Table S1. Comparison of engineered EVs with established therapeutic delivery platforms.

Comparative dimension	Engineered EVs	Lipid nanoparticles	Viral vectors	Synthetic polymeric/inorganic nanocarriers
Representative cargo	Proteins, RNAs, mRNAs, gene-editing components, small molecules, or multimodal cargo combinations	Primarily nucleic acids, including mRNA, siRNA, antisense oligonucleotides, and gene-editing components	DNA, RNA, or genome-editing components, depending on vector type	Small molecules, proteins, nucleic acids, imaging agents, or combination payloads
Delivery and biological activity	Variable; limited by cargo loading, targeting, cytosolic delivery, and product heterogeneity	Clinically validated for nucleic acid delivery, particularly RNA-based therapeutics	Often high gene-transfer efficiency and durable expression in permissive tissues	Highly formulation-dependent; delivery efficiency and release kinetics vary by material and route
Manufacturing and regulatory maturity	Emerging; limited EV-specific precedent, with challenges in scalable production, purity, potency assays, and batch consistency	Relatively mature for RNA delivery, with established formulation, scale-up, and clinical precedents	More established for gene therapy, but manufacturing capacity, cost, and quality consistency remain challenging	Some precedent from nanomedicines, but regulatory assessment remains product- and material-specific
Main advantages	Biocompatibility, multimodal cargo potential, surface engineering flexibility, and suitability for local or niche-level modulation	Scalable formulation, efficient nucleic acid encapsulation, transient expression, and strong clinical validation	Efficient gene transfer, long-term expression when desired, and established gene therapy experience	Precise physicochemical tunability, scalable synthetic chemistry, and broad formulation flexibility

Comparative dimension	Engineered EVs	Lipid nanoparticles	Viral vectors	Synthetic polymeric/inorganic nanocarriers
Main limitations	Heterogeneity, variable cargo loading, difficult potency definition, immature GMP workflows, and limited regulatory precedent	Limited efficiency and specificity for delivery beyond the liver, reactogenicity, repeat dosing constraints, and formulation-dependent toxicity	Cargo-size limits, immunogenicity, redosing barriers, genotoxic concerns for some systems, and manufacturing complexity	Limited biological specificity, protein corona–mediated alteration of targeting and biodistribution, systemic clearance, material-dependent toxicity, and variable clinical translation

Table S2. Limitations of unmodified EVs and corresponding engineering solutions.

Limitation of Natural EVs	Impact on Therapeutic Efficacy	Engineering Solution & Rationale
Low Loading Efficiency	Inadequate therapeutic cargo (e.g., mRNA, proteins) reaches the target site, leading to suboptimal effects.	Genetic Engineering of Parental Cells: Forces the overexpression and selective packaging of therapeutic molecules into EVs during biogenesis, significantly increasing cargo yield.
Poor Target Specificity	Off-target distribution reduces therapeutic index and increases the risk of side effects. Batch-to-Batch Variability: Inherent heterogeneity in EV sources complicates manufacturing standardization and dosing.	Surface Modification: Display of targeting ligands (e.g., peptides, antibodies) via genetic fusion or chemical conjugation promotes EV binding or uptake through specific receptors on target cells[1-3]. Synthetic Biology Approaches: Cell-free production and bottom-up assembly of EV mimetics offer greater control over composition and reproducibility.
Rapid Systemic Clearance	Short circulatory half-life limits bioavailability and requires frequent dosing, as EVs are rapidly cleared[4,5].	Biomaterial-Assisted Delivery: Encapsulation of EVs in hydrogels or scaffolds provides localized, sustained release, reducing premature loss and enhancing retention at the target site[6-10].
Inefficient Cytosolic Delivery	Even upon reaching the target cell, therapeutic cargo (especially nucleic acids) can be trapped and degraded in endosomes, preventing functional activity[11].	Endosomal Escape Enhancement: Engineering EVs to express fusogenic proteins (e.g., VSV-G) promotes the release of cargo from endosomes into the cytoplasm, a critical step for enabling efficient in situ translation of mRNA[12].

Table S3. Representative endogenous loading strategies for therapeutic cargo delivery by engineered EVs.

Strategy Classification	Specific Method	Mechanism	Typical Cargo	Representative evidence
Parental-cell engineering	Protein overexpression	Target proteins are overexpressed in producer cells and incorporated into EVs during vesicle biogenesis.	Therapeutic proteins (such as EPO), reporter proteins (TGL)	Comparative studies have reported greater protein-loading capacity in large EV fractions than in the corresponding small EV fractions, although these properties depend on the separation strategy and producer cell[13].
RNA-protein interaction	MS2-MCP system	MCP fused to EV-associated proteins recruits RNAs containing MS2 hairpins into EVs.	mRNA, shRNA, sgRNA-containing RNPs	MS2-MCP engineering can enrich target RNA cargo in EVs[14].
Virus-like delivery system	VEDIC/VFIC system	Combines VSV-G-mediated membrane fusion with intein-based cargo release to enhance intracellular delivery.	Full-length mRNA, CRISPR/Cas9 RNP	Supports functional intracellular delivery of protein and RNA cargo, including in vivo delivery in mouse models[12].

Table S4. Representative post-isolation loading strategies for therapeutic cargo delivery by engineered EVs.

Strategy Classification	Specific Method	Mechanism	Typical Cargo	Advantage	Disadvantage	References
Passive Loading	Incubation	Cargo enters EVs mainly through passive diffusion.	Hydrophobic small molecules	Simple; minimal structural disruption	Very low loading efficiency; unsuitable for large cargos such as proteins or mRNA	[15]
Physical Methods	Electroporation	Electrical pulses create transient membrane pores.	siRNA, miRNA, mRNA	Broadly applicable to charged molecules	May cause EV/cargo aggregation and impair membrane proteins	[15,16]
	Sonication	Ultrasound temporarily disrupts and reseals EV membranes.	Proteins, small molecule drugs	Can improve loading efficiency	May damage EV integrity or cargo activity; requires optimization	[15,17]
	Extrusion	EV-cargo mixtures are forced through nanoporous membranes.	Proteins, nucleic acids	Produces relatively uniform vesicles	May alter EV membrane properties and therefore requires careful optimization.	[15]
Chemical Methods	Saponin Permeabilization	Saponin forms temporary pores by interacting with membrane cholesterol.	Proteins, small molecules	Relatively mild and efficient	Residual saponin may be cytotoxic and must be removed	[17]

	Sodium carbonate treatment	Transient alkaline pH increases EV membrane permeability.	Proteins, small molecules	Maintains EV size, morphology, composition, and uptake ability; protects cargo from degradation	Relatively low protein loading; may affect peripheral proteins	[18]

Table S5. Strategies for enhancing cytoplasmic delivery and translation efficiency.

Strategy	Specific Methods	Core Mechanism	Key Effects	References
Enhance endosomal escape	VSV-G	Promotes EV–endosomal membrane fusion under acidic endosomal conditions.	Enhances cytosolic cargo release and functional Cre recombinase delivery.	[12]
	Aurein 1.2 and GALA	Promote endosomal escape through membrane-disruptive or fusogenic activity.	Enhance cytosolic protein delivery.	[19,20]
Optimizing mRNA Translation	Nucleotide Modifications	Modified nucleotides, such as pseudouridine, reduce innate immune recognition.	Improves mRNA stability, prolongs protein expression, and reduces cytotoxicity.	[21]
	UTR engineering	Optimized 5′ and 3′ UTRs improve translation efficiency.	Can enhance protein expression levels.	[22]
	Terminal structure optimization	5′ cap and 3′ poly(A) tail protect mRNA and promote translation initiation.	Enhances mRNA stability and translation.	[23]

Table S6. The following table compares genetically engineered EV surface display strategies.

Anchoring Protein	Targeting Ligand	Target Receptor/Pathway	Primary Application Context	Key Outcome/Quantitative Data
Lamp2b	RVG peptide	Nicotinic acetylcholine receptor	Neurodegenerative diseases (e.g., Alzheimer's, Parkinson's)	Enhanced brain accumulation compared with non-targeted EVs in preclinical models.
Lamp2b / pDisplay	GE11 peptide	EGFR	Cancer (e.g., breast, lung)	Enhanced EGFR-mediated cellular uptake.
GPI anchor	EGa1 nanobody	EGFR	Cancer (e.g., neuroblastoma)	15–25% of EVs conjugated; improves binding specificity to EGFR.
PDGFR Transmembrane Domain	RGD/iRGD peptide	Integrins (αvβ3, αvβ5)	Cancer (angiogenesis, metastasis)	Enhanced cellular uptake in endothelial and cancer cells.
PTGFRN	HaloTag / Antibody fragments	Various (e.g., GLP1 receptor)	Versatile platform for diverse targeting	Successful targeting to primary human hepatocytes and GLP1R-overexpressing cells.

Table S7. The following table compares the main chemical and physical surface modification strategies.

Modification Method	Mechanism	Key Advantages	Limitations / Considerations	Typical Conjugation Efficiency / Ligand Density
Click Chemistry (SPAAC)	DBCO reacts with azide groups on EVs	High specificity, biocompatible, versatile	Requires metabolic engineering of parent cells to introduce azide groups	High conjugation efficiency reported in some studies
Lipid Insertion	Spontaneous insertion of DSPE-PEG-ligand conjugates into the EV membrane	Simple procedure; no genetic modification required; suitable for post-isolation modification.	Potential micelle formation, ligand stability may vary	Rapid surface functionalization with high efficiency
Covalent Conjugation (EDC/NHS)	Reaction between surface amines and ligand carboxyl groups	Direct modification, applicable to various amine ligands	May modify internal cargo proteins; potential EV aggregation	Ligand density can be adjusted, depending on EV source and reaction conditions.
Covalent Conjugation (Maleimide-thiol)	Reaction between maleimide and thiols (-SH) on ligands	High specificity for thiols; stable bond	Ligands must have accessible thiols	Case-dependent; commonly used for antibody fragments

Table S8. Selected hydrogel platforms for sustained EV delivery.

Hydrogel Material	Producer cell/source	Application	Key Findings	Reference
Supramolecular HPMC-C12/C18	Mesenchymal stem cells (MSCs)	Anti-inflammatory therapy	Non-covalent EV–polymer interactions enabled sustained release; enhanced anti-inflammatory effect compared to bolus EV administration	[6]
Gelatin Methacrylate (GelMA)	MSCs	Wound healing, Bone regeneration	Sustained EV release from GelMA promoted angiogenesis and wound repair in diabetic wound models.	[9]
PEG-based hydrogel	MSCs	Liver regeneration	Controlled EV release improved therapeutic efficacy in chronic liver injury models relative to free EVs	[7]
Silk Fibroin (SF) / Sodium Alginate (SA) bioink	MSCs	3D bioprinting for tissue engineering	Hybrid bioink enabled controlled EV release and enhanced biological performance of 3D-printed scaffolds	[24]
Cationized Gelatin Hydrogel	MSCs	Regenerative therapy	Prolonged EV retention and controlled release dependent on cross-linking; released EVs maintained immunomodulatory activity	[25]
dECM-based bioink	Chondrocytes	Cartilage repair	Tissue-specific scaffold combined with EVs promoted chondrogenesis via 4E-BP/Smad2 signaling pathway	[10]
Microfiber- Reinforced GelMA Hydrogel	hiPSCs	Promoting angiogenesis	Fiber reinforcement and higher GelMA concentration delayed EV release, reduced burst, and supported endothelial cell internalization up to ~14 days	[8]

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