	% ^b	% ^b	% ^b	% ^b	% ^b	% ^b	% ^b	% ^b	% ^b
Sterols	47.1 ^e	42.1	15.3 ^d	43 ^a	43 ^a	43 ^a	43 ^a	42.5	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a
Chol esters																	
GP	23.5	(20.3) ^c	27.95	14.82	26.7	(24.225) ^c	(24.51) ^c	0.08	20.52	33	22.23	26.15	0.07 ^c	0.17	0.15	0.23	1.35
PS	5.9	(20.3) ^c	(15.25) ^c	(10.83) ^c	14.9	(24.225) ^c	(24.51) ^c	15.28	14.25	1.1	1.93	1.02	16.82	32.6	34.26	28.16	31.64
PE	12.6	(14.6) ^c	22.82	14.82	10.9	20.235	15.96	11.66	7.41	4	8.70	7.65	10.98	1.25	1.2	0.8	0.1
PE ethers		(14.6) ^c						5.78					10.98	3.82	4.33	1.94	0.96
PC ethers								3.27		0.8				1.14	1.14	1.25	
PG								0.81			2.51	1.88	0.55				
PA		(20.3) ^c						0.17			0.01	0.01	0.006				
PI	2.4	(20.3) ^c	(15.25) ^c	(10.83) ^c		(24.225) ^c	(24.51) ^c	0.16	3.42	2.3	0.56	0.35	0.87	2.62	2.85	1.25	0.06
CL								0.13			0.01	0.03	0.55				
SP	8.4 ^c	(23) ^c	11.4	11.4	8.2	(12.54) ^c	(16.53) ^c	16.26	9.69	12.5	18.22	14.55	3.41	14.14	11.69	20.63	17.18
Cer								0.32	0.285	0.2	0.38	0.22	0.37	1.37	1.43	2.91	0.17
HexCer								0.76		0.02			0.48				
LacCer								0.12									
Sulfatide																	
Gangliosides	8.4 ^c	(23) ^c				(12.54) ^c	(16.53) ^c	0.086					0.3				0.02
MG											1.19	2.14	0.31				0.02
TG											0.12	0.11	0.33				4.63
DAG								1.52					0.14				0.04

^aChol not reported and set at 43% for comparison.

^bPercentages of GPs and SPs recalculated to allow for Chol.

^cSum for all classes shown in parentheses and have the same number.

^dChol estimated from Chol:Phospholipid ratio = 0.18:1.

^eChol estimated from Chol:Phospholipid ratio = 0.89:1.

TABLE 2 | Lipid class composition of sEVs from different cell types.

Lipid class	B-lymphocytes (Vidal et al. 1989)		Mast cells (Laulagier et al. 2004)		Dendritic cells (Laulagier et al. 2004)		Oli-neu cells (Trajkovic et al. 2008)		Melanoma + Basic Acidic (Parolini et al. 2009)		Melanoma + PC-3 cells (Llorente et al. 2013)		Platelets (Pieni-maeki-Roemer et al. 2015)		HepG2/C3a (Chapuy-Regaud et al. 2017)		Adipocytes (Durcin et al. 2017)		HOSEPIC (Cheng et al. 2020)		SKOV-3 (Cheng et al. 2020)		Brain derived (Su et al. 2021)		Colon epithelial cells (Elmallah et al. 2022)		Nonmetastatic colon cancer cells (Elmallah et al. 2022)		Mesenchymal stem cells (Amaro-Prellezo et al. 2024)	
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
Sterols	47.1	42.1	15.3	43 ^a	43 ^a	43 ^a	43.00 ^a	43 ^a	43.00 ^a	43 ^a	43.6	46.6	43 ^a	43 ^a	43 ^a	43 ^a	43.00 ^a	43 ^a	43 ^a	43 ^a	43.00 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	43 ^a	
Glycerophospholipids	44.4	34.9	66.07	40.47	52.5	44.46	40.47	37.26	39.3	41.2	45.6	41.2	36.28	38.1	49.73	41.43	43.78	33.4	32.76											
Sphingolipids	8.4	23	11.86	11.4	8.2	12.54	16.53	17.546	12.9	12.72	18.6	14.76	7.42	15.51	13.12	23.54	17.35													
Glycerolipids								1.52																						

^aChol not reported and set at 43% for comparison.

2.2 | Sterol Composition

The three predominant classes of lipids which compose sEVs (Sterols, GPs and SPs) can be further classified into the specific lipids which make them up. The sterols are primarily composed of cholesterol, with cholesterol esters (CEs) playing a minor role. Six studies identified CEs and in five of these CEs contributed less than 1% to the total sterols. Platelet derived sEVs were found to contain a larger amount of CEs but this was still less than 10% of the total sterols. Additionally, as CEs are mainly found in lipid droplets and lipoproteins, high amounts could indicate contamination of the sample (Skotland et al. 2020). Therefore, the primary sterol component of sEVs is cholesterol.

2.3 | Sphingolipid Composition

Similar to sterols, SPs were found to be predominantly composed of one lipid, this being sphingomyelin (SM) with it making up >85% of SPs in almost all sEVs investigated. The studies investigating melanoma-derived sEVs under basic and acidic conditions grouped SM with gangliosides, so it was therefore not possible to separate the two. However, in the brain-derived sEVs, SM only made up 45% of the total SPs, with gangliosides contributing ~39%. Only one other study investigated the levels of gangliosides within the sEVs, and this was those derived from PC-3 cells, which contributed to <0.5% of the total SPs. As a result, despite only two studies investigating the ganglioside composition, a large degree of variation exists (<0.5%–45%). This warrants further investigation of the exact sphingolipid composition to identify if there is correlation between the composition of SM, gangliosides and the cell type. The higher composition of gangliosides in brain-derived sEVs is most likely due to the high abundance of gangliosides in the CNS (Yoon et al. 2022), but this again highlights the differential composition of sEVs derived from the CNS, possibly indicating the ability to harness this for brain delivery. While the sterols and SPs are primarily composed of 1–2 lipids a greater diversity of lipid composition is found within GPs.

2.4 | Glycerophospholipid Composition

As seen in Table 3 the primary GPs identified were phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI). These made up >85% of the total GPs in 14 of the studies with only oli-neu cell derived sEVs not reporting on PI. In addition to these, eight studies reported on PE ethers, PC ethers, phosphatidylglycerol (PG) and phosphatidic acid (PA) which were identified in lower levels. PC was the most abundant GP found in sEVs with it ranging from 36.62% in those derived from dendritic cells, to 94.15% from MSCs. Although, the majority of sEV's GPs were composed of between 40% and 55% PC. However, three studies reported PC along with other lipids so its exact mol % could not be determined. Interestingly, three of the five studies which presented PC levels greater than 55% were all derived from colon cell lines, with colon epithelial cells at 78.68%, nonmetastatic colon cancer cells at 78.26%, and metastatic colon cancer cells at 84.3%. These are significantly higher than the PC levels found in other cell lines suggesting a characteristic profile

TABLE 3 | Glycerophospholipid composition of sEYs from different cell types.

Glycerophospholipid	Reticulo-lymphocytes (Vidal et al. 1989)		B-lymphocytes (Wubboldts et al. 2003)		Mast cells (Laulagnier et al. 2004)		Dendritic cells (Laulagnier et al. 2004)		Oli-neu cells (Trajkovic et al. 2008)		Melanoma + Basic (Parolini et al. 2009)		Melanoma + Acidic (Parolini et al. 2009)		PC-3 cells (Llorente et al. 2013)		Platelets (Pieni et al. 2015)		HepG2/C3a (Chapuy-Regaud et al. 2017)		Adipocytes (Durcin et al. 2017)		HOSEPiC 3 (Cheng et al. 2020)		SKOV-3 derived (Su et al. 2021)		Brain epithelial cells (Elmallah et al. 2022)		Nonmetastatic colon cancer cells (Elmallah et al. 2022)		Metastatic colon cancer cells (Amaro-Prellezo et al. 2024)					
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%					
PC	52.79		38.37		36.62		50.86		41.01		40.46		45.00		80.10		61.27		68.63		40.12		78.68		78.26		84.30		94.15							
PS	13.33				28.38		28.38		31.29		26.72		31.25		2.67		5.31		2.68		33.83		3.03		2.73		2.39		0.31							
PE	28.43		31.4		36.62		20.76		15.51		7.89		16.25		9.71		23.98		20.08		22.08		9.22		9.90		5.80		2.85							
PE Ethers									8.78		8.14				1.94								2.75		2.60		3.75									
PC Ethers									2.17		3.56																									
PG									0.46																											
PA									0.43																											
PI	5.46								0.35		13.23		7.50		5.58		1.54		0.91		1.75		6.33		6.51		3.75		0.17							
LPC																																				
LPE																																				
CL																																				
PC + PS + PA + PI		58.17																																		
PE + PE Ethers		41.83																																		
PS + PI			20.22		26.76																															
PC + PS + PI												54.70		60.56																						

for sEVs derived from the colon. Additionally, adipocytes had a high PC content with it making up 80.1% of the total GPs. This similarity in lipid composition with colon cancer cell sEVs is interesting as recently it has been shown that adipocytes may assist in the induction of colorectal cancer cells progression (Olszańska et al. 2023). Therefore, this similarity in sEV lipid composition could allow for enhanced communication between adipocytes and colorectal cancer cells. Additionally, MSCs also had a high composition of PC and this similarity could be due to the fact that they are a major source for adipocyte generation (Matsushita and Dzau 2017). Following PC the second most common GP varies, PE is the second most common in eight studies, while PS is in five. In three studies PS is grouped together with other lipids and as a result cannot be compared to PE. PE however, is reported on separately in 17 studies (grouped with PE ethers in b-lymphocytes) and it ranges from 2.85% in MSC sEVs to 45.3% in sEVs derived from melanoma cells under basic conditions. Interestingly, a correlation between colon, and adipocyte cell lines is again seen in PE composition with adipocytes, colon epithelial cells, nonmetastatic and metastatic colon cancer cells producing sEVs with 9.71%, 9.22%, 9.9% and 5.8% PE, respectively. These are all low levels of PE and this similarity is in conjunction with the fact that 11 of the 13 other studies all have PE levels greater than 15% and 9 of 13 have levels greater than 20%. PS is grouped with other GPs in five studies and could not be analysed on its own, but in the 13 other studies it ranged from 0.31% in MSC sEVs to 33.83% in brain-derived sEVs. Similar to PE, the correlation between colon and adipocyte cell lines is again seen in PS, with all four having levels below 3.1%. Whereas in brain-derived and oli-neu sEVs (CNS originating sEVs) the PS levels are much higher at 33.83% and 28.38%. Only nine studies reported on PI separately, but it tends to make up a much smaller component of sEV lipid composition, with it ranging from 3.75% in metastatic colon cancer to 13.23% in platelets, and eight of these nine studies had PI \leq 7.5%. Overall, these results highlight the fact that sEVs can have differing lipid profiles depending on their cell of origin, and this could be playing into some of their beneficial characteristics, like their tropism. Further research into the lipid profile of sEVs originating from similar areas of the body (i.e., CNS vs. Colon) could elucidate a characteristic lipid profile for these regions and enhance the delivery of therapeutics to these areas.

2.5 | Molar Composition of sEVs

As seen, it is difficult to create an overall average molar composition for sEVs as there is a large degree of variation in their lipid profile, which could suggest different profiles for different tissues. Therefore, future research into the sEV lipid composition of therapeutic targets could lead to enhanced delivery of lipid-based nanocarriers. For example, in the studies investigating CNS-derived sEVs, an average sEV could have a molar ratio of 43:23:16:11:6:1 (Chol:PC:PS:PE:SM:PI). Whereas, in colon and adipocyte derived sEVs the average molar ratio would be 43:32:1:4:15:2:2:1 (Chol:PC:PS:PE:SM:PI:Cer:PE ethers). However, these both require further research as cholesterol is assumed at 43% in all of these studies and in addition the oli-neu sEVs only reported on SM, while gangliosides made up ~39% of

the SPs in brain-derived sEVs. Additionally, another issue may arise from the degree of complexity seen within lipid species. A lipid species may have multiple variations of the same lipid with the length of the carbon chain and degree of saturation all having the ability to change and this may impact the role lipid classes play. As a result, techniques which produce EV-mimetics from natural sEVs may be able to maintain this lipid complexity and maintain their beneficial characteristics. Therefore, investigating these techniques and sEVs derived from target areas and undertaking thorough lipid analyses of them will provide a much finer degree of detail when designing and producing EV-mimetics and synEVs.

3 | Role of Lipids in sEV Nanocarrier Function

As mentioned above, the exact molar composition of sEVs varies depending on their cell of origin, among other factors. However, all sEVs analysed are composed of at least Chol, PC, PS, PE, PI and SM. Each of these lipids plays role in the function of sEVs, and these will be discussed here as well as how these could be harnessed in the production of synEVs. Although more detailed analyses elucidated further lipids that contributed to sEV membranes at levels greater than 1% like PE/PC ethers, ceramides and gangliosides, only the dominant lipids will be discussed here. A brief review of the role of the different lipids in sEVs can be found in Figure 1.

3.1 | Cholesterol

Chol is enriched in sEVs and contributes the largest portion to their membranes of any individual lipid (Table 1). It helps to provide stability to lipid bilayers and has been shown to improve the stability of LNPs in the presence of serum proteins (Sakurai et al. 2001). This, as a result, would make Chol an important component of sEVs, as while in circulation, it reduces the accumulation of surface-bound proteins, which improves the circulation half-life (Semple et al. 1996). Chol is an exchangeable molecule and as a result, while in circulation, has been shown to accumulate in liposomes (Rodriguez et al. 1993), therefore when including Chol at an equimolar amount to endogenous membranes in formulations it would prevent its net influx or efflux and maintain membrane integrity (Hald Albertsen et al. 2022). Due to Chol's structure, it helps to fill spaces between lipids, and subsequently, high levels of Chol in the membrane can act as a barrier and decrease the permeability of small molecules passing through the membrane (Lippincott-Schwartz and Phair 2010). In addition to Chol's role in membrane stability and integrity, it has been shown to outperform DOPE as a helper lipid for gene delivery in vivo, despite having a lower fusogenicity (Dabkowska et al. 2012). Chol can also work with other lipids to enhance its functionality. This is seen in the sEV-mimicking liposomes developed by Lu et al. (2018), which contained lipid-raft-like domains, enriched with Chol and SM, and these domains may help to enhance intracellular delivery by fusing directly with cells (Montecalvo et al. 2012). Further to this, when used in combination with PC, the presence of Chol produces stable lipid bilayers, highlighting its functionality with additional lipids (Semple et al. 1996).

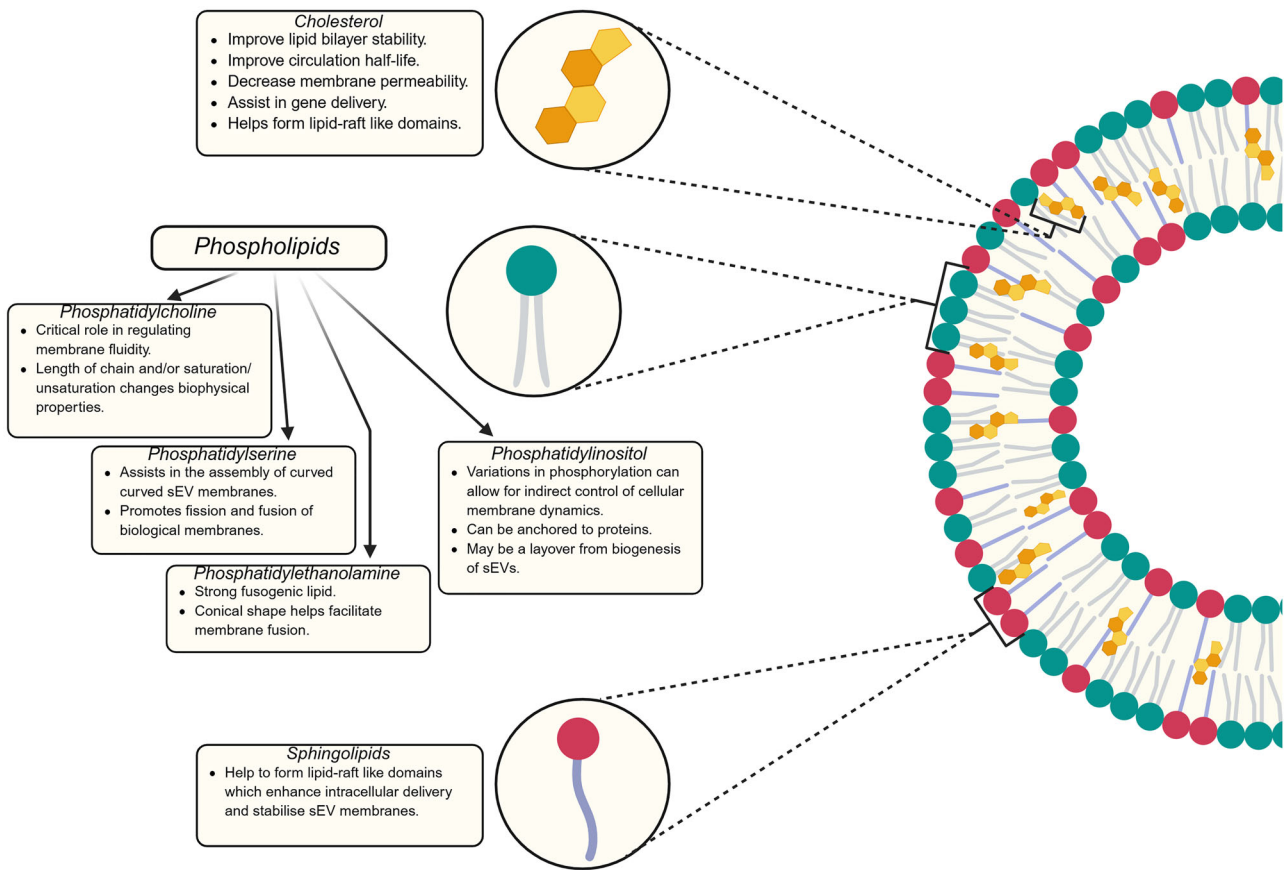


FIGURE 1 | Role of lipids in sEV membranes.

3.2 | Phosphatidylcholine

PC is a primary natural component of cellular membranes and while contributing a lower mol % in sEVs membranes compared to cells (Cheng and Lee 2016) generally it is still the second most common lipid in sEV membranes (Table 1). PC plays a critical role in regulating the fluidity of membranes (Kanno et al. 2007) and due to its cylindrical geometry, it favours the formation of bilayers (Thewalt and Bloom 1992). It also plays a key role in maintaining membrane integrity. In cellular membranes (and sEV membranes) the most common form of PC is the diacyl-subclass; however, within this subclass further variations in the length of the fatty acyl chains and the degree of saturation versus unsaturation can impact its biophysical properties (McMaster 2018). Most PC molecules have chains of 16 or 18 carbons long but can range from 14 to 26. Those with shorter fatty acyl chains have less surface area for stabilisation via van der Waals forces with neighbouring hydrophobic fatty acids and are as a result less stiff and viscous than those with longer chains (Taneva et al. 2003). The number of double bonds also changes the shape and subsequent biophysical properties, with saturated forms resulting in a less fluid but thicker membrane, while the introduction of double bonds increases the membrane fluidity but reduces its rigidity (McMaster 2018). Multiple variations of PC have been used in liposome/LNP formulations, these include the saturated forms like distearoylphosphatidylcholine (DSPC), and hydrogenated soybean PC (HSPC) as well as the unsaturated forms, egg PC and DOPC (Cheng and Lee 2016). Due to their structure, the saturated forms have been shown to produce highly

stable LNPs which subsequently have greater in vivo serum stability but are unable to facilitate endosomal release which may hinder cargo release (Cheng and Lee 2016). However, it was found that when adding varying forms of PC into hybrid liposome-sEV nanoparticles, that ‘hard vesicles’ (with more saturated forms of PC) were able pass through the epithelial cell barrier in the intestinal track by moving from the endosome to the Endoplasmic Reticulum (ER) and Golgi apparatus and being released by the ER-Golgi exocytosis pathway (Xiao et al. 2025). The unsaturated forms on the other hand are in a more fluid state and at a greater risk of protein opsonisation (Yan et al. 2005), but with the inclusion of Chol these membranes could be stabilised which could allow for a balance between stability and transfection efficiency (Cheng and Lee 2016). It was also recently shown that PC facilitated vesicles’ efficient cellular uptake in vitro (Li et al. 2025). Interestingly, lysophosphatidylcholine (LPC) could be relevant to brain delivery as it has been shown to be the preferential carrier of fatty acids across the BBB through the interaction with a specific LPC receptor (Semba 2020). However, it was found at very low levels in the brain-derived sEVs (<1%) (Su et al. 2021). Although further research into its content in sEVs and its potential ability to cross the BBB could provide a new avenue for the development of ‘brain-targeting’ nanoparticles.

3.3 | Phosphatidylserine

PS is a negatively charged phospholipid which, under physiological conditions, is generally found in the inner leaflet of cell

membranes (Perez et al. 2023). Based on the biogenesis of both ectosomes and exosomes, it would be assumed that PS would also be found on the inner leaflet of sEVs. However, initiation of the apoptotic cascade causes a reversal of the asymmetrical distribution of PS, with it being expressed on the outer leaflet. Externalised PS then acts as an 'eat me' signal to macrophages, promoting the clearance of apoptotic bodies (Segawa and Nagata 2015). This is supported by the fact that masking PS on the surface of apoptotic bodies inhibits their uptake by macrophages (Asano et al. 2004). Although constitutive exposure of PS on viable cells does not induce phagocytosis, suggesting macrophages can differentiate between apoptotic and viable cells (Segawa et al. 2011). The negative charge of PS in sEV membranes is believed to be involved in this recognition and clearing via macrophages (Matsumoto et al. 2017). The role PS plays in the immune system is highlighted as its expression on sEVs can act as a signal to activate CD8+ T cells (adaptive immune system) (Rausch et al. 2023) and PS-rich EVs even provide protection against viral infection (Groß et al. 2024). While these characteristics may seem detrimental to synEVs, with longer circulation times being preferred, additional features provide more beneficial qualities. The natural conical shape of PS may aid in the assembly of curved sEV membranes (Maxfield and McGraw 2004). It has also been shown to be crucial in facilitating the fission and fusion of biological membranes through various methods, with it also being critical in the viral envelope to promote membrane fusion with host cells for HIV, Zika and Ebola (Chua et al. 2019; Whitlock and Chernomordik 2021). The introduction of PS into synEVs could therefore improve their membrane fusion with target cells and promote their cargo release, and this was shown by Sakai-Kato et al. (2020) who produced synEVs and through the addition of DOPS into the formulation was able to improve cellular internalisation. However, the concentrations used of PS would have to be carefully controlled to prevent rapid clearance. Although, as mentioned previously, given that sEVs expressing PS can activate CD8+ T cells this could potentially be utilised to target and activate the adaptive immune system and improve the efficacy of vaccinations. It has also been shown that the synergistic combination of PS with PE was able to enhance lysosomal/endosomal escape ability, and the specific combination of PS with SM was also able to assist vesicles in penetrating into the nucleus (Li et al. 2025).

3.4 | Phosphatidylethanolamine

In sEV membranes PE alternates with PS between being the 2nd and 3rd most common GP behind PC. Generally PE is found in both the inner and outer leaflets of EVs as EVs lack the membrane asymmetry of PE found in parental cells due to the presence of phospholipid scramblase in the EV membrane (Laulagnier et al. 2004; Donoso-Quezada et al. 2021). As previously mentioned it can be used synergistically with PS to enhance lysosomal/endosomal escape (Li et al. 2025). PE is composed of two fatty acid acyl chains esterified to a glycerol backbone with an ethanolamine head group linked to the glycerol (Amaro and Almeida 2013). Due to the relative volume differences between the small ethanolamine head group and long acyl chains, PE adopts a conical shape. This conical shape provides beneficial characteristics to nanocarriers, with it facilitating the negative

curvature of membranes which can enhance membrane fusion (Ridgway 2021). Typically, one of the major limiting steps in the delivery of genetic medicine is getting the cargo across the endosomal membrane. PE's geometry can stabilise the non-bilayer hexagonal phase which is found in transitional structures during membrane fusion (Hattori et al. 2005). This allows it to promote membrane fusion and subsequent cargo release into cells and as a result is usually described as a fusogenic lipid. PE has been frequently used in LNPs in the form DOPE, however in vivo interactions with blood serum proteins reduces the stability and fusogenicity of DOPE-based LNPs while in circulation (Sakurai et al. 2001). Therefore, despite its benefits towards membrane fusion and subsequent cargo delivery, this could explain why it is only present in relatively low mol %'s in sEVs (1.94%–24.57%).

3.5 | Phosphatidylinositol

PI is composed of a diacylglycerol chain attached to an inositol head group via a phosphate group, and they usually only make up a small component of sEV membranes (0.13%–5.2%). They exist as an anionic lipid at physiological pH and are predominantly found in the inner leaflet of cellular (and assumably sEV) membranes. PI's low abundance indicates that it may only play a minor structural role within membranes (Stillwell 2016). However, within cellular membranes it plays a critical role in several cell functions including cell signalling and regulation (Vivanco and Sawyers 2002). Within cellular (and sEV) membranes PI can exist in its phosphorylated form (PIP) (Di Paolo and De Camilli 2006). PI kinases can phosphorylate the 3-, 4- and -5 hydroxyl groups of the inositol head either singularly or in combinations, creating distinct PIPs. These PIPs can recruit effectors which then allows for the regulation of cellular membrane dynamics through the indirect control of signalling pathways, cytoskeletal dynamics, membrane contact sites or ion channels, as well as vesicular transport (Skotland et al. 2019; Schink et al. 2016). Therefore, PIs exist as an inert storage form of the biological signalling molecules, PIPs (Stillwell 2016). These roles in membrane dynamics and vesicular transport which PIPs hold, unsurprisingly implicates this lipid in sEV secretion and evidently through manipulation of PI kinases the secretion of sEVs can be altered (Hessvik et al. 2016). They also have the ability to specify organelle identity which suggests exosomes and microvesicles could have different compositions of specific PIPs (Skotland et al. 2020). Additionally, PIs can also be involved in binding a variety of proteins to the membrane surface via a glycosyl bridge forming glycosyl-phosphatidylinositol (GPI)-anchored proteins (Ferguson and Williams 1988). The presence of PIs in sEVs could then just be a layover from the processes required in their formation and may not be required for their function. Although the possibility for the formation of GPI-anchored proteins may mean they play a role in the formation of the surface proteome of sEVs.

3.6 | Sphingolipids

All members of the sphingolipid family contain the parent amino alcohol sphingosine group at C-18 (Stillwell 2016). Ceramide

is synthesised from sphingosine and through further modifications can be used to yield SM and glycosphingolipids, like the gangliosides (Slotte 2013). For sEVs the most common of the sphingolipids is SM, with ceramides and gangliosides making up smaller components, although each class (ceramides, gangliosides and SM) are all significantly enriched into sEVs when compared to their parent cells (Skotland et al. 2019). Sphingomyelin is composed of the sphingosine backbone bonded to one fatty acid at the NH₂ function, and a polar head group like phosphocholine or phosphoethanolamine at the CH₂OH function (Zhang 2015). As a result a large degree of variation can be found within SM with at least 18 different molecular species identified within fibroblast cells (Slotte 2013). With Palmitic Acid (16:0) being the most common N-linked acyl chain in mammalian peripheral cells, while stearic acid (18:0) is the most common in neural tissue (Barenholz 1984; Zhou et al. 2012). The ability of the sphingolipids to form many hydrogen bonds facilitates the formation of sphingolipid (or SM) enriched domains within membranes. This is particularly important due to cholesterol's preferred interactions with SM which allows for the formation of lipid-raft like domains (Slotte 2013). As mentioned previously, these domains may enhance the intracellular delivery of sEVs by fusing directly with cells to release their cargo (Montecalvo et al. 2012). In cellular membranes SM is mainly localised into these lipid-rafts (Jiang et al. 2018) so it could be hypothesised then this could be the same in sEVs and the formation of these lipid-raft domains is its primary role. In addition to SM and cholesterol, the gangliosides (GM1, GM2, GM3, etc.) are also well-known components of lipid-rafts in cellular membranes (Sezgin et al. 2017). These could then be assumed to also play a role in these sEV lipid-raft like domains. This is seen with gangliosides like GM3 acting in synergy with Chol and SM to act as stabilisers of sEV membranes and shield the vesicles against blood components (Kooijmans et al. 2012). However, this may only be relevant in lower concentrations as high concentrations of GM3 induces membrane segregation and leakage of cargo (Yokoyama et al. 2003). As mentioned previously, this evident synergy of SM with many lipids is seen in its ability to promote nuclear penetration in combination with PS (Li et al. 2025). Ceramide generation has been shown to be a crucial component in the formation and secretion of sEVs (Dinkins et al. 2016), this is probably due to the ability of ceramide to form micro-domains which induce negative membrane curvature and allow for cargo sorting and exosomal budding into MVBs to occur (Ghadami and Dellinger 2023). Ceramide is then unique in that it is required for both exosome formation and secretion but it can also assist in the formation of the lipid-raft domains and play a further role in intracellular signalling (Elsherbini and Bieberich 2018). Interestingly, it was also shown that tumour cells utilise the sphingolipids within EV membranes which are released into the tumour microenvironment (TME) to promote tumour progression and T-cell exhaustion. This is achieved by packaging the enzymatically active sphingosine kinase-1 into EVs, which upon release, cleave the sphingosine present within the membrane into sphingosine-1-phosphate (S1P) leading to the rupture of the EVs and release of S1P into the TME, inducing T-cell exhaustion and promoting tumour progression (Gupta et al. 2022). This further highlights how the lipid composition of EVs can have various biological roles.

4 | EV-Mimetics and synEVs as Nanocarriers in Biomedical Applications

As previously mentioned, sEVs provide many advantageous characteristics with their widespread biodistribution, biocompatibility, ability to cross the BBB, and express tissue tropism (Wiklander et al. 2015; Kim et al. 2017; Batrakova and Kim 2015). However, despite these beneficial qualities, the widespread clinical application of sEVs has been hindered by low extraction yields and low encapsulation efficiencies of cargo. This is in addition to the fact that most isolation techniques are labour-intensive, complex, inefficient and expensive (Sadeghi et al. 2023). Despite this, over 200 clinical trials are listed (at the time of writing) which utilise 'exosomes' as their intervention. These range from treatments of hair loss to osteoarthritis and stroke. These include multiple studies which have either completed or are currently in Phase 3, including in the treatment for androgenic alopecia and acute respiratory distress syndrome utilising mesenchymal stromal cell and bone marrow MSC derived EVs, respectively (ClinicalTrials.gov 2024a; ClinicalTrials.gov 2024b). This highlights the broad interest and potential of sEVs, however in addition to the previously mentioned challenges, natural sEVs have a very complex and diverse array of interactions within the body. With much still to learn about sEVs, the potential exists for unforeseen interactions which could further hinder their progression into the clinic, like the finding that repeated intravenous administration of EVs into non-human primates lead to reduced circulation times, potentially due to the formation of an EV specific antibody response (Driedonks et al. 2022). As a result, the current gold standard for nanocarriers remain as LNPs with high encapsulation efficiencies, low-cost and ease of production. However, as mentioned, only 1% of their cargo can escape the endosome and be delivered to cells (Pei and Buyanova 2019), as opposed to sEVs which have been shown to achieve up to 24.5% delivery of their cargo to cells once internalised (Joshi et al. 2020). LNPs also only distribute to a small range of tissues with the vast majority localising within the liver, spleen, and kidney (Chen et al. 2016; Veiga et al. 2023). In addition to the immune response elicited by PEGylated lipids (Ju et al. 2022). Therefore, by combining these two approaches it could allow for the efficient and cost-effective production of a sophisticated class of nanoparticles. This gap between sEVs and LNPs has been attempted to be bridged by EV-mimetics and synEVs. Within this article techniques which utilise natural sources and attempt to maintain or mimic the structure and function of sEVs will be referred to as EV-mimetics. While as described earlier, nanoparticles which are produced entirely synthetically while attempting to maintain or mimic the natural structure and function of sEVs will be referred to as synEVs.

4.1 | Extrusion Approach to EV-Mimetics

Various techniques have been utilised to produce EV-mimetic nanoparticles. These include the direct extrusion of cells through porous membranes, microfluidic devices, the coating of nanoparticle cores with exosomal or cellular membranes, and the hybrid membrane fusion of sEVs with liposomes. The extrusion of cells approach produces sEV sized nanoparticles which attempt

to maintain the plasma membrane and protein profile from the original parent cells (Severic et al. 2021; Yu et al. 2023; Qian et al. 2024). This was utilised by Zhu et al. (2022) who took human umbilical mesenchymal cells and repeatedly extruded these through progressively smaller pores to produce EV-mimetics, these were then added into a hydrogel and were able to improve wound healing in vitro and in vivo and even did so better than standard sEVs in vitro, however in vivo they produced similar results. Extrusion also was recently used with red blood cells (RBCs), which provide the advantage of having no internal nuclear or mitochondrial membranes allowing for a much ‘cleaner’ preparation, and these were able to be packaged efficiently and deliver their cargo (Biagiotti et al. 2024). Further modification can be performed to these EV-mimetics, like that utilised by Kang et al. (2024) who incorporated PS into the membranes of the EV-mimetics to treat osteoporosis, as PS-receptors are highly expressed on the surface of osteoclasts (Harre et al. 2012), and were able to protect against bone loss in an ovariectomised mouse model. This additionally further highlights the potential role the lipid profile may play in the biodistribution of sEVs.

4.2 | Microfluidic Approach to EV-Mimetics

Microfluidic approaches utilise a technique similar to that used in the production of LNPs (Figure 3A). Briefly, when used in the production of LNPs, the microfluidic approach uses two inlets, one containing lipids and the other the therapeutic cargo. These two lanes are mixed and packaged LNPs are produced. In terms of EV-mimetics this was first used with whole cells suspended in PBS which were forced through microchannels in a microfluidic chip to form nanoparticles and these were able to successfully transfer RNA to the recipient cells (Jo et al. 2014). However, these EV-mimetics weren’t specifically packaged with any cargo and thus relied on the contents of the original parent cell. Additionally, while the EV-mimetics were produced utilising microfluidics, the actual technique more closely resembled extrusion, with the cells being stretched over and forced through the microchannels present on the microfluidic chip. Recently extrusion was modified by Wang et al. (2024) who developed a novel microfluidic chip utilising four inlets containing cell plasma membrane, therapeutic cargo, surfactant mixture and PBS buffer. The cell plasma membranes were extracted through repeated high-speed homogenisation and centrifugation, before being resuspended in PBS via sonication, all steps were conducted at 4°C to maintain the biological properties of the cell membrane proteins. Then by including the surfactant mixture, it allowed for the cell plasma membranes to be ‘opened up’ so the therapeutic cargo could be packaged inside before the membranes reassembled around the therapeutic cargo at ~50 nm in size. These EV-mimetics were then able to be specifically packaged and successfully delivered their cargo.

4.3 | Coating of Nanoparticle Core Approach to EV-Mimetics

Another technique involves the coating of synthetic nanoparticle cores with exosomal or cellular membranes. This involves the packaging/attachment of the desired therapeutic to a nanoparticle (gold, mesoporous silica, iron oxide, etc.) which is then coated

in the cellular or exosomal membrane (Lopes et al. 2023; Pei et al. 2021). This allows for much higher levels of packaging while also maintaining the beneficial biological features of sEVs like their increased circulation time and immune evasion (Duan et al. 2023). Recently nanoparticles coated with antigen presenting cell membranes have been produced, which maintained the beneficial characteristic of tropism, and were able to directly interact with and modulate T cells responses (Li et al. 2023).

4.4 | Membrane Fusion Approach to EV-Mimetics

The hybrid membrane fusion of sEVs with liposomes attempts to combine the ease of packaging of liposomes with the beneficial membrane characteristics of sEVs. Multiple methods can be used to form the hybrid nanoparticles, briefly, these involve the production and packaging of liposomes with the desired therapeutic. Then fusion of the liposomes with sEVs can be achieved through various methods, in one study the LNPs are extruded with sEVs to fuse the nanoparticles together, forming the hybrid sEV-liposomes (Wang et al. 2024). While another technique incubates the liposomes with the sEVs and performs multiple freeze-thaw cycles to fuse the membranes (Wu et al. 2023). These hybrid nanoparticles have even been shown to package large plasmids and deliver the CRISPR/Cas9 system to MSCs (Lin et al. 2018). Membrane-fusion approaches have been used extensively in the formulation of nanocarrier systems and has been shown to demonstrate some targeting ability, with it being used to target tumours through the fusion of liposomes and macrophage-derived sEVs (Zhou et al. 2025). Interestingly, it has been shown that performing membrane fusion appears to maintain the availability of the surface EV proteins and their enzymatic activity is unaffected (Bader et al. 2025). This could be beneficial in maintaining some of the lipid bilayer arrangement in the hybrid particles along, although with the addition of new lipids from the liposomes/LNPs the overall structure of the lipid profile will be significantly changed. Although the encapsulation efficiency of cargo into the hybrid nanoparticles still remains fairly low at ~30%–40% (Xiao et al. 2025; Bader et al. 2025), with the number of particles which can be generated through this method, the desired therapeutic effect can still be achieved.

4.5 | Limitations of EV-Mimetics

The various techniques used in EV-mimetics can produce sophisticated nanoparticles, however, they all still rely on cell culture for either cellular or sEV membranes to be used in manufacturing process. The previously mentioned limitations for mass clinically relevant sEV production, hinders the use of sEV membranes. Using cellular membranes provides a more clinically relevant alternative for production, however distinct differences are found in the lipid profile of sEVs when compared to their parental cells, with cholesterol and SM for example being usually 2–3× enriched in sEVs (Skotland et al. 2019), and as a result could limit the translation of some of their beneficial characteristics. As a greater knowledge is built around the composition of sEV’s plasma membranes this could be used to produce sophisticated nanoparticles. A summary of the different techniques used in creating EV-mimetics can be found in Figure 2 and the various uses of EV-mimetics can be found in Table 4.

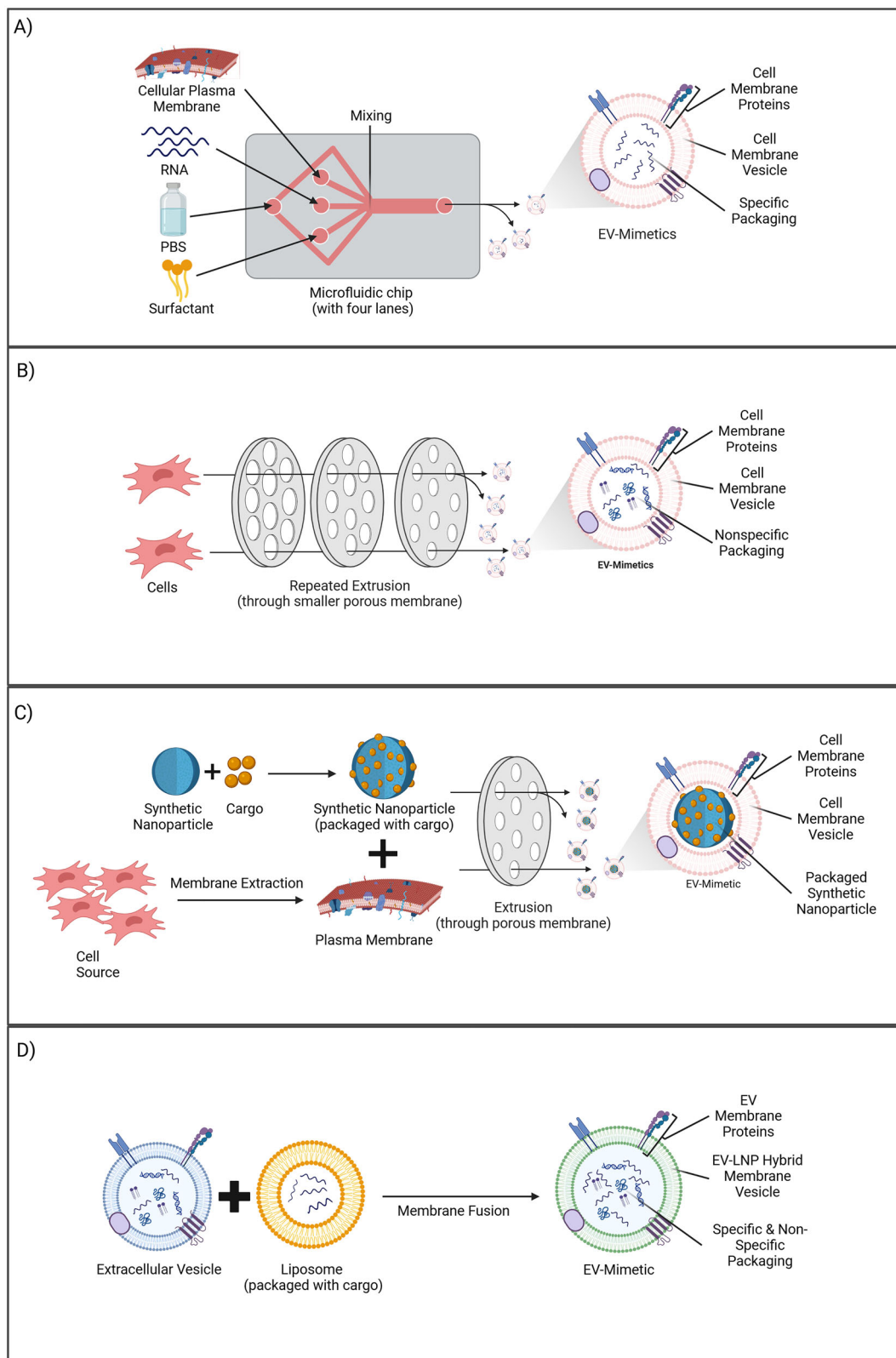


FIGURE 2 | Generalised schematics of the different techniques used for the formulation of EV-mimetics. (A) Utilises four channel microfluidics based on Wang et al. (2024) to package and form EV-mimetics from cell plasma membranes. (B) Utilises multiple extrusion steps to generate EV-mimetics from whole cells. (C) Utilises extrusion to encapsulate synthetic nanoparticle cores with a cellular plasma membrane. (D) Utilises membrane fusion to combine natural EVs with pre-packaged liposomes.

TABLE 4 | Summary of the EV-mimetics and synEVs as nanocarriers in biomedical applications.

Technique	Source of EVs	Packaged cargo	Targeted cells/tissue	Lipid or surface modifications or composition	Average size (n.m)	Limitations/ concerns	Reference
Extrusion	U937 monoblastic cells derived extracellular vesicles (EVs)	No packaged cargo was mentioned	Advanced prostate cancer cells (PC)	Anti prostate-specific membrane antigen (PSMA) peptide, WQPDTAHHWATL on surface	170–180 nm by DLS and 133 and 135 nm by NTA	Low tissue specificity	Yokoyama et al. (2003)
Extrusion	Polymorphonuclear neutrophils (PMNs) and activated PMNs (aPMNs) derived EVs	Vascular endothelial growth factor (VEGF)	Chronic diabetic wounds	N/A	160 nm	The generation of PMN EVs was low, which limited its application in therapeutic settings VEGF has low stability, poor targeting ability, and is prone to degradation and diffusion in vivo	Dinkins et al. (2016)
Gradient extrusion	Mesenchymal stem cell (MSC) derived EVs	mTOR agonist MHY1485	Renal ischemia-reperfusion injury	Reactive oxygen species (ROS) sensitive lipids, Thioketal (TK), embedded in the MSC membrane	271.73 ± 19.12 nm	Low yield and purity of MSC EVs	Ghadami and Dellinger (2023)
Serial extrusion	Human umbilical mesenchymal stem cells (hUMSCs) derived EVs	Mitochondrial-derived oxidative phosphorylation-related proteins	Wounds	N/A	100–200 nm	Differences in proteomic composition between EVs and mimetics could affect therapeutic outcomes Potential immune responses or off-target effects due to proteomic differences have not been fully investigated	Elsherbini and Bieberich (2018)

(Continues)

TABLE 4 | (Continued)

Technique	Source of EVs	Packaged cargo	Targeted cells/tissue	Lipid or surface modifications or composition	Average size (n.m)	Limitations/ concerns	Reference
Soft extrusion	Red blood cell-derived EVs (RBCEVs)	miR-210 and dextran polymers	Human umbilical vein endothelial cells (HUVECs) and peripheral blood mononuclear cells (PBMCs)	N/A	100–300 nm	N/A	Gupta et al. (2022)
Thin film hydration and extrusion	Mouse bone marrow- MSCs derived EVs	AMG487, a CXCR3 antagonist, which is a chemokine receptor inhibitor	Macrophages (RAW264.7 cells & bone marrow-derived macrophages) and osteoporotic bone, in an ovariectomy (OVX)-induced osteoporosis mouse model	Phosphatidylserine lipids (PS)	sEV mimetics (EMs):135.2 nm by NTA, phosphatidylserine-incorporated sEV mimetics (PS-EMs): 144.1 nm by NTA and 186.1 nm by DLS	PS-EMs exhibited cytotoxicity at high phosphatidylserine (PS) concentrations, limiting the amount that could be incorporated Study did not observe a significant additional effect of AMG487 over PS-EMs alone in reducing bone loss, indicating that the osteoclast inhibition effect of PS-EMs might have been dominant.	Sadeghi et al. (2023)
Microfluidics	Murine embryonic stem cells (ES cells)	mRNAs (Oct3/4 and Nanog, key stem cell markers), Intracellular proteins, Plasma membrane proteins (ICAM-1)	NIH-3T3 fibroblasts	N/A	60–120 nm	Low yield, size variability, cellular damage and storage and stability concerns	ClinicalTrials.gov (2024)

(Continues)

TABLE 4 | (Continued)

Technique	Source of EVs	Packaged cargo	Targeted cells/tissue	Lipid or surface modifications or composition	Average size (n.m)	Limitations/ concerns	Reference
Microfluidics	Macrophage plasma membranes of RAW 264.7 cells (murine macrophages)	Indocyanine Green (ICG)	Glioma cells (U-87 MG-Luc2) and orthotopic gliomas in a mouse model	No additional synthetic modifications	~51 nm	Batch-to-batch variation, toxicity concerns and stability and storage	Driedonks et al. (2022)
Coating of nanoparticle core	M2 macrophage-derived EV membranes from bone marrow-derived macrophages (BMDMs)	Smart silencer containing 3 interfering RNAs (siRNAs) and 3 antisense oligonucleotides (ASOs) targeting Dnmt3aos gene	M2 macrophages	PEI (polyethyleneimine) to improve sEV membrane fusion	EV membrane-coated polylactic-co-glycolic acid (EM-PLGA@Dnmt3aos Smart Silencer); 137 nm	Scalability and stability concerns The efficiency of Dnmt3aos knockdown was ~46%, indicating room for further optimization	Severic et al. (2021)
Coating of nanoparticle core	Macrophage membranes derived from THP-1 human monocytic leukemia cell membrane	No specific cargo was loaded	Pulmonary tissue in LPS-induced lung injury in mice and alveolar macrophages	Proline-Alanine-Serine (PAS) chains were genetically expressed on macrophage membranes before coating with no synthetic lipid modifications	~108 nm	Long-term immune effects of genetically modified membranes require further study	Yu et al. (2023)
Coating of nanoparticle core	Whole cell membrane from bone marrow-derived dendritic cells (BMDCs) from BALB/c mice	Not loaded with a specific drug but Ovalbumin (OVA) peptide was incorporated into some nanoparticles for antigen-specific T cell activation	Naïve and activated T cells and alloreactive T cells in 4C mice	Biotinylation of MCNPs allowed conjugation with SA-FasL	~389–412 nm	Size and charge variability	Qian et al. (2024)

(Continues)

TABLE 4 | (Continued)

Technique	Source of EVs	Packaged cargo	Targeted cells/tissue	Lipid or surface modifications or composition	Average size (n.m)	Limitations/ concerns	Reference
Membrane fusion	EVs derived from normal MRC-5 cells and TGF- β 1-activated MRC-5 cells	Cryptotanshinone (CTS) and fluorescent dyes	Lung myofibroblasts and fibrotic lung lesions in a bleomycin-induced pulmonary fibrosis rat model	Fibronectin-binding peptide (CREKA) and cell-penetrating peptide (TAT)	~130 nm	Further studies are needed to confirm safety for long-term pulmonary delivery	Zhu et al. (2022)
Extrusion and membrane fusion	EVs derived from HEK293T cells	ALKBH5 mRNA	colorectal cancer cells and colitis-associated cancer model mice	DOPE, DOTAP and DSPE-PEG-FA	235 \pm 4 nm	Unavoidable distribution of ALKBH5 mRNA to healthy tissues, therapeutic effects of ALKBH5 mRNA sEV are likely to depend on the cancer type, and ALKBH5 promotes tumorigenesis in some of other cancers	Biagiotti et al. (2024)
Membrane fusion	EVs derived from HEK293FT cells	CRISPR/Cas9 expression plasmids including: (pEGFP-C1, sgRNA-dCas9 targeting Runx2 gene and sgRNA-Cas9 targeting CTNNB1 gene)	MSCs and Bone marrow-derived MSCs	No additional surface modifications	~150 nm	Low encapsulation efficiency of large DNA plasmids in EVs and lipofectamine caused mild cytotoxicity in MSCs	Kang et al. (2024)

(Continues)

TABLE 4 | (Continued)

Technique	Source of EVs	Packaged cargo	Targeted cells/tissue	Lipid or surface modifications or composition	Average size (n.m)	Limitations/ concerns	Reference
Thin-film hydration and microfluidics	Synthetic	Genistein	N/A	DPPC, sphingomyelin (SM), DOPS, and cholesterol. Genistein was incorporated into the lipid bilayer	60–340 nm	The two methods yielded different particle sizes, with thin-film hydration producing smaller particles	Harre et al. (2012)
Lipid film hydration and size-exclusion chromatography	Synthetic	Curcumin	Neuronal cells (SH-SY5Y neuroblastoma cells) and zebrafish embryos	DPPC Sphingomyelin Cholesterol PEG-ceramide DODAP	Below 200 nm.	Curcumin-loaded exo-liposomes were stable for up to 3 months, while empty ones remained stable for 6 months. Curcumin incorporation increased the particle size and slightly affected homogeneity	Jo et al. (2014)
Thin-film hydration and Ionic gelation technique	Synthetic	Anti-VEGF siRNA	Glioblastoma (U87 MG) cells and Lung carcinoma (A549) cells	Membrane proteins (Cx43) into lipid bilayers, DOPC, DOPS, SM, Cholesterol, DOPE	L/CS-siRNA NPs: ~119 nm Cx43/L/CS-siRNA NPs: ~120 nm After incubation in serum: ~130 nm	Cx43/L/CS-siRNA NPs had lower siRNA delivery efficiency compared to Lipofectamine 2000, size variation	Wang et al. (2024)
Thin-film hydration and extrusion	Synthetic	No therapeutic cargo was mentioned	HeLa cells	DSPC, Cholesterol, DOPS and SM	100–148 nm.	The internalisation of liposomes was dependent on lipid composition and membrane fluidity. The liposomal stiffness decreased at higher temperatures	Groß et al. (2024)

(Continues)

TABLE 4 | (Continued)

Technique	Source of EVs	Packaged cargo	Targeted cells/tissue	Lipid or surface modifications or composition	Average size (n.m)	Limitations/ concerns	Reference
Double emulsion method	Synthetic	Histone A and Pigment Epithelium-Derived Factor (PEDF) plasmid DNA (pDNA)	Pulmonary vascular endothelial cells (ECs) and hypoxia-induced vascular remodeling and endothelial injury	PC, SM, PS, PE and Cholesterol	102 ± 2 nm	N/A	McMaster (2018)
Thin-film hydration and extrusion	Synthetic	VEGF siRNA	A549 (human lung carcinoma cells) and HUVEC	DOPC, DOPE, DOPS, SM and Cholesterol	118.87 nm	sEV-mimicking liposomes exhibited 31.5% encapsulation efficiency, lower than DOTAP liposomes (69.6%). Their uptake was higher than PC-Chol liposomes but varied by cell type. While they delivered more siRNA than PC-Chol liposomes, their efficiency was lower than DOTAP liposomes. Internalisation occurred via caveolae-mediated endocytosis in HUVEC cells and macropinocytosis + membrane fusion in A549 cells	Wang et al. (2023)

Abbreviations: DLS, dynamic light scattering; NTA, nanoparticle tracking analysis; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DPPC, dipalmitoylphosphatidylcholine; DODAP, 1,2-dioleoyl-3-dimethylammonium-propane; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPS, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine.

4.6 | Synthetics EVs

synEVs attempt to combine the low cost, ease of production and high encapsulation efficiencies of LNPs while attempting to mimic the bodies natural nanocarrier system. This is achieved by using synthetic lipids which match that of sEVs, and combining these at a molar ratio to mimic them. The lipid mixture can then be used to form nanoparticles through any method which is used in liposome or LNP production (Sakai-Kato et al. 2020; Tsakiri et al. 2024). As liposomes and LNPs are already in use in the clinic this could allow for a much more direct approach to their broader application. Additionally, with the potentially strong effect the lipid composition may be playing in sEVs it could maintain many of their beneficial characteristic and allow for much easier and cost-effective production. synEVs have been used to deliver curcumin to neuronal cells which exerted a neuroprotective effect (Fernandes et al. 2021) and have been packaged with VEGF siRNA which were then able to successfully be taken up by cells and exert a silencing effect. This was in addition to also showing a greater serum stability than traditional liposomes. Lu et al. (2018) completed this by formulating synEVs with a molar ratio of 30/21/17.5/17.5/14 (Chol/DOPC/SM/DOPE/DOPS), which was based off an 'average' sEV. These showed different uptake rates depending on the cell type and as there are no surface proteins present on these nanoparticles this could then be due to the differential lipid composition of the cells interacting with the lipids of the synEVs. Therefore, if a cell specific sEV molar ratio was used for the synEVs, as opposed to an average, this could possibly allow for an enhanced uptake. More recently Li et al. (2025) also used this technique to deliver PEDF-pDNA packaged within synEVs to the lungs of mice. These nanoparticles however were formulated to mimic HUVEC sEVs with a molar ratio of 10/10/15/10/35 (Chol/SM/PC/PS/PE). The Chol molar % seemed quite low in this formulation and this was based off a lipidomic analysis of HUVEC-derived sEVs, which had Chol at 4.1%, however it is not reported how these results were acquired and as they are significantly different to most other reported Chol levels this could be an error. Despite this, these synEVs were able to be packaged with, and deliver PEDF-pDNA and induce PEDF protein expression in vitro and in vivo. Additionally, Skotland et al. (2020) highlighted potential lipid species which may produce the best mimic of the lipids in sEVs, with formulations containing PC with one saturated and one monounsaturated fatty acyl group and a 16 or 18 length carbon chain, and the most common SM and glycosphingolipids being C16:0, C24:0 and C24:1 as the N-amidated fatty acyl chain. Following on from their work Lu et al. (2019) utilised cell-free protein synthesis systems to incorporate Connexin 43 into the synEVs. Although the incorporation of connexin 43 into the synEVs increased uptake efficiency in U87 MG cells it had no difference in A549 cells. The same result was also seen when comparing standard sEVs to synEVs without connexin 43. Therefore, while proteins can assist in improving the uptake in certain cell types, as similar uptakes in A549 cells are seen in sEVs, synEVs w/ connexin 43, and synEVs w/o connexin 43, this could suggest that the proteomic profile may not be crucial for the development of synEVs. However, with the development of cell free synthesis systems as well as a greater knowledge of the lipid composition of specific sEV populations this could allow for the production of sophisticated nanoparticle systems. A brief visualisation of how synEVs can be formulated can be seen in

Figure 3 and a summary of the use of synEVs for biomedical application can be found in Table 4.

5 | Future Directions

While the potential of EV-mimetics and synEVs is promising there are many aspects of these which require further research to elucidate their long-term therapeutic relevance. The role of lipids in contrast to proteins in the beneficial characteristics of sEVs is one such area which requires a deeper knowledge. It has been suggested that proteins play a dominant role in the beneficial characteristics of sEVs (like membrane fusion) (Kooijmans et al. 2012) and when pre-treating sEVs with paraformaldehyde, which causes cross-linking of proteins on the sEV membrane, their membrane fusion was reduced by ~20% compared to untreated sEVs. However, when treating sEVs with filipin, a molecule known to perturb the membrane composition of cells, a 50% reduction in membrane fusion occurred, suggesting that lipids may be playing a dominant role (Parolini et al. 2009). Although, proteins may still play a role as when sEVs were treated with octylglucoside and reconstructed through dialysis (removing all membrane proteins) they were unable to fuse with cells at all. However, octylglucoside is also known to cause disruption of lipid bilayer membranes at concentrations higher than the critical micelle concentration of 25 mM (Morandat and El Kirat 2007; Uratani and Hoshino 2000) and in the experiment performed by Parolini et al. (Parolini et al. 2009) sEVs were solubilised with 60 mM octylglucoside. This therefore would suggest that both the removal of membrane proteins and the disruption of the bilayer membrane would have occurred leading to the complete lack of membrane fusion. This therefore highlights the uncertainty around the role of lipids versus proteins in the function of sEVs. As LNPs and synEVs have been shown to effectively deliver to cells without any membrane proteins it is possible that they may not be an essential requirement in the development of future sEV-based delivery systems. However, as these synEVs were able to have their delivery to some specific cells enhanced when proteins were added to the membrane it is expected that proteins may be able to play a role in enhancing future nanoparticle systems. To further elucidate the role of lipids versus proteins in the beneficial characteristics of sEVs a greater knowledge of the lipid composition of various cell-derived sEVs is required. Once a greater level of knowledge is achieved this could allow for the development of highly specific synEVs which could mimic the lipid composition of the target sEV much closer. Additionally, as previously mentioned the various combinations of lipids can have significant effects in the ability of vesicles to escape the endosome and penetrate the nucleus. Future research could then determine exactly how many of the beneficial characteristics of sEVs are maintained when solely mimicking the lipid composition and minutely adjusting the lipid composition to determine the synergistic effects other lipids may have, which could be done by comparing synEVs to native sEVs in membrane fusion assays, in vitro biodistribution studies, ability to cross the BBB, serum stability, and so forth. If these synEVs could maintain many beneficial characteristics of sEVs then their much simpler and cost-efficient production could provide a much more streamlined approach to the clinic. However, the large degree of variation within molecular species of lipids could

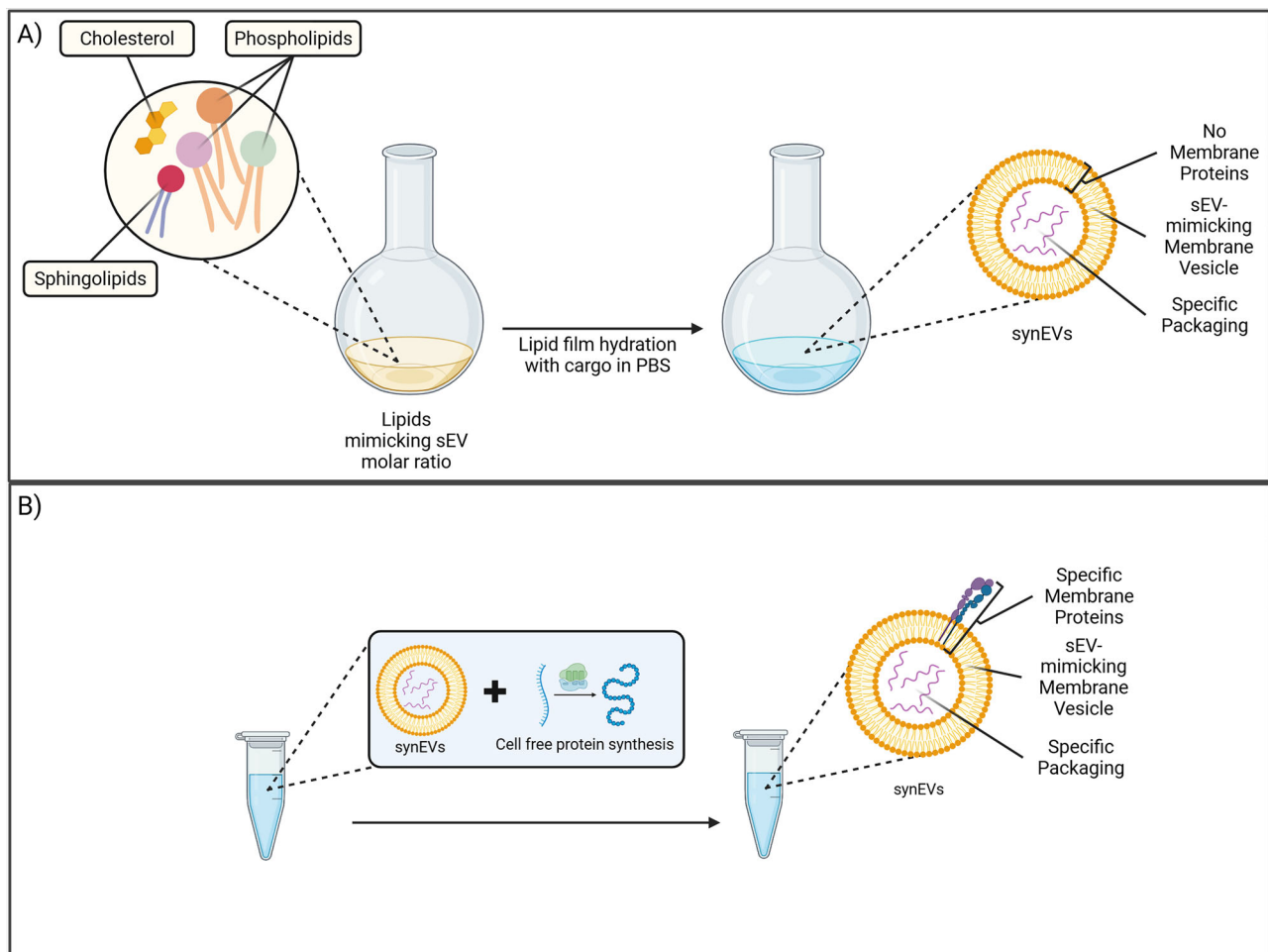


FIGURE 3 | Techniques used for the formulation of synthetic EVs by Lu et al. (2018). (A) Formulation of synEVs with synthetic lipids mimicking sEV molar ratios. (B) Use of cell free protein synthesis systems can allow for the introduction of proteins into synEV membranes.

make producing a formulation to exactly mimic sEVs difficult and the most common molecular species may have to be used. The asymmetrical distribution of lipids within each leaflet of natural EV membranes also presents another difficult problem for synEVs as mimicking this distribution is not possible with simple LNP formulation techniques. However, further research into the role different lipids play within each leaflet of the membrane may elucidate the degree of functional change which occurs with this as well as determining the degree of asymmetry which is present in sEV membranes following release. As the presence of phospholipid scramblase in sEV membranes (Laulagnier et al. 2004) may alter this. Additionally, with the development of cell-free protein synthesis systems, the inclusion of specific proteins which may enhance the function of synEVs could allow for an efficient, low-cost and sophisticated nanoparticle.

6 | Conclusion

The potential of sEVs as a therapeutic delivery vehicle is evident with their innate biodistribution and biocompatibility and their ability to avoid the MPS to extend their circulation time, cross the BBB, and exhibit tissue tropism. However, their therapeutic capability is limited by the labour-intensive, complex, inefficient and expensive techniques used in their harvesting and packaging

and as a result LNPs remain as the gold standard. EV-mimetics and synEVs attempt to bridge the gap between sEVs potential and the success of LNPs. However, EV-mimetics still rely on the harvesting and isolation of sEVs which is labour intensive and expensive, synEVs therefore represent a promising alternative which could combine the beneficial characteristics of sEVs (by mimicking their specific lipid composition) with the ease of production and low cost of LNPs. With a greater knowledge of the lipid composition of sEVs from different cell types more specific and sensitive nanoparticles could be formulated. Currently the major lipids found in sEVs are Chol, PC, PS, PE and SM, and each play unique and specific roles in the function of sEVs. However, the role of lipids found in smaller quantities, like LPC's suggested role in crossing the BBB, need to be further elucidated. Further research also needs to be undertaken to determine the effect lipids versus proteins has on the beneficial characteristics of sEVs to identify if proteins are a key requirement in these characteristics or can be used as modifiers to enhance the already present function of the lipids.

Author Contributions

Austin Brent: conceptualization, writing–original draft, writing–review and editing. **Paniz Shirmast:** writing–original draft, visualization. **Nigel**

A. J. Mcmillan: funding acquisition, writing–review and editing, conceptualization.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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