

REVIEW ARTICLE

Organoid-derived extracellular vesicles: From organoid, to organoid

Ting Cheng^{1,2,3,4} , Wojciech Chrzanowski^{5,6*} , Han Liu^{1,2,3,4,7,8*} , and Jiacan Su^{1,2,3,4,9*} 

¹Institute of Translational Medicine, Shanghai University, Shanghai, China

²MedEng-X Institutes, Shanghai University, Shanghai, China

³Organoid Research Center, Shanghai University, Shanghai, China

⁴National Center for Translational Medicine (Shanghai), Shanghai University, Shanghai, China

⁵Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Camperdown, New South Wales, Australia

⁶Department of Materials Science and Engineering, Division of Biomedical Engineering, Uppsala University, Uppsala, Sweden

⁷Suzhou Innovation Center of Shanghai University, Suzhou, Jiangsu, China

⁸Sanming Second Hospital, Sanming, Fujian, China

⁹Department of Orthopedics, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

*Corresponding authors: Wojciech Chrzanowski (wchrzanowski@sydney.edu.au); Han Liu (liuhanqiu@shu.edu.cn); Jiacan Su (drsujacan@163.com)

Citation: Cheng T, Chrzanowski W, Liu H, Su J. Organoid-derived extracellular vesicles: From organoid, to organoid. *Organoid Res.* 2025;1(4):025450031. doi: 10.36922/OR025450031

Received: November 5, 2025

Revised: December 24, 2025

Accepted: December 25, 2025

Published online: December 30, 2025

Copyright: © 2025 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, which provided that the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Organoids are miniature, three-dimensional tissue constructs generated from stem cells or primary cells that recapitulate key structural and functional features similar to those of *in situ* organs. Extracellular vesicles (EVs) are non-replicating nanocarriers enclosed by a phospholipid bilayer (20–400 nm) that mediate the delivery of bioactive substances. Organoid-derived EVs (OEVs) are easier to produce than conventional EVs and exhibit enhanced biological functions. Given that organoids retain stem cell-like characteristics, the transportation of bioactive substances by OEVs shows broad prospects in medical applications. This article examines the development, concepts, construction methods, and applications of organoids, providing an overview of their types, research progress, and advantages. The review also outlines the basic biological features of EVs and their potential applications in disease treatment and intervention. Furthermore, we examine the key distinctions between OEVs and conventional EVs. Finally, this review summarizes the advantages and challenges of OEVs and outlines their future prospects in disease treatment.

Keywords: Organoids; Organoid-derived extracellular vesicles; Cell-free therapy; Regenerative medicine

1. Introduction

Organoids are miniature, three-dimensional (3D) organ models formed *in vitro* through the self-organization and self-assembly of stem cells, including pluripotent, embryonic, and adult stem cells, as well as primary tissue fragments, under defined culture conditions. Given that organoids recapitulate key structural and functional features of *in vivo* organs, they are likely to secrete and utilize extracellular vesicles (EVs) throughout their

development.^{1,2} This technology has been rapidly adopted for research in stem cell biology, organogenesis, and the study of human diseases. The term “organoid” originally refers to various types of *in vitro* cultures resembling organs, including tissue explants and organ-on-a-chip systems. Over the past few decades, developmental and stem cell biology have elucidated the molecular mechanisms underlying stem cell self-renewal and differentiation along specific lineages; regenerative medicine has demonstrated

that dissociated stem cells can reconstruct organs *in vivo*.³⁻⁵ These advancements have collectively advanced the concept of reconstructing intact organs *in vitro*. In recent decades, while classical two-dimensional (2D) cell lines have driven numerous medical breakthroughs, their limited differentiation capacity and restricted lineage diversity have made it challenging to simulate complex disease mechanisms or organ development processes, rendering them increasingly inadequate for contemporary precision medicine needs.⁶ With the rise of stem cell technology, organoids have emerged as 3D culture systems in which stem cells self-assemble into microphysiological systems, successfully bridging the gap between traditional cell lines and animal models.⁷ Organoids comprise multiple cell types, and the interactions between cells are relatively complex. They can reproduce disease characteristics for drug screening and mechanism research, serving as a more effective research platform than 2D models. In recent years, researchers have begun to isolate EVs from organoid culture media.

EVs are non-replicating, nanoscale, membrane-bound messengers (typically 20–400 nm in diameter) that mediate intercellular and interorgan communication by transporting bioactive molecules.^{2,8} Due to their unique properties, including nanoscale dimensions, minimal toxicity, elevated capacity for drug loading, excellent biocompatibility, straightforward functionalization and modification capabilities, as well as the potential for large-scale industrial production, EVs are increasingly recognized as a highly promising platform for next-generation delivery systems in biomedicine.⁹

Compared to traditional 2D culture-derived EVs, organoid-derived EVs (OEVs) retain the nanoscale size and biocompatibility of EVs while containing a greater diversity and higher abundance of bioactive molecules due to the 3D microenvironment, thereby demonstrating enhanced regulatory potential.¹⁰

This review introduces for the first time the closed-loop intervention concept of “from organoids to organoids,” while proposing that “organoid microenvironment specificity” determines the molecular fingerprint and functional advantages of OEVs. It lays the theoretical and technical foundation for OEVs to advance toward new paradigms of personalized exosomal therapeutics and *in situ* regeneration induction.

In this study, we review the development, conceptual framework, construction methods, and applications of organoids. We comprehensively examine the types, research progress, and advantages of EVs, and introduce the fundamental biological principles underlying OEVs. We explore their potential in therapeutic interventions across a range of diseases and highlight the distinctions between

OEVs and conventional EVs. Finally, we summarize the advantages and challenges of OEVs, outline future prospects for their use in disease treatment, and comment on the potential of using OEVs in organoid construction and functional testing, including drug screening and developmental biology.

2. Overview of organoids

2.1. History and development of organoids

Organoids are 3D multicellular tissue structures cultivated *in vitro* that resemble *in vivo* organs. Their development can be traced back to the 1970s, when Yi *et al.*¹¹ successfully formed layered squamous epithelial colonies similar to human epidermis through the co-cultivation of primary human keratinocytes and 3T3 fibroblasts.¹¹ This achievement laid the foundation for the subsequent development of organoid technology; however, the cultivation methods at that time were primarily based on 2D surfaces, which limited research into the 3D organizational behavior of cells.¹² Subsequently, developmental biologists studied the morphogenesis processes of various model organisms (such as sponges, amphibians, chicks, and mice) and demonstrated the proficiency of cells to autonomously organize and re-cluster into structures resembling tissues. These studies emphasized the key role of self-organization in morphogenesis and provided theoretical support for the development of organoid technology.^{13,14} In the late 1980s, Jiang *et al.*¹⁵ observed that a gel rich in laminin could serve as a substitute for the basement membrane, promoting the differentiation and morphogenesis of breast epithelial cells. This discovery enables the use of the extracellular matrix (ECM) for 3D culture.¹⁵ By the 1990s, studies had shown that ECM components not only provided physical support but also regulated gene expression through integrin adhesion. In 2009, the Anania team¹⁶ cultured single intestinal stem cells in an ECM substitute and cultivated intestinal organoids with crypt–villus structures. This milestone promoted the development of organoid technology, ushering in the organoid era and enabling the construction of numerous organ models, including the stomach, liver, pancreas, prostate, and brain, based on adult stem cells. Meanwhile, the replication of physiological microenvironments has led to the development of angiogenesis technology, assisting in the transport of oxygen and nutrients. By the end of the 2020s of the 21st century, in-depth understanding of organogenesis mechanisms and advances in biomaterials and bioengineering led to the customization of organoid technology.¹⁷ Scientists have developed customizable hydrogel pads to precisely regulate key extracellular factors in organoid development. Advances in induced pluripotent stem cells (iPSCs) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technology enable the

construction of accurate disease model organoids through specific gene mutations, which enhances physiological relevance and promotes widespread applications in organ-level biology, disease modeling, and regenerative medicine. Research in developmental and stem cell biology has elucidated the molecular behaviors of stem cells and progenitor cells, such as self-renewal and differentiation into specific tissue lineages, laying a theoretical foundation for reconstructing organs with stem cells *in vitro*.¹⁸ Over the past few decades, organoid technology has undergone rapid development in the study of tissue and organ biology, with applications ranging from basic research to clinical practice.

2.2. The concept and construction strategies of organoids

2.2.1. The concept of organoids

Organoids are 3D cell clusters formed by stem cells. They self-organize through differentiation in the laboratory to simulate the spatial and functional characteristics of natural organs; however, they do not correspond to real human organs. The term “organoid” is derived from “organ” and the suffix “-oid,” meaning “similar,” reflecting the structural and functional resemblance of organoids to native organs.¹⁹ The development of organoids is limited by lineage identification, cell sorting, specific culture conditions, and cytokine requirements.¹⁰ Organoids are simplified models of organs, offering great potential in tissue development, disease modeling, personalized medicine, drug screening, and cell therapy.

2.2.2. The construction strategies of organoids

There are three steps in organoid construction: creating a matrix gel, building biological scaffolds, and utilizing regulatory factors. It creates an environment similar to the natural body, helping cells self-assemble and differentiate, and ultimately forms organoids with specific organ properties and structures.

Active matrix gels mimic the ECM to support cell growth and differentiation by simulating the matrix environment required for normal cell growth. These gels can be selected or optimized to adapt to the characteristics of specific organs, such as the microenvironmental signals for breast epithelial cell regeneration, or the types of gels used for liver and neural organoids.²⁰ Moreover, tuning the physical properties of matrix gels enables simulation of organ-specific microenvironments, thereby promoting cell growth and functional maturation.

Bioactive scaffolds are crucial in tissue engineering for providing mechanical support and spatial characteristics that mimic those of natural organs. They offer cells a 3D structural framework that replicates the mechanical

properties and spatial features of tissues. Scaffold construction involves selecting appropriate materials, including natural polymers such as collagen and gelatin, as well as synthetic ones like polylactic acid-hydroxyacetic acid copolymers. Scaffold design is further tailored to meet organ-specific needs, with soft scaffolds used for soft tissue organoids, such as the liver and kidney, and stiff compositions for hard tissue organoids, like bone.¹⁰ Recent progress has been made in the field of biomedical engineering, leading to the innovation of composite bioactive scaffold materials. These materials can better mimic the microstructures of natural organs and enhance their functions in medical applications, such as tissue engineering.

Growth regulatory factors, such as cytokines, play a crucial role in the development of bone organoids. They interact with receptors on the cell surface, activate signaling pathways to shape cell behavior, and affect cell differentiation and function. The Wnt/ β -catenin pathway is important for the development of intestinal organoids, and the bone morphogenetic protein pathway is crucial for the development of skeletal organoids.^{21,22} Researchers regulate the concentration and exposure duration of signaling molecules to direct cell differentiation along specific lineage pathways, enabling the *in vitro* cultivation of organoids that recapitulate the structural and functional characteristics of native organs.

2.3. Applications of organoids

Organoids are a key cell culture method in biomedicine, used to simulate tissue-like structures, support long-term culture, and enable the construction of 3D architectures. These characteristics enable them to have numerous biological and clinical applications, and can be used as miniature models of human organs to conduct mechanistic research. The importance of organoids in biobanks lies in their ability to preserve complex cellular structures, thereby providing support for future research and treatment. They have great potential in the field of precision medicine and can develop personalized treatment plans tailored to individual patients. Organoids are also of great significance in regenerative medicine, offering new ways to repair or regenerate damaged tissues and bringing hope for the advancement of medical treatment (Figure 1).

Organoids are utilized in various applications, including genetic disease modeling, drug screening, personalized cancer treatment, gene repair and transplantation, biobank construction, and regenerative medicine. Since 2009, patient-derived organoids (PDOs)—particularly rectal organoids carrying *CFTR* mutations—have been widely used to model cystic fibrosis. With the subsequent development of genome-editing tools such as CRISPR/

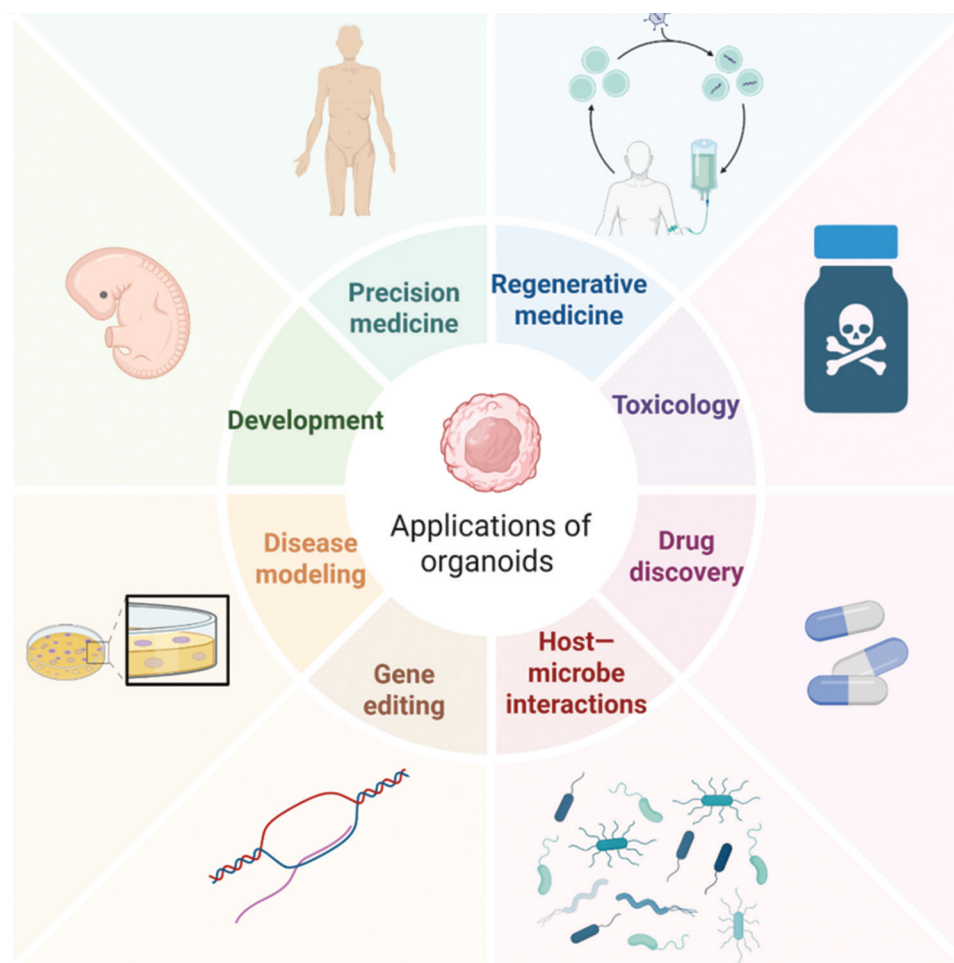


Figure 1. Applications of organoid technology. The chart presents the current status of organoid technology across various fields, including developmental biology, disease modeling, precision medicine, generative medicine, toxicology, drug discovery, host-microbiome interactions, and gene editing. Created with BioRender. Bigbone, B. (2026) <https://BioRender.com/7fgijk4>.

Cas9 and base editors, pathological phenotypes have been reproduced in diverse organoid models of genetic diseases, including polycystic kidney disease, Fabry disease, retinitis pigmentosa, microcephaly, and Down syndrome.²³ The “mutation-repair-recovery” closed-loop process can be completed within 7–14 days, providing a basis for dosage and delivery parameters.²⁴ Liver, heart-lung-liver multi-organ chips, brain, intestine, and cardiac muscle organoids have been integrated into a high-throughput platform, enabling the screening of hundreds of drugs for metabolism, toxicity, infection, or arrhythmia within 48–72 h, significantly reducing the risk of failure in later stages.²⁵ In oncology, studies based on biobanks comprising more than 500 PDOs, which retain 95% of the primary mutation load, have demonstrated that organoid-tumor-infiltrating lymphocyte co-culture or single-cell multi-omics approaches can accurately predict the efficacy of programmed cell death protein 1 inhibitors, protein arginine methyltransferase 5 inhibitors, enzalutamide, epidermal growth factor receptor tyrosine kinase inhibitors, and antibody-drug conjugates.

These platforms have achieved an 85% consistency rate and have progressed into prospective clinical trials.²⁶ In terms of gene repair, CRISPR/Cas9 or adenine base editors achieved more than 90% homologous repair in intestinal, retinal, lung, and brain organoids. When combined with vascular endothelial co-culture or immune-modulating hydrogels, this approach maintains function for over 6 months and has been shown to reduce infarct volume by 40%. PDOs serve as a living biological sample bank, spanning 10 types of tumors and non-tumor diseases.^{27,28} They maintain genetic fidelity and tumor heterogeneity even after continuous passage, enabling the discovery of new targets, the optimization of drug combinations, and the prediction of sensitivity to radiotherapy and chemotherapy. In regenerative medicine, organoids are increasingly viewed as a scalable, autologous transplant option. Intestinal, liver, bile duct, and neural organoids have been shown to reconstruct functional crypts-villus architecture, alveolar-like structures, and neural synaptic networks in animal models, alleviating conditions such

as short bowel syndrome, acute liver failure, and certain neurodegenerative diseases.²⁹ Moreover, CRISPR-mediated gene correction strategies are being expanded to complex conditions, such as Parkinson's disease.

3. Overview of EVs

3.1. Definition of EVs

EVs are nanoscale structures bounded by a membrane that are actively secreted by nearly all eukaryotic and prokaryotic cells. These vesicles typically exhibit diameters ranging from 30 nm to 1 μ m and are enclosed in a phospholipid bilayer membrane.³⁰ EVs are primarily classified into three principal types based on their size and the pathways through which they are formed: (i) Exosomes, which measure between 30 and 150 nm in diameter and arise from the intricate process of fusion between multivesicular bodies and the plasma membrane; (ii) Microvesicles, with diameters ranging from approximately 100–1,000 nm, which are generated by direct budding off of the plasma membrane, and carry diverse biological signals; and (iii) Apoptotic bodies, which are notably larger than 1,000 nm and are formed through fragmentation during the later phases of apoptosis, facilitating controlled clearance of cellular debris.³¹ EVs carry cell-specific components, such as proteins, lipids, messenger RNA (mRNA), microRNA (miRNA), DNA fragments, and metabolites, forming a stable molecular fingerprint. Integrins, glycoproteins, and ligands on the surface facilitate tissue targeting and participate in communication between cells, organs, and the host microenvironment, regulating biological processes such as immune response, inflammation, angiogenesis, tumor metastasis, and tissue regeneration.^{32–34} EVs are regarded as promising new cell-free therapies and precise delivery systems. It has low immunogenicity, minimizing adverse immune risk, good compatibility with biological systems, and is able to achieve body integration and function. In addition, it can be engineered for targeted delivery of drugs or nucleic acids, thereby improving therapeutic effects and exploring new medical applications.

3.2. Structure of EVs

EVs have a phospholipid bilayer membrane and show a spherical or cup-shaped morphology. Their diameter ranges from 30 nm to 1 μ m, and the thickness of the membrane is about 4–6 nm.^{35,36} The membrane contains cholesterol, sphingomyelin, and phosphatidylserine, forming a rigid and negatively charged shell. Transmembrane proteins, such as cluster of differentiation (CD) 63, CD81, CD9, integrins, and their ligands, are embedded in the membrane surface, enabling the cell to exhibit target recognition and adhesion.^{37–39} There are specific proteins, lipids, mRNAs, miRNAs, DNA fragments, and metabolites from the parent cell, serving as a relatively stable “cargo” storage

place.⁴⁰ EV subpopulations are derived from multivesicular bodies, which contain numerous small vesicles, whereas microvesicles display a more uniform structure. This structure enables EVs to resist enzymatic degradation, pass through physical barriers, and deliver bioactive molecules to targets, which is important in intercellular communication and biological function^{6,41,42} (Figure 2).

3.3. Classification of EVs

EVs are divided into three subclasses based on biogenesis, size, and biochemical properties. Exosomes are a distinct type, with a diameter of 30–150 nm, that are released into the extracellular space after the fusion of multivesicular bodies with the plasma membrane, carrying substances such as transmembrane proteins (e.g., CD63, CD81, CD9) and nucleic acids (e.g., mRNA, miRNA). They are used for intercellular communication and have therapeutic potential.^{43,44} Microvesicles (with a diameter of 100–1,000 nm) bud from the plasma membrane and have markers such as integrins on their surface for cell signaling. Apoptotic bodies (with a diameter >1,000 nm) are derived from the fragmentation of the cell membrane during apoptosis and contain nucleosomes, histones, and DNA fragments of the dead cells.^{45,46} In addition, EVs derived from bacteria can be classified into different subtypes based on the type of bacterial cell wall. Outer membrane vesicles of Gram-negative bacteria contain lipopolysaccharides, which play a crucial role in interactions with host cells and the immune response. In contrast, cell membrane vesicles from Gram-positive bacteria lack lipopolysaccharides, which influences their biological functions. Bacteria-derived EV subtypes also differ in size, membrane composition, cargo, and targeting properties. This diversity provides potential opportunities for the diagnosis and treatment of various diseases.⁴⁷

4. Overview of OEVs

OEVs secreted by organoids are similar to EVs generated through 2D cell culture. OEVs facilitate the exchange of information and the transport of substances between cells in organoids, serving as key mediators of intercellular communication. However, due to the unique 3D structure and diverse cell types of organoids, OEVs differ from traditional EVs, as OEVs more closely resemble EVs extracted from human biological fluids (Figure 3).

4.1. Sources, structure, composition, and internalization of OEVs

4.1.1. Sources of OEVs

OEVs originate from three release pathways of donor cells and follow a highly conserved and regulated “endocytosis–sorting–secretion” cascade. First, microvesicles (100–1,000 nm) are directly pinched off through local outward

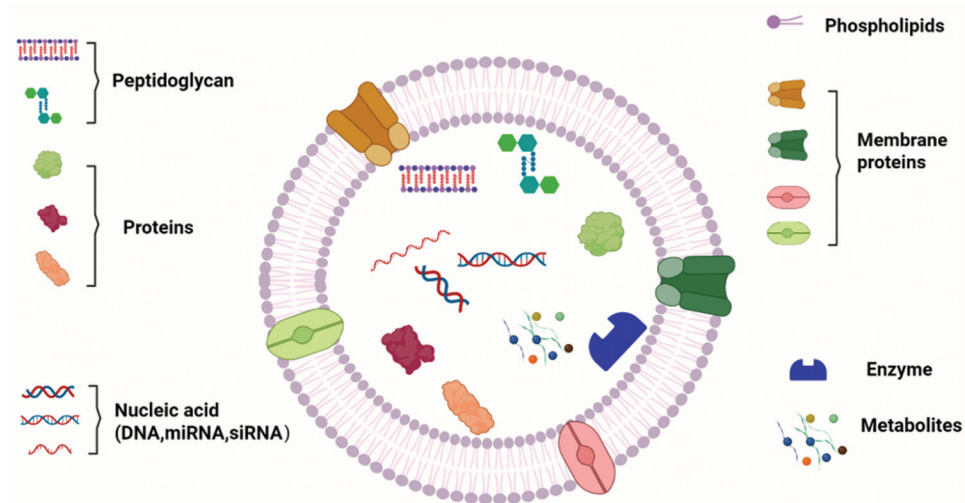


Figure 2. Structure of EVs. EVs are nanospheres with a diameter of 30–1 μm . They have a phospholipid bilayer membrane with a thickness of approximately 4–6 nm and transmembrane proteins on its surface. They serve as stable cargos for specific proteins, lipids, microRNAs, DNA fragments, and metabolites. Created with BioRender. Bigbone, B. (2026) <https://BioRender.com/srzj412>.

Abbreviation: EVs: Extracellular vesicles.

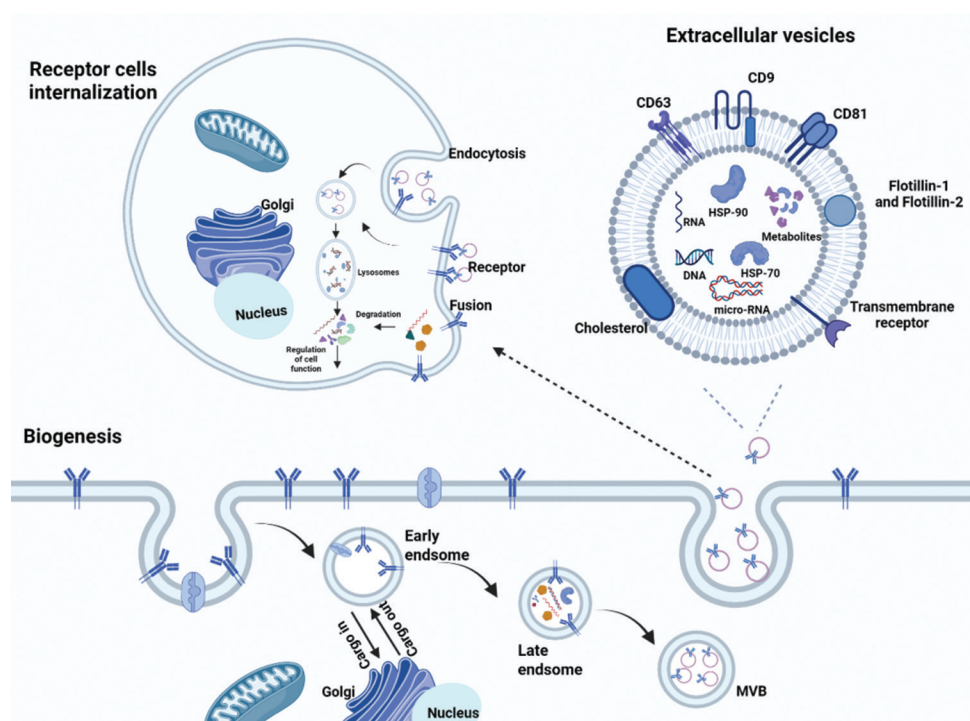


Figure 3. The biogenesis of OEVs. The biogenesis of OEVs is a complex process, beginning with plasma membrane invagination to form early endosomes, which mature into late endosomes through the exchange of cargo with the Golgi. Late endosomes accumulate intraluminal vesicles and can either fuse with lysosomes for degradation or merge with the plasma membrane to release EVs. OEVs are phospholipid bilayer vesicles that transport RNA, DNA, and proteins. Recipient cells internalize them via membrane fusion, endocytosis, or receptor-mediated pathways. Created with BioRender. Bigbone, B. (2026) <https://BioRender.com/g607k0s>.

Abbreviations: CD: Cluster of differentiation; EVs: Extracellular vesicles; MVB: Mammalian extracellular vesicles; OEVs: Organoid-derived extracellular vesicles.

budding of the plasma membrane, which is dependent on actin remodeling and the small guanosine triphosphatase adenosine diphosphate ribosylation factor 6, and are rapidly released into the culture medium. Second, exosomes

(30–150 nm) are formed from early endosomes through double invagination to create intraluminal vesicles. The endosomal sorting complex required for transport-0, I, II/III complexes recognize ubiquitinated cargo and drive

membrane curvature, followed by the maturation of early endosomes into multivesicular bodies (MVBs). The fate of MVB is regulated by small guanosine triphosphatases such as Rab27a/b, Rab35, and RalA, as well as fusion molecules such as cortactin and synaptotagmin-7. If MVBs migrate along microtubules and dock with the plasma membrane, soluble NSF attachment protein receptor complexes mediate the release of exosomes, thereby achieving paracrine/autocrine signaling. In contrast, if MVBs fuse with lysosomes or autophagosomes, their contents are degraded, and signaling is terminated. The third source is apoptotic bodies (50–5,000 nm), which are generated during late programmed cell death by cellular fragmentation and carry nuclear DNA and histones; however, research often focuses on the exosome subtype. In summary, OEVs production comprises four key steps: endocytosis, sorting, transport, and membrane fusion. The proportion of subtypes and release efficiency depend on the microenvironment of the organoid, its metabolic state, and genetic background, laying the foundation for subsequent functional studies.⁴

4.1.2. Structure of OEVs

OEVs exhibit a typical lipid bilayer vesicle structure, with membrane components that are highly similar to those of the donor cell membrane and are more specialized.⁴⁸ Exosomal subpopulation membranes are enriched in ceramides, gangliosides, and unsaturated lipids, and the ratio of phosphatidylcholine and diacylglycerol is relatively low.⁵ The outer leaflet has more cholesterol and phosphatidylserine. This asymmetric distribution contributes to membrane rigidity and facilitates endocytosis by target cells through “eat me” signals. EVs are formed in the multivesicular bodies of endosomes, distinguishing them from plasma membrane-derived microvesicles. Their membranes contain less polyunsaturated glycerophosphoserine, suggesting that the biogenesis pathway determines the membrane’s characteristics. Consequently, the Laurdan fluorescence anisotropy of EV membranes is higher than that of cell membranes, reflecting increased order and rigidity. Notably, decreases in microenvironmental pH reversibly reduce membrane rigidity and increase fluidity, which is beneficial for fusion with the target cell membrane. The activity of transmembrane flippases in the EV membrane is higher than that in the cell membrane, allowing rapid lipid exchange between the bilayer leaflets.⁹ Sphingomyelin is closely associated with cholesterol and performs corresponding functions to resist phospholipase and oxidative stress in the circulation.^{9,49} This structure–function coupling enables OEVs to maintain morphological and functional integrity over long distances and for extended periods in 3D culture media, while also providing predictable physical parameters for downstream engineering modifications.

4.1.3. Composition of organoid-derived microvesicles

Components in the OEVs can be divided into three categories—lipids, nucleic acids, and proteins—which reflect the characteristics of their parent organoids and should be interpreted within the specific biological context.² In addition to structural phospholipids, lipids in EVs also include metabolic enzymes (such as fatty acid synthase, phospholipase D/A2) and bioactive substances (such as arachidonic acid, prostaglandin E₂, ceramide). Under hypoxic stress, these substances reshape the lipid metabolism of receptor cells and induce apoptosis.^{5,50} At the nucleic acid level, the intestinal virus RNA primarily consists of small RNA (<200 nucleotides), miRNA, and transfer RNA, accounting for approximately 15% of the total RNA content. Long-chain coding and non-coding RNAs, as well as repetitive sequences (such as endogenous retrovirus (HERV), Alu, and L1 elements) are selectively packaged into the OEVs. This sorting is mediated by the specific motif (CUGCC) in the 3′ untranslated region or the hnRNPA2B1 recognition element.⁵⁰ EV DNA consists of double-stranded genomic fragments ranging from 100 to 2.5 kilobases, with a mutation spectrum consistent with that of the parental organoid genomic DNA, making it a potential biomarker for liquid biopsy.² Protein complexes in OEVs reflect biological pathways. Exosomal membranes contain transmembrane proteins such as CD63, CD81, CD82, major histocompatibility complex class II, the endosomal sorting complex required for transport complex (Alix and TSG101), and molecular chaperones (Hsc70 and Hsp90). Microvesicles contain plasma membrane proteins, including integrins, P-selectin, and adenosine diphosphate ribosylation factor 6, among others, whereas apoptotic bodies contain histones but lack glycoproteins.¹ Functionally, OEVs can deliver proteins, such as phosphoinositide 3-kinase, mitogen-activated protein kinase, and erythroblastic oncogene B family kinases, as well as oncogenic or matrix remodeling factors, including epidermal growth factor receptor VIII, hypoxia-inducible factor 1 α , and matrix metalloproteinase/A disintegrin and metalloproteinase 10, to recipient cells. This cargo can activate signaling pathways such as mitogen-activated protein kinase/protein kinase B, downregulate E-cadherin, or degrade the ECM, thereby promoting invasive phenotypes. Overall, the molecular composition of OEVs not only precisely reflects the physiological and pathological state of donor cells but also provides a rich and programmable biochemical resource for EV-based organoid–host communication, disease modeling, and drug delivery.

4.1.4. Internalization of organoid-derived microvesicles

The internalization of OEVs is a multistep, receptor-ligand-mediated, and bidirectionally cell-type-determined

dynamic process. First, OEVs in circulation recognize and dock to target cells via surface molecules. Integrin $\alpha\beta1/\beta4$ directs OEVs toward lung fibroblasts and epithelial cells, $\alpha v\beta5$ targets liver Kupffer cells, and the $\alpha4\beta1$ -Tspan8 complex precisely targets pancreatic cells.⁵¹ CD63-positive OEVs target neurons and glial cells, while CD63-negative OEVs only bind to neuronal dendrites. Fibronectin mediates the directed binding of OEVs to oligodendrocyte precursor cells through heparan sulfate proteoglycans in microvascular endothelium. Phosphatidylserine is recognized by the T-cell immunoglobulin and mucin domain-containing 4 receptor, mediating macrophage phagocytosis. Glycans, such as free oligosaccharides, synergize with chemokine (C-C motif) ligand 18 to attract OEVs to C-C chemokine receptor Type 8-positive glioblastoma cells.⁵² Subsequently, OEVs enter cells through four main pathways. Clathrin-mediated endocytosis dominates in bone marrow mesenchymal stem cells (MSCs), while caveolin-dependent endocytosis promotes uptake in some cases and may also negatively regulate it. Macropinocytosis, which depends on actin polymerization, extensively takes up OEVs from the culture medium. Macrophages efficiently internalize OEVs through phagocytosis. Several reports suggest that OEVs can form a direct fusion stalk with the plasma membrane through hemifusion, ultimately achieving complete fusion and directly injecting the cargo into the cytoplasm. Internalized OEVs are sorted into early endosomes, late endosomes, or lysosomes, depending on the functionality of their contents. Due to the diversity of uptake pathways and differences in receptor expression profiles, the functional output of OEVs highly depends on the cell types and microenvironments of the producer and recipient cells. Future engineered OEVs require precise matching of target ligands and uptake receptors to enhance the delivery efficiency of therapeutic cargo.⁹

4.2. Extraction and purification of OEVs

For the extraction and purification of OEVs, the standard procedure begins by replacing normal culture medium with conditioned medium within 24–48 h before collection to minimize interference. The culture medium is then harvested, and EV isolation is completed within 24 h.⁵³ The process typically involves removing cells by low-speed centrifugation, filtering through a 0.22 μm filter membrane, and then ultrafiltration through a 100 kDa filter membrane. Subsequently, OEVs are separated through two rounds of ultra-high-speed centrifugation. Downstream analyses, such as fluorescence-based detection of surface markers, are performed to confirm the identity and purity of the vesicles. Accurate results depend on careful consideration of the donor organoid source. This workflow represents an evolution of conventional EV isolation methods⁵⁴ (Figure 4).

4.3. Differences between OEVs and EVs

Organoids and traditional cell cultures differ in structural organization. Organoids have a 3D structure, while traditional cell cultures are 2D structures. The interactions between cells in a 3D environment are more complex and exhibit a greater range of characteristics compared to those in a 2D environment. For instance, human MSCs grown in a 3D environment produce EVs in large quantities with high levels of CD63 expression.⁵⁵ A study demonstrated that 3D MSCs released EVs at approximately 28-fold higher concentrations than conventional 2D cultures, accompanied by increased expression of CD81 and CD9. These vesicles enhanced neurite formation and branching, supporting brain regeneration.⁵⁶ Compared to 2D EVs, 3D MSC EVs exhibit enhanced biological functions and therapeutic potential for Alzheimer's disease, with notable differences in miRNA and protein levels.⁵⁷ Research on EVs from gastric cancer cell lines cultured in 2D and 3D environments demonstrated downregulated adenosine diphosphate ribosylation protein 6 and elevated miRNA expression, with 3D cultures yielding more smaller EVs.⁵⁸ In addition, studies on 3D MSC EVs for cardiac repair showed superior cardioprotective effects compared to 2D EVs, indicating enhanced EV efficacy through 3D systems.⁵⁹ Moreover, investigations into the bone marrow MSC secretome have revealed benefits for corneal wound healing, suggesting that 3D culture augments secretome effectiveness and optimizes culture conditions.⁶⁰ To monitor the recovery of traumatic brain injury after injection, mice were injected with MSC derivatives from 2D and 3D cultures. It was found that mice treated with MSCs cultured in advanced 3D organoids exhibited improved recovery, particularly in terms of angiogenesis and neural regeneration. The cell structure cultured in 3D more closely resembles the *in vivo* physiological state and can promote the secretion of EVs, compared to a 2D culture. In this study, we focused on EVs derived from 3D cultures, rather than OEVs from complex multicellular organoids. 3D-cultured EVs demonstrate superior biological activity and yield compared with 2D EVs,⁶¹ particularly in mediating cell-cell communication. Organoids, with their greater diversity of cell subtypes and a larger contact area, present an even more complex microenvironment, suggesting that OEVs are likely to be more intricate and precise in their signaling than 3D-cultured EVs.

Recent evidence indicates that organoids produce higher quantities of OEVs than conventional EVs obtained from the same cell types. OEVs exhibit enhanced biological functions, which may be attributed to the properties of the parental organoid stem cells, as key characteristics are retained and reflected in their EVs. It is also possible to mass-produce OEVs based on the characteristics of the original organoids and customize them for specific applications (Table 1).

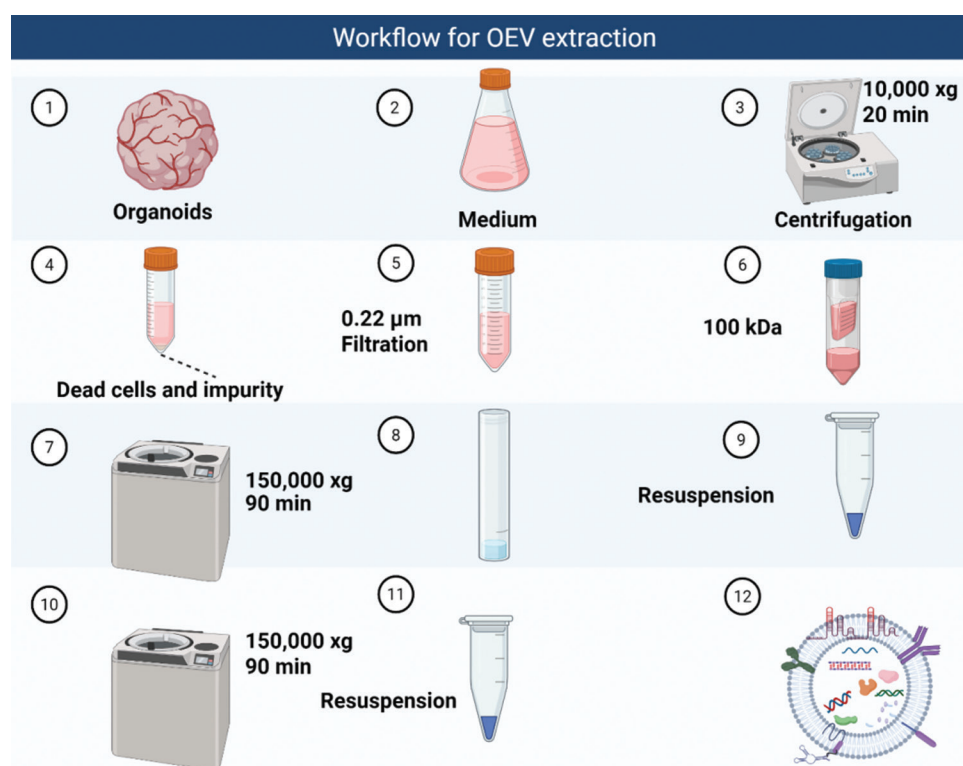


Figure 4. Extraction workflow for OEVs. The organoid culture medium is harvested, followed by centrifugation at $10,000 \times g$ for 20 min at 4°C to remove debris. The supernatant is then filtered through a $0.22 \mu\text{m}$ filter membrane, using a 100 kDa ultrafiltration membrane. Subsequently, another ultracentrifugation is performed at $150,000 \times g$ for 90 min at 4°C to harvest exosome-like OEVs. Created with BioRender. Bigbone, B. (2026) <https://BioRender.com/4ry146c>.

Abbreviation: OEVs: Organoid-derived extracellular vesicles.

Table 1. Differences between 2D, 3D, and OEVs

Feature	2D EV	3D EV	OEV
Structural organization	Arranged in a flat, 2D format	Exhibits a complex 3D architecture	Produced by complex organoid structures with multiple cell types
Intercellular interactions	Limited complexity in cell interactions	Enhanced interactions due to the 3D environment	Significantly amplifies intercellular communication by increasing cell subtype variety and contact area
EV production	Low quantity of EVs produced	High quantity, with 28 times more concentration than 2D methods ⁶¹	Significantly larger quantities compared to conventional EVs from the same number of cells ⁶¹
Biological functionality	Limited therapeutic potential	Enhanced biological functions and therapeutic potential, especially for neurological recovery	Expected to outperform 3D EVs in biological functions and yields
CD63, CD81, and CD9 expression	Low expression levels ^{55,56}	High expression levels	Anticipated to exhibit very high expression levels due to stem cell origin ⁵⁶
MicroRNA and protein levels	Less variation in microRNA and protein levels ⁵⁷	Significant variations compared to 2D EVs ^{57,60}	Inherits key stem cell-derived properties, leading to enhanced functionalities
Application potential	Limited scalability and application specificity ⁵⁷	Potential for tailored applications based on organoid characteristics ⁵⁷	Opens possibilities for manufacturing OEVs at scale for specific applications

Abbreviations: 2D: Two-dimensional; 3D: Three-dimensional; CD: Cluster of differentiation; EVs: Extracellular vesicles; OEVs: Organoid-derived extracellular vesicles.

5. Layer-specific EV release and signaling

Organoid structures simulate the spatial arrangement of natural tissues and determine the physiological functional characteristics of cells. The layered structure results in

heterogeneity, with different cell populations present in distinct regions. For example, there are proliferating stem cell-like cells in the inner layer and more differentiated cells in the outer layer. This spatial composition affects cell behavior, vesicle release, and signal transduction. Each

layer contributes to the vesicle pool, and the accessibility or fate of vesicles varies due to different origin locations within the organoid.

In organoids, EVs are often released from inner cell layers and primarily mediate signaling to adjacent or outer layers. This means that EVs from inner layers can influence neighboring cells, contributing to localized cellular communication. Conversely, EVs arising from outer layers are typically directed into the extracellular space. These externally released EVs facilitate broader communication pathways, enabling interactions with a larger network of cells. Inner vesicles can affect the biogenesis and secretion of upper vesicles. These are “secondary” vesicles, which are released in response to inner signals. It is also plausible that some of the EVs from the inner layer may cross cellular barriers and be released to extracellular spaces.

5.1. The necessity of understanding organoid structure

Caution is required when interpreting OEVs. For instance, the release of specific miRNAs in EVs into the extracellular environment may not directly reflect their expression levels in the parent cells, but instead could elicit systemic responses in adjacent cells or tissues. In addition, it is essential to comprehend the dynamic changes in EV signals between the buried layer and the outer layer for clinical research or diagnosis purposes.

Research shows that when the cell density or vitality in the organoid layer changes, different EVs will be secreted, which reflects the metabolic status of the origin.^{62,63} Zhou *et al.*⁶² reported that the EVs of human retinal organoids contain small miRNAs that can alter the gene expression of recipient cells, indicating a connection between the function of EVs and the structure of organoids. When evaluating the impact of EVs from cellular organoids in regenerative medicine and disease models, it is necessary to describe the structure of cellular organoids.

5.2. Influence of necrosis on the composition of EVs

When organoids grow to over 400–500 μm , limited diffusion of nutrients and oxygen can lead to the formation of a necrotic core. Cells in the interior experience nutrient and oxygen deprivation, resulting in necrosis and the development of a central region of stressed or dead cells.⁶⁴ Gradient guides organoid differentiation and maturation; there are viable cells around the injury in the necrotic core.¹² It is possible to trigger the release of EVs, which may carry damaged or inflammatory contents. These EVs can reach the upper tissues, possessing damaged contents such as proteins and RNA that show metabolic abnormalities, and can interfere with the functions of cells in the viable tissue layer.⁶⁵ Stress signals from EVs may

damage neighboring healthy cells, trigger inflammation, or affect metabolism.⁶⁶ Notably, EVs produced through necrosis or those contaminating the extracellular space can alter the characteristics and compromise the integrity of the vesicles. When evaluating EVs for downstream use, the pathological state of the internal organoids must be taken into consideration (Figure 5).

5.3. Autophagy in organoids and its effects on EV signaling

Autophagy is a key cellular stress response that regulates the degradation and recycling of components. In organoids, autophagy affects the composition of exosome biogenesis,

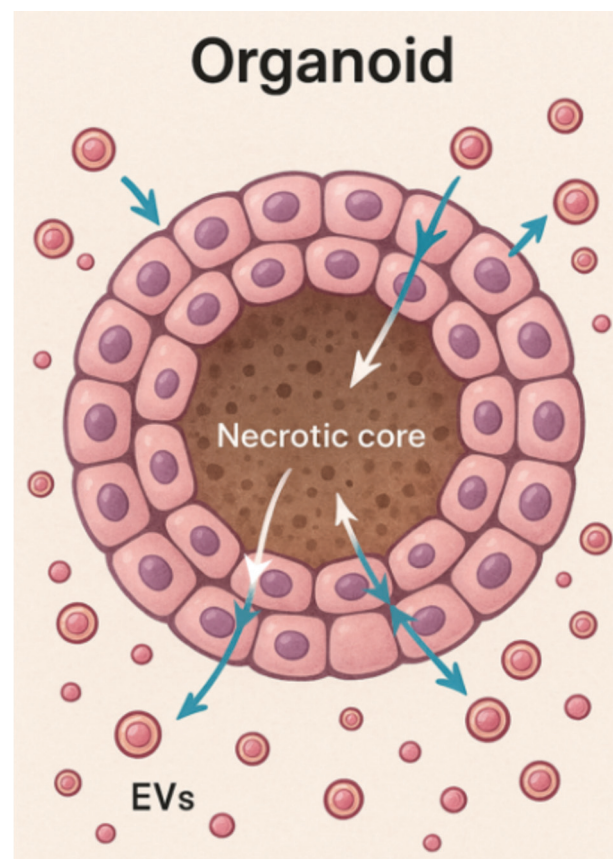


Figure 5. Formation of necrotic cores in large organoids and pathological signaling mediated by EVs. When the diameter of organoids exceeds 400–500 μm , the diffusion of nutrients and oxygen will be restricted, leading to the formation of a necrotic core. Cells in the core will undergo necrosis due to starvation, hypoxia, genomic stress, or death signals. Although signals may guide differentiation and maturation, the necrotic core will damage living cells, triggering the release of EVs that carry damaged inflammatory markers. These EVs may migrate upward to the living cell layer, carrying damaged protein RNA to disrupt the function of living cells, possibly causing inflammation and interfering with metabolism. EVs released from necrotic cells can contaminate the EV pool, alter its molecular profile, and compromise vesicle function. Therefore, the pathological state of organoids should be carefully considered before downstream applications. Created with BioRender. Bigbone, B. (2025) <https://BioRender.com/hbeh5q2>. Abbreviation: EVs: Extracellular vesicles.

thereby affecting intercellular signal transduction and possibly altering cellular homeostasis.⁶⁷ Organoids feature unique physiological microenvironments with heterogeneous cell states, and the dynamic interplay between autophagy and EV production is critical.

Stress factors, such as nutrient deficiency, hypoxia, and organelle accumulation, can trigger the autophagy process. Autophagy helps cells survive by not only clearing debris but also providing substrates for metabolism during stress. Recent studies have shown that autophagy regulates the secretion of EVs in various ways. Proaño-Pérez *et al.*⁶⁸ found that autophagy affects the composition of EVs in glioblastoma tumor organoids, and subsequently, autophagy-related pathways regulate the characteristics of immunomodulatory signaling biomolecules. Alterations in the relationship between autophagic activity and EV RNA packaging lead to changes in cargo composition, which can serve as indicators of cellular stress.⁶⁹

Autophagy-related EVs are different from EVs derived from healthy cells. When analyzing the exosome profile, it is necessary to distinguish between these two populations. Autophagy-related EVs may cause relatively large interference to the research on EVs produced by organoids.

6. Application of OEVs in organoid research

EVs serve as cell-free platforms for transporting bioactive substances and facilitating intercellular communication. They are excellent therapeutic agents and delivery vehicles, and have great potential in modern medicine. OEVs are promising in treating diseases such as cancer and genetic disorders, and can also deliver genetic material to target specific cells, thereby enhancing therapeutic efficiency. Moreover, OEVs can reflect the growth conditions of organoids. Combined with organoid technology, they can enhance drug screening methods and facilitate research into diseases and cancers. Moreover, OEVs are capable of overcoming intrinsic physiological barriers within the human body, thereby positioning them as exceptional vehicles for drug delivery.

6.1. Applications in organoid disease models

6.1.1. Intestinal OEVs for immune regulation

EVs amplify the inflammatory response through the inflammasome pathway, which is the core mechanism. Research indicates that cells can package apoptosis-associated speck-like proteins, NLRP3, activated caspase-1, and mature interleukin 1 β into EVs for release. Upon endocytosis by recipient cells, these vesicles carrying a “re-assembled core” can trigger or enhance inflammasome assembly with a lower upstream stimulation threshold, facilitating cross-cell inflammatory transmission. In gut organoid-immune co-culture systems, low-dose, sustained

stimulation of interleukin 1 β and tumor necrosis factor alpha induces epithelial and immune cells to synergistically release apoptosis-associated speck-like protein-rich OEVs. Over time, the spatial heatmaps of immune infiltration and the fragmentation index in barrier imaging showed a synchronous increase, with inflammation permeating bidirectionally across both the luminal and basal surfaces, exhibiting a “leaping expansion” phenomenon that is difficult to explain solely by soluble factors.

EVs from various origins have been demonstrated in numerous studies to possess immune-regulating properties. For instance, intestinal epithelial cell-derived EVs have the ability to control regulatory T cells.⁶⁹ Moreover, the EVs secreted by intestinal organoids derived from human and/or murine intestinal crypt stem cells exhibit potent immunoregulatory effects, effectively reducing the synthesis of various inflammatory mediators induced by lipopolysaccharides. Furthermore, it has been observed that the immunomodulatory capabilities of OEVs diminish when morphine is employed as an intervention in organoid cultures.⁸ Several miRNAs, in particular Let-7, were identified as essential elements of OEVs’ immunomodulatory capabilities following analysis and comparison of OEVs before and after morphine therapy.

6.1.2. Salivary gland OEVs for epithelial repair

Salivary gland OEVs exhibit specific advantages in tissue regeneration and repair, as pure OEVs demonstrate more potent regenerative effects than organoid-derived organoids or MSC EVs. In an *in vitro* model of traumatic epithelial injury, EVs derived from dental pulp MSCs showed only a marginal improvement in epithelial repair efficiency, approximately 15%. In contrast, direct transplantation of salivary gland organoids (SGOs) yielded a 25% increase in epithelial repair efficiency. Purified OEVs delivered locally via perfusion devices further enhanced epithelial cell proliferation by up to 60%.

Chansaenroj *et al.*⁷⁰ developed a magnetic 3D bioassembly platform to create functional SGOs and to non-invasively culture human dental pulp stem cells using magnetic nanoparticles.⁷⁰ The successful construction of SGOs using the platform was demonstrated by the broad expression of salivary gland-specific markers, including aquaporin 1, mucin 7, keratin 5, keratin 14, cholinergic receptor muscarinic 3, and tubulin beta 3 class III.⁷⁰ Subsequently, EVs derived from 3D-cultured human dental pulp stem cells and OEVs from SGOs were isolated. Their reparative potential was evaluated using an epithelial injury model, with epithelial cell proliferation and neural regeneration as key outcome measures.⁷⁰ Direct SGO transplantation demonstrated 25% therapeutic efficacy, 3D human dental pulp stem cell-derived EV showed 15% therapeutic efficacy, and SGO-OEV achieved

60% therapeutic efficacy. Proteomic analysis revealed an abnormal expression of 99 distinct proteins in SGo-OEV, many of which are intimately linked to adhesion, bio-regulation, biogenesis, development, and cell proliferation. This finding is particularly noteworthy, as it distinctly illustrates the disparities between 3D EVs and OEVs, with OEVs displaying markedly enhanced biological functions compared to 3D EVs.⁷¹ The therapeutic efficacy of OEVs surpasses that of traditional SGo transplantation; however, it is currently unclear whether this is a unique phenomenon or a more generalized trend.

6.1.3. Epidermal OEVs for epithelial injury repair

Studies show that EVs produced by iPSC-derived 3D epidermal organoids can effectively stimulate the proliferation and migration of human dermal fibroblasts, contribute to wound healing, and promote the formation of vascular-like lumens in human umbilical vein endothelial cells. miR-146a-5p, miR-31-5p, and miR-21-3p are highly expressed in EVs and are associated with diverse regulatory effects. The miRNA-rich vesicles produced by EVs play a role through these pathways, promoting epithelial cell differentiation via the phosphatase and tensin homolog/miR-146a-5p axis, enhancing cell migration through the focal adhesion kinase/Rac1/miR-31-5p axis, and promoting inflammation resolution via the nuclear factor kappa B/miR-21-3p axis. Experimental results demonstrated that EVs derived from local ovarian cancer significantly accelerated wound healing in a full-thickness skin defect mouse model. Histological analysis revealed that wounds in the EV-treated group exhibited denser granulation tissue, a thicker regenerated epithelial layer, and increased vascular density, as evidenced by elevated expression of vascular endothelial growth factor (VEGF), fibroblast growth factor 2, and CD31⁺ markers. Masson trichrome staining revealed a more orderly organization of collagen fibers and enhanced collagen deposition, indicating that EVs derived from ovarian cancer play a crucial role in vascular reconstruction and ECM repair *in vivo*. Furthermore, OEVs induce keratinocytes to undergo a phenotype shift similar to an epithelial-mesenchymal transition, thereby promoting re-epithelialization. Specifically, during the early wound healing phase, OEVs activate transforming growth factor beta/Smad and Wnt/ β -catenin signaling pathways in keratinocytes at the wound margin, enhancing their migration and synthesis to facilitate wound closure. During the later stages of wound healing, the effects of OEV activation gradually diminish, allowing cells to regain their epithelial phenotype and reconstruct the epithelial basement membrane barrier function.

In the investigations conducted by Kwak *et al.*,⁷² iPSCs were effectively employed to create epidermal organoids (iEpiO). When epidermal cells were cultured

in a 2D environment, key characteristics of organoids were markedly diminished. EVs from iEpiO were isolated from both 2D and 3D cultures for a comparative analysis. Nanoparticle tracking analysis showed similar EV particle sizes between the two conditions; however, EV yield from 3D cultures was approximately twofold higher. Notably, 3D EVs exhibited reduced expression of ALG-2-interacting protein X and tumor susceptibility 101 protein, alongside increased CD9 levels. VEGF was highly enriched in 3D EVs and significantly upregulated VEGF mRNA in human umbilical vein endothelial cells, thereby promoting angiogenesis, as confirmed by polymerase chain reaction and tube formation assays. Small RNA sequencing indicated higher expression of 16 specific miRNAs in 3D EVs, highlighting regulatory roles in cellular communication.⁷² In a mouse skin wound-healing model, the therapeutic efficacy of 3D EVs was confirmed. Mice treated with 3D EVs demonstrated wound closure rates 1.6-fold higher than those of the control group on days 3, 5, and 7. Immunohistochemical observation revealed enhanced expression of VEGF at the wound site, indicating a robust biological response. This discovery holds great significance for the field of regenerative medicine and highlights a key limitation of organoid technology: many hallmark features of organoids, particularly their complex 3D structures, are lost upon transition to 2D culture, thereby restricting therapeutic applicability.

Differences in marker expression profiles between 2D and 3D states result in substantial alterations in EV biological activity. OEVs have great medical application potential, offering solutions to the low yield and limited functionality associated with conventional EV extraction methods. Importantly, OEVs have more pronounced physiological effects than the parent organoids. Continued advances in organoid technology are therefore expected to accelerate OEV research. Complex organoid models, such as vascularized and neuralized organoids, are likely to produce OEVs with specific medical functions, offering promising strategies for treating neurodegenerative diseases and improving angiogenesis in ischemic tissues. Integrating 3D bioprinting with CRISPR/Cas9 gene editing technology will enhance OEVs, thereby enabling the development of customized treatment models for various diseases.

As organoid technologies mature, they will inevitably drive progress in OEV-related research. These nanoscale vesicles are poised to play an increasingly influential role in the future of biomedicine.

6.2. Applications in organoid drug delivery

OEVs have unique advantages in drug delivery. They are natural nanoscale carriers with biocompatibility and targeted delivery capabilities, and can also be utilized as new biomarkers for assessing drug toxicity. In drug

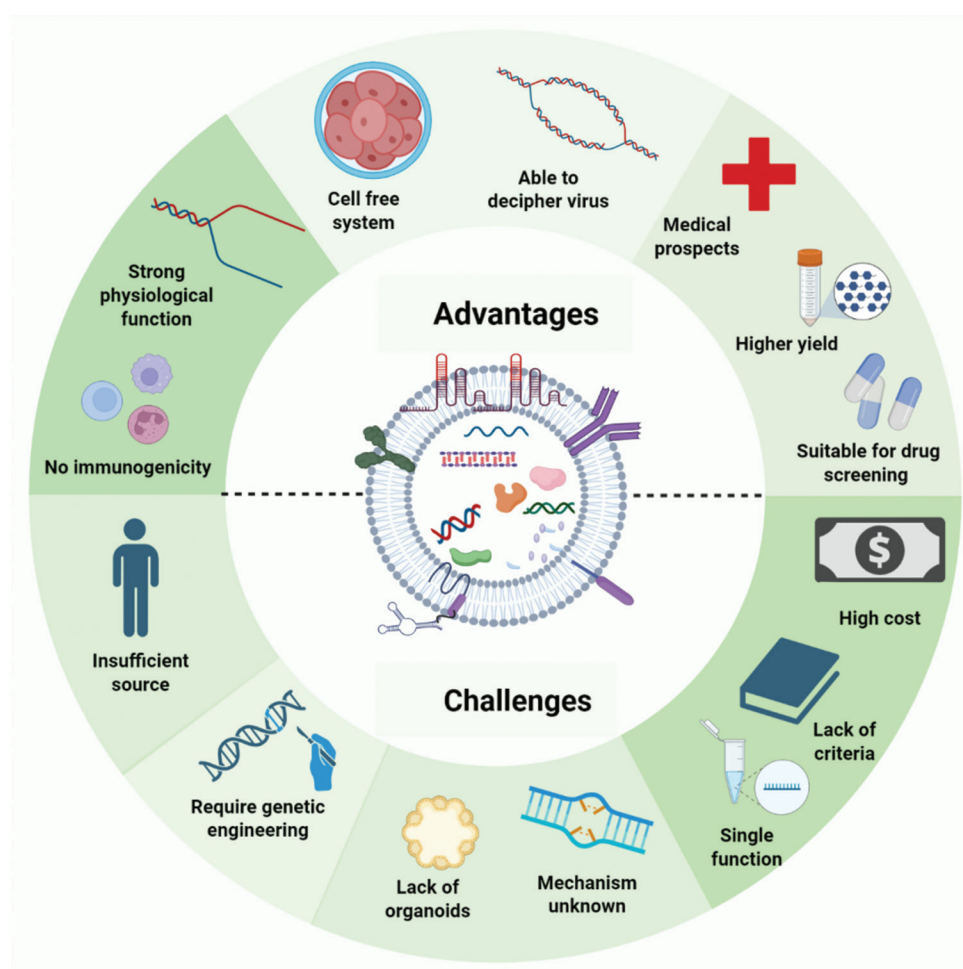


Figure 6. Advantages and challenges of organoids and OEVs. Organoids offer benefits like a cell-free system and applications in drug screening, but face challenges such as insufficient source and high costs. OEVs provide advantages like more production and better drug delivery, but struggle with unclear mechanisms and require genetic engineering. Created with BioRender. Bigbone, B. (2026) <https://BioRender.com/v796f3n>. Abbreviation: OEVs: Organoid-derived extracellular vesicles.

delivery, EVs have inherent homing ability due to the specificity of the source cells. In the organoid system, EVs, due to their lipid bilayer structure, natural origin, and low immunogenicity, have emerged as ideal “biological delivery carriers.”⁷³ EVs can pass through physiological barriers such as the blood–brain barrier and organoid basement membranes. Surface charges, glycan chains, and membrane proteins endow them with tissue-selective uptake ability, enabling enrichment in specific sub-regions of organoids. Small molecules, nucleic acids, or proteins can be actively encapsulated into EVs through electroporation, transfection, or co-culture, thereby avoiding issues such as low drug solubility, short half-lives, and non-target toxicity. For example, encapsulating doxorubicin into EVs derived from macrophages can precisely target the tumor microenvironment in organoids and significantly reduce nephrotoxicity.⁷⁴ Parental cells undergo genetic engineering operations, or after separation, the surface antibodies of vesicles are coupled with the surfaces of ligands, and EVs

obtain the “intelligent navigation” function. For example, programmed cell death protein 1-negative EVs secreted by chimeric antigen receptor T cells can avoid cytokine storm. Surface engineering operations are carried out using fluorescent peptides, dendrimers, polyethylene glycol, or albumin binding domains to extend the circulating half-life of EVs in organoid perfusion fluid. Local release is also achieved through continuous methods, such as external fields, including magnetic fields or photodynamic therapy.⁷⁵ The microenvironment of organoids can exert feedback on the uptake of EVs and drug release. It is necessary to monitor the integrity of vesicle membranes, drug loading efficiency, and functional indicators of organoids to establish a standardized quality control system. EVs in organoid drug delivery have multiple advantages, such as penetration, targeting, sustained release, and tracing. They may also accelerate the process of transforming pretreatment strategies from 2D cells to 3D organoids and *in vivo* applications.

7. Advantages and challenges of OEVs in organoid applications

Organoids secrete OEVs, which are nanoscale phospholipid vesicles. These nanoscale phospholipid vesicles have garnered attention in the biomedical field due to their unique characteristics and diverse applications. Compared to traditional EVs, they have advantages such as low immunogenicity and reduced immune risk, and they perform well in cell-free environments. In addition, the delivery mechanism of OEV-like vesicles is relatively efficient, as they are rich in bioactive molecules (including proteins, lipids, and nucleic acids), which can affect the gene expression of target cells and create new treatment approaches for various diseases.⁷⁶ The yield and physiological activity of EVs are superior to those of traditional EVs, and their properties can further be improved by genetic or chemical methods, making them more suitable for both research applications and therapeutic use.⁷⁷ However, the development of OEVs is restricted due to inherent limitations. The therapeutic mechanism is unclear, as the protein and RNA molecular pathways for enhancing functions have not been identified, and production is also limited due to the size of organoids and technical issues.⁷⁸ Organoid and OEV technologies are still in the early stages. The lack of standard protocols is a significant obstacle to the widespread application of OEVs in research and clinical practice, making it challenging to transition OEVs from basic research to clinical application.

To promote the standardization of EVs and their clinical application, it is necessary to construct a technical framework of “culture–isolation–identification–verification.” The sources of initial cells, the selection of matrix gel, and the culture protocol must be standardized and maintained consistently. When isolating EVs, standards need to be determined based on size, density, or surface markers, and minimum requirements for exosome vesicle concentration, purity, and key functional molecules must be established. At the same time, shared standard functional verification models need to be developed to evaluate the biological activity of EVs from different sources. Through cross-laboratory research and data sharing, an open “exosome fingerprint” database needs to be constructed. This will lay the groundwork for production processes and quality control systems that comply with Good Manufacturing Practice (GMP) standards, thereby addressing the current shortcomings in technical standardization (Figure 6).

8. Conclusion

OEVs are natural nanocarriers that have been re-recognized with the rise of organoid technology, combining the 3D physiological complexity of organoids with the intercellular communication capabilities of EVs.⁷⁹ Over the past 5 years,

research has systematically mapped their “endocytosis–sorting–secretion” biogenesis pathway, elucidated their highly context-dependent composition of lipids, proteins, nucleic acids, and metabolites, and developed purification paradigms based on differential-ultracentrifugation combined with density gradient, immunoprecipitation, and microfluidics. Functionally, OEVs have been shown to precisely mimic the stem cell niche of their source tissues: intestinal OEVs suppress lipopolysaccharide-induced inflammatory cascades via miRNAs such as Let-7, salivary gland OEVs synergistically promote epithelial–neuronal complex repair through 99 differentially expressed proteins, retinal OEVs protect photoreceptor cells through fatty acid metabolism-related proteins, and epidermal OEVs accelerate wound vascularization via VEGF-rich cargo. Compared to 2D/3D EVs, OEVs have a higher yield, more potent physiological activity, and lower immunogenicity. It has been applied in genetic disease models, such as cystic fibrosis and Alzheimer’s disease, as well as in tumor PDO drug sensitivity testing, and has demonstrated regenerative effects comparable to or even superior to cell transplantation in animal models of myocardial infarction, liver resection, and spinal cord injury.⁷ Meanwhile, as a “cell-free preparation,” OEVs also possess delivery carrier properties, enabling targeted delivery of small molecules, nucleic acids, and proteins via electroporation, gene editing, or surface conjugation, thereby overcoming physiological barriers such as the blood–brain and blood–retinal barriers. There are challenges in translating laboratory findings to the clinic, and the preparation of organoids is still in its early stages. Key bottlenecks include poor scalability, a high cost of 3D culture systems such as Matrigel, difficulty in maintaining organoid homogeneity and functional stability within large-volume bioreactors, the complex and low-yield process of isolating and purifying EVs from large-scale culture supernatants, and the absence of standardized production workflows and GMP compliance. These limitations result in insufficient exosome yields to meet the requirements for systemic administration, confining applications to localized delivery or *in vitro* model construction.

Looking ahead, OEV’s research and translation will focus on three key directions: scalability, intelligence, and standardization. First, leveraging perfusion bioreactors, 3D bioprinting, and synthetic hydrogel scaffolds can establish scalable and quality-controlled organoid production platforms and achieve a significant increase in OEVs yield by activating Rab27a/b or inhibiting TSG101 through CRISPR at secretory nodes. Second, by using multi-omics–artificial intelligence joint analysis as the core, a “molecular fingerprint–functional map” database for OEVs from various tissue sources can be established.³ By combining organ-on-a-chip and microphysiological systems, we can track the spatiotemporal distribution and transcriptional/

post-translational dynamics of OEVs in target tissues in real-time, thereby identifying the “key cargo” that determines therapeutic efficacy and its functional networks. Third, developing modular engineering strategies to introduce light-sensitive, magnetically responsive, or enzyme-cleavable release elements at the organoid stage would enable targeted and controlled release of OEVs. Utilizing cell membrane biomimetic coating and immune-modulating hydrogel microcapsules can reduce reticuloendothelial system clearance and extend circulation half-life. In addition, there is an urgent need for an international consensus organization to lead the development of specialized guidelines for OEVs, standardize culture substrates, separation processes, characterization metrics, and efficacy evaluation systems, and establish a traceable GMP-grade production chain.⁸⁰ In the long run, OEVs may be combined with gene, cell therapy, and tissue engineering to form “personalized exosome drugs” for rare diseases, degenerative diseases, and tissue repair. This may lead to a new *in situ* regenerative model, offering affordable, accessible, and sustainable solutions for addressing organ shortages and chronic disease burdens during the global aging period.

Organoids and EVs are advanced models in biomedical research and also have great therapeutic potential, which can provide new strategies and insights for disease treatment. However, before these technologies reach commercial viability, further optimization of production processes is needed. Future research should focus on improving organoid culture technology, enhancing functional diversity, and utilizing innovative methods to construct functionally integrated organoids that can meet the needs of clinical transplantation treatment. At the same time, through in-depth exploration of the growth and development mechanisms of organoids, the generation methods of organoids can be diversified. This not only helps us understand the development process of human tissue organs but also can provide an ideal model for drug screening and personalized medicine. The application of expanded organoid technology can accelerate drug discovery and enhance treatment outcomes. The continuous progress of organoid technology will directly promote the development of OEVs, enabling improvements in yield and quality, as well as the enhancement of functions. This will further broaden the potential applications in the biomedical field. Overall, this review highlights the significant potential of these cutting-edge technologies and promotes further investigation and development within this growing research area.

Acknowledgments

None.

Funding

This work was supported by the National Natural Science Foundation of China (82202344), the Jiangsu Province Natural Science Foundation project (BK20241808), the Fujian Province Natural Science Foundation project (2024J01221), and the Jiangsu Province Youth Science and Technology Talent Support Project (JSTJ-2024-097).

Conflict of interest

Jiacan Su is one of the Editors-in-Chief of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. The other authors declare they have no competing interests.

Author contributions

Conceptualization: Wojciech Chrzanowski, Han Liu, Jiacan Su

Visualization: Ting Cheng

Writing—original draft: Ting Cheng

Writing—review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Cano A, Ettcheto M, Bernuz M, *et al.* Extracellular vesicles, the emerging mirrors of brain physiopathology. *Int J Biol Sci.* 2023;19(3):721-743.
doi: 10.7150/ijbs.79063
2. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells.* 2019;8(7):727.
doi: 10.3390/cells8070727
3. Liu H, Geng Z, Su J. Engineered mammalian and bacterial extracellular vesicles as promising nanocarriers for targeted therapy. *Extracell Vesicles Circ Nucleic Acids.* 2022;3(2):63-86.
doi: 10.20517/evcna.2022.04
4. Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: Unique intercellular delivery vehicles. *Trends Cell Biol.* 2017;27(3):172-188.
doi: 10.1016/j.tcb.2016.11.003
5. Kumar MA, Baba SK, Sadida HQ, *et al.* Extracellular vesicles as tools and targets in therapy for diseases. *Signal Transduct Target Ther.* 2024;9(1):27.

- doi: 10.1038/s41392-024-01735-1
6. Chutkan H, MacDonald I, Manning A, Kuehn MJ. Quantitative and Qualitative Preparations of Bacterial Outer Membrane Vesicles. In: *Methods in Molecular Biology*. Humana Press; 2012:259-272.
doi: 10.1007/978-1-62703-245-2-16
 7. Ji N, Wang F, Wang M, Zhang W, Liu H, Su J. Engineered bacterial extracellular vesicles for central nervous system diseases. *J Control Release*. 2023;364:46-60.
doi: 10.1016/j.jconrel.2023.10.027
 8. Su Y, Sun X, Liu X, *et al*. hUC-EVs-ATO reduce the severity of acute GVHD by resetting inflammatory macrophages toward the M2 phenotype. *J Hematol Oncol*. 2022;15(1):99.
doi: 10.1186/s13045-022-01315-2
 9. Zaborowski MŁP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: Composition, biological relevance, and methods of study. *Bioscience*. 2015;65(8):783-797.
doi: 10.1093/biosci/biv084
 10. Zhou G, Li R, Sheng S, *et al*. Organoids and organoid extracellular vesicles-based disease treatment strategies. *J Nanobiotechnol*. 2024;22(1):679.
doi: 10.1186/s12951-024-02917-3
 11. Yi SA, Zhang Y, Rathnam C, Pongkulapa T, Lee KB. Bioengineering approaches for the advanced organoid research. *Adv Mater*. 2021;33(45):2007949.
doi: 10.1002/adma.202007949
 12. Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. *Nat Rev Genet*. 2018;19(11):671-687.
doi: 10.1038/s41576-018-0051-9
 13. Garreta E, Kamm RD, Chuva De Sousa Lopes SM, *et al*. Rethinking organoid technology through bioengineering. *Nat Mater*. 2021;20(2):145-155.
doi: 10.1038/s41563-020-00804-4
 14. Mei J, Liu X, Tian HX, *et al*. Tumour organoids and assembloids: Patient-derived cancer avatars for immunotherapy. *Clin Transl Med*. 2024;14(4):e1656.
doi: 10.1002/ctm2.1656
 15. Jiang X, Oyang L, Peng Q, *et al*. Organoids: Opportunities and challenges of cancer therapy. *Front Cell Dev Biol*. 2023;11:1232528.
doi: 10.3389/fcell.2023.1232528
 16. Anania C. Human intestinal immuno-organoids. *Nat Genet*. 2024;56(9):1765.
doi: 10.1038/s41588-024-01927-z
 17. Yang Q, Li M, Yang X, *et al*. Flourishing tumor organoids: History, emerging technology, and application. *Bioeng Transl Med*. 2023;8(5):e10559.
doi: 10.1002/btm2.10559
 18. Sakaguchi H. Self-organization and applications of neural organoids. *Eur J Cell Biol*. 2025;104(2):151496.
doi: 10.1016/j.ejcb.2025.151496
 19. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: Construction, analysis, and application. *Bioact Mater*. 2023;31:525-548.
doi: 10.1016/j.bioactmat.2023.09.005
 20. Srivastava V, Huycke TR, Phong KT, Gartner ZJ. Organoid models for mammary gland dynamics and breast cancer. *Curr Opin Cell Biol*. 2020;66:51-58.
doi: 10.1016/j.ceb.2020.05.003
 21. Liang X, Li C, Song J, *et al*. HucMSC-exo promote mucosal healing in experimental colitis by accelerating intestinal stem cells and epithelium regeneration via wnt signaling pathway. *Int J Nanomedicine*. 2023;18:2799-2818.
doi: 10.2147/IJN.S402179
 22. Liu H, Song P, Zhang H, *et al*. Synthetic biology-based bacterial extracellular vesicles displaying BMP-2 and CXCR4 to ameliorate osteoporosis. *J Extracell Vesicles*. 2024;13(4):e12429.
doi: 10.1002/jev2.70095
 23. Yang Y, Kong Y, Cui J, Hou Y, Gu Z, Ma C. Advances and applications of cancer organoids in drug screening and personalized medicine. *Stem Cell Rev Rep*. 2024;20(5):1213-1226.
doi: 10.1007/s12015-024-10714-6
 24. Corrò C, Novellademunt L, Li VSW. A brief history of organoids. *Am J Physiol Cell Physiol*. 2020;319(3):C575-C582.
doi: 10.1152/ajpcell.00120.2020
 25. Verstegen MMA, Coppes RP, Beghin A, *et al*. Clinical applications of human organoids. *Nat Med*. 2025;31(2):409-421.
doi: 10.1038/s41591-024-03489-3
 26. Tang XY, Wu S, Wang D, *et al*. Human organoids in basic research and clinical applications. *Signal Transduct Target Ther*. 2022;7(1):168.
doi: 10.1038/s41392-022-01024-9
 27. Gopallawa I, Gupta C, Jawa R, *et al*. Applications of organoids in advancing drug discovery and development. *J Pharm Sci*. 2024;113(9):2659-2667.
doi: 10.1016/j.xphs.2024.06.016
 28. Hong ZX, Zhu ST, Li H, *et al*. Bioengineered skin organoids: From development to applications. *Mil Med Res*. 2023;10(1):40.
doi: 10.1186/s40779-023-00475-7
 29. Clevers H. Modeling development and disease with organoids. *Cell*. 2016;165(7):1586-1597.
doi: 10.1016/j.cell.2016.05.082

30. Liu H, Zhang Q, Wang S, *et al.* Bacterial extracellular vesicles as bioactive nanocarriers for drug delivery: Advances and perspectives. *Bioact Mater.* 2022;14:169-181.
doi: 10.1016/j.bioactmat.2021.12.006
31. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologicals. *Biochim Biophys Acta.* 2014;1841(1):108-120.
doi: 10.1016/j.bbailip.2013.10.004
32. Schwechheimer C, Kuehn MJ. Outer-membrane vesicles from Gram-negative bacteria: Biogenesis and functions. *Nat Rev Microbiol.* 2015;13(10):605-619.
doi: 10.1038/nrmicro3525
33. Pérez-Cruz C, Delgado L, López-Iglesias C, Mercade E. Outer-inner membrane vesicles naturally secreted by gram-negative pathogenic bacteria. *PLoS One.* 2015;10(1):e0116896.
doi: 10.1371/journal.pone.0116896
34. Almousa S, Kim S, Kumar A, *et al.* Bacterial nanovesicles as interkingdom signaling moieties mediating pain hypersensitivity. *ACS Nano.* 2025;19(3):3210-3225.
doi: 10.1021/acsnano.4c10529
35. Xu H, Li W, Yue H, *et al.* S100B induces angiogenesis via the clathrin/FOXO1/ β -catenin signaling pathway and contributes to Bevacizumab resistance in epithelial ovarian cancer. *J Adv Res.* 2025.
doi: 10.1016/j.jare.2025.05.060
36. Moghaddam ZS, Dehghan A, Halimi S, *et al.* Bacterial extracellular vesicles: Bridging pathogen biology and therapeutic innovation. *Acta Biomater.* 2025;200:1-20.
doi: 10.1016/j.actbio.2025.05.028
37. Toyofuku M, Schild S, Kaparakis-Liaskos M, Eberl L. Composition and functions of bacterial membrane vesicles. *Nat Rev Microbiol.* 2023;21(7):415-430.
doi: 10.1038/s41579-023-00875-5
38. Melo-Marques I, Cardoso SM, Empadinhas N. Bacterial extracellular vesicles at the interface of gut microbiota and immunity. *Gut Microbes.* 2024;16(1):2396494.
doi: 10.1080/19490976.2024.2396494
39. Skotland T, Llorente A, Sandvig K. Lipids in extracellular vesicles: What can be learned about membrane structure and function? *Cold Spring Harb Perspect Biol.* 2023;15(8):a041415.
doi: 10.1101/cshperspect.a041415
40. Liu JH, Chen CY, Liu ZZ, *et al.* Extracellular vesicles from child gut microbiota enter into bone to preserve bone mass and strength. *Adv Sci (Weinh).* 2021;8(9):2004831.
doi: 10.1002/advs.202004831
41. Prados-Rosales R, Brown L, Casadevall A, Montalvo-Quirós S, Luque-García JL. Isolation and identification of membrane vesicle-associated proteins in Gram-positive bacteria and mycobacteria. *MethodsX.* 2014;1:124-129.
doi: 10.1016/j.mex.2014.08.001
42. Vanaja SK, Russo AJ, Behl B, *et al.* Bacterial outer membrane vesicles mediate cytosolic localization of LPS and caspase-11 activation. *Cell.* 2016;165(5):1106-1119.
doi: 10.1016/j.cell.2016.04.015
43. Lee EY, Bang JY, Park GW, *et al.* Global proteomic profiling of native outer membrane vesicles derived from *Escherichia coli*. *Proteomics.* 2007;7(17):3143-3153.
doi: 10.1002/pmic.200700196
44. Manabe T, Kato M, Ueno T, Kawasaki K. Flagella proteins contribute to the production of outer membrane vesicles from *Escherichia coli* W3110. *Biochem Biophys Res Commun.* 2013;441(1):151-156.
doi: 10.1016/j.bbrc.2013.10.022
45. Jang KS, Sweredoski MJ, Graham RL, Hess S, Clemons WM Jr. Comprehensive proteomic profiling of outer membrane vesicles from *Campylobacter jejuni*. *J Proteomics.* 2014;98:90-98.
doi: 10.1016/j.jprot.2013.12.014
46. Xie J, Li Q, Haesebrouck F, Van Hoecke L, Vandenbroucke RE. The tremendous biomedical potential of bacterial extracellular vesicles. *Trends Biotechnol.* 2022;40(10):1173-1194.
doi: 10.1016/j.tibtech.2022.03.005
47. Díaz-Garrido N, Badia J, Baldomà L. Microbiota-derived extracellular vesicles in interkingdom communication in the gut. *J Extracell Vesicles.* 2021;10(13):e12161.
doi: 10.1002/jev2.12161
48. Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol.* 2018;19(4):213-228.
doi: 10.1038/nrm.2017.125
49. Zhao X, Wei Y, Bu Y, Ren X, Dong Z. Review on bacterial outer membrane vesicles: Structure, vesicle formation, separation and biotechnological applications. *Microb Cell Fact.* 2025;24(1):27.
doi: 10.1186/s12934-025-02653-9
50. Clua-Ferré L, Suau R, Vañó-Segarra I, Ginés I, Serena C, Manyé J. Therapeutic potential of mesenchymal stem cell-derived extracellular vesicles: A focus on inflammatory bowel disease. *Clin Transl Med.* 2024;14(11):e70075.
doi: 10.1002/ctm2.70075
51. Joshi BS, De Beer MA, Giepmans BNG, Zuhorn IS. Endocytosis of extracellular vesicles and release of their cargo from endosomes. *ACS Nano.* 2020;14(4):4444-4455.
doi: 10.1021/acsnano.9b10033
52. Zhu G, Yang F, Wei H, *et al.* 90 K increased delivery efficiency of extracellular vesicles through mediating internalization.

- J Control Release.* 2023;353:930-942.
doi: 10.1016/j.jconrel.2022.12.034
53. Geng Z, Sun T, Yuan L, Zhao Y. The existing evidence for the use of extracellular vesicles in the treatment of osteoporosis: A review. *Int J Surg.* 2025;111(5):3414-3429.
doi: 10.1097/JS9.0000000000002339
 54. Haertinger M, Weiss T, Mann A, Tabi A, Brandel V, Radtke C. Adipose stem cell-derived extracellular vesicles induce proliferation of Schwann cells via internalization. *Cells.* 2020;9(1):163.
doi: 10.3390/cells9010163
 55. Yuan X, Sun L, Jeske R, *et al.* Engineering extracellular vesicles by three-dimensional dynamic culture of human mesenchymal stem cells. *J Extracell Vesicles.* 2022;11(6):e12235.
doi: 10.1002/jev2.12235
 56. Carter K, Lee HJ, Na KS, *et al.* Characterizing the impact of 2D and 3D culture conditions on the therapeutic effects of human mesenchymal stem cell secretome on corneal wound healing *in vitro* and *ex vivo*. *Acta Biomater.* 2019;99:247-257.
doi: 10.1016/j.actbio.2019.09.022
 57. Sun L, Ji Y, Chi B, *et al.* A 3D culture system improves the yield of MSCs-derived extracellular vesicles and enhances their therapeutic efficacy for heart repair. *Biomed Pharmacother.* 2023;161:114557.
doi: 10.1016/j.biopha.2023.114557
 58. Sugimoto A, Okuno T, Miki Y, *et al.* EMMPRIN in extracellular vesicles from peritoneal mesothelial cells stimulates the invasion activity of diffuse-type gastric cancer cells. *Cancer Lett.* 2021;521:169-177.
doi: 10.1016/j.canlet.2021.08.031
 59. Prieto-Vila M, Yoshioka Y, Kuriyama N, *et al.* Adult cardiomyocytes-derived EVs for the treatment of cardiac fibrosis. *J Extracell Vesicles.* 2024;13(7):e12461.
doi: 10.1002/jev2.12461
 60. Yang L, Zhai Y, Hao Y, Zhu Z, Cheng G. The regulatory functionality of exosomes derived from hUMSCs in 3D culture for Alzheimer's disease therapy. *Small.* 2020;16(3):e1906273.
doi: 10.1002/smll.201906273
 61. Zinger A, Cvetkovic C, Sushnitha M, *et al.* Humanized biomimetic nanovesicles for neuron targeting. *Adv Sci (Weinh).* 2021;8(19):2101437.
doi: 10.1002/advs.202101437
 62. Zhou J, Flores-Bellver M, Pan J, *et al.* Human retinal organoids release extracellular vesicles that regulate gene expression in target human retinal progenitor cells. *Sci Rep.* 2021;11(1):21128.
doi: 10.1038/s41598-021-00542-w
 63. Arthur P, Kan doi S, Sun L, *et al.* Biophysical, molecular and proteomic profiling of human retinal organoid-derived exosomes. *Pharm Res.* 2023;40(4):801-816.
doi: 10.1007/s11095-022-03350-7
 64. Alhasan L, Qi A, Al-Abboodi A, *et al.* Rapid enhancement of cellular spheroid assembly by acoustically driven microcentrifugation. *ACS Biomater Sci Eng.* 2016;2(6):1013-1022.
doi: 10.1021/acsbomaterials.6b00144
 65. Villard A, Boursier J, Andriantsitohaina R. Microbiota-derived extracellular vesicles and metabolic syndrome. *Acta Physiol (Oxf).* 2021;231(4):e13600.
doi: 10.1111/apha.13600
 66. Xing X, Han S, Cheng G, Ni Y, Li Z, Li Z. Proteomic analysis of exosomes from adipose-derived mesenchymal stem cells: A novel therapeutic strategy for tissue injury. *Biomed Res Int.* 2020;2020:6094562.
doi: 10.1155/2020/6094562
 67. Schuster M, Braun FK, Chiang DM, *et al.* Extracellular vesicles secreted by 3D tumor organoids are enriched for immune regulatory signaling biomolecules compared to conventional 2D glioblastoma cell systems. *Front Immunol.* 2024;15:1388769.
doi: 10.3389/fimmu.2024.1388769
 68. Proaño-Pérez E, Serrano-Candelas E, Guerrero M, *et al.* MITF regulates autophagy and extracellular vesicle cargo in gastrointestinal stromal tumors. *Mol Biomed.* 2025;6:92.
doi: 10.1186/s43556-025-00329-9
 69. Upadhya R, Zingg W, Shetty S, Shetty AK. Astrocyte-derived extracellular vesicles: Neuroreparative properties and role in the pathogenesis of neurodegenerative disorders. *J Control Release.* 2020;323:225-239.
doi: 10.1016/j.jconrel.2020.04.017
 70. Chansaenroj A, Adine C, Charoenlappanit S, *et al.* Magnetic bioassembly platforms towards the generation of extracellular vesicles from human salivary gland functional organoids for epithelial repair. *Bioact Mater.* 2022;18:151-163.
doi: 10.1016/j.bioactmat.2022.02.007
 71. Roush S, Slack FJ. The let-7 family of microRNAs. *Trends Cell Biol.* 2008;18(10):505-516.
doi: 10.1016/j.tcb.2008.07.007
 72. Kwak S, Song CL, Lee J, *et al.* Development of pluripotent stem cell-derived epidermal organoids that generate effective extracellular vesicles in skin regeneration. *Biomaterials.* 2024;307:122522.
doi: 10.1016/j.biomaterials.2024.122522
 73. Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. *Nat Nanotechnol.* 2021;16(7):748-759.

- doi: 10.1038/s41565-021-00931-2
74. Zhang Y, Dou Y, Liu Y, *et al.* Advances in therapeutic applications of extracellular vesicles. *Int J Nanomedicine*. 2023;18:3285-3307.
doi: 10.2147/ijn.s409588
 75. Elsharkasy OM, Nordin JZ, Hagey DW, *et al.* Extracellular vesicles as drug delivery systems: Why and how? *Adv Drug Deliv Rev*. 2020;159:332-343.
doi: 10.1016/j.addr.2020.04.004
 76. Liu H, Zhang H, Han Y, Hu Y, Geng Z, Su J. Bacterial extracellular vesicles-based therapeutic strategies for bone and soft tissue tumors therapy. *Theranostics*. 2022;12(15):6576-6594.
doi: 10.7150/thno.78034
 77. Wu Y, Song P, Wang M, Liu H, Jing Y, Su J. Extracellular derivatives for bone metabolism. *J Adv Res*. 2024;66: 329-347.
doi: 10.1016/j.jare.2024.01.011
 78. Liu H, Su J. Organoid extracellular vesicle-based therapeutic strategies for bone therapy. *Biomater Transl*. 2023;4(4): 199-212.
doi: 10.12336/biomatertransl.2023.04.002
 79. Nemati M, Singh B, Mir RA, *et al.* Plant-derived extracellular vesicles: A novel nanomedicine approach with advantages and challenges. *Cell Commun Signal*. 2022;20(1):69.
doi: 10.1186/s12964-022-00889-1
 80. Rutter BD, Innes RW. Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins. *Plant Physiol*. 2017;173(1):728-741.
doi: 10.1104/pp.16.01253