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Organoid-derived extracellular vesicles: From organoid, to organoid

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Abstract

Organoids are models of miniature organs formed by three-dimensional (3D) culture of stem cells or primary tissue cells. Their structure or function is highly similar to that of in-situ organs. Extracellular vesicles (EVs) are non-replicating nanocarriers with a phospholipid bilayer (20 - 400 nanometers) used to deliver bioactive substances. Organoid-derived extracellular vesicles (OEVs) are easier to form than conventional EVs and have enhanced biological functions. Organoids have the characteristics of stem cells; the transportation of bioactive substances by OEVs has broad prospects in medical applications. This article expounds the development, concept, construction methods and applications of organoids, describes the types, research progress and advantages of EVs, then outlines the concept of the basic biology of EVs, and explores their potential applications in disease treatment and intervention. Furthermore, we examine the distinctions that differentiate OEVs from conventional EVs. Finally, this paper summarises 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, adult stem cells, and primary tissue fragments, under defined culture conditions. Because organoids recapitulate key structural and functional features of *in vivo* organs, it is natural to expect that they also secrete and utilise extracellular vesicles (EVs) during their development.[1,2] This technology has been rapidly adopted for research in stem cell biology, organogenesis, and human diseases. The term "organoid" originally referred 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 lack of differentiation capacity and limited lineage diversity have made it difficult 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 emerged: through 3D culture, stem cells self-assemble into microphysiological systems, successfully bridging the gap between traditional cell lines and animal models.[7] Organoids have multiple cell types, and the interactions between cells are relatively complex. They can reproduce disease characteristics for drug screening and mechanism research, and are a better research platform than 2D models. In recent years, researchers have further isolated EVs from organoid culture media.

EVs are non-replicating, nanoscale, membrane-bound messengers (typically 20 - 400 nm in diameter) that mediate intercellular and inter-organ 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 the realm of biomedicine.[9]

Compared to traditional 2D culture-derived EVs, organoid-derived Extracellular Vesicles (OEVs) inherit the nanoscale size and biocompatibility of EVs while enriching a greater variety and higher abundance of active molecules due to the 3D microenvironment, demonstrating stronger 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 towards new paradigms of "personalised exosomal therapeutics" and "*in situ* regeneration induction".

In this review, 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 summarise 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 organs *in vivo*. Their development can be traced back to the 1970s, when Howard Green and his colleagues successfully formed layered squamous epithelial colonies similar to human epidermis through 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, limiting research into the 3D organisational behaviour of cells.[12] Subsequently, developmental biologists studied the morphogenesis processes of various model organisms (such as sponges, amphibians, chicks, mice) and demonstrated the proficiency of cells to autonomously organize and re-cluster into structures resembling tissues. These studies emphasised the key role of self-organisation phenomena in morphogenesis and provided theoretical support for the development of organoid technology.[13,14] In the late 1980s, Bissell and her colleagues observed that a gel rich in laminin could serve as a basement membrane, promoting the differentiation and morphogenesis of breast epithelial cells. This discovery enables the extracellular matrix (ECM) to be used for 3D culture.[15] By the 1990s, studies showed that ECM components not only provided physical support but also regulated gene expression through integrin adhesion. In 2009, the Clevers team cultured single intestinal stem cells in an ECM substitute and cultivated intestinal organoids with crypt-villus structures, promoting the development of organoid technology, initiating the organoid era, and soon constructing numerous organ models such as stomach, liver, pancreas, prostate, brain, etc. based on adult stem cells. Meanwhile, angiogenesis technology developed, using the replication of physiological microenvironments to assist in the transport of oxygen and nutrients. By the end of the 2020s of the 21st century, due to in-depth understanding of organogenesis mechanisms and great progress in biomaterials and bioengineering, organoid technology entered the customization era.[16] Scientists have developed customizable hydrogel pads to precisely regulate key extracellular factors in organoid development. Advances in induced pluripotent stem cells (iPSCs) and CRISPR/Cas technology make it feasible to construct 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 clarified the molecular behaviors of stem cells and progenitor cells, such as self-renewal and differentiation into specific tissue lineages, which lays a theoretical foundation for reconstructing organs with stem cells *in vitro*.[17] In the past few decades, organoid technology has developed rapidly in the study of tissue and organ biology, showing applications 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, but they do not belong to real human organs. "Organoid" is composed of "organ" and the suffix "-oid" meaning "similar", just because of this similarity.[18] The development of organoids is limited by lineage identification, cell sorting, as well as specific culture conditions and the requirements of cytokines.[10] Organoids are simplified organ models and have great potential in tissue development, disease modeling, personalized medicine, drug screening, and cell therapy.

2.2.2. The construction strategies of organoids

There are 3 steps in organoid construction: making matrix gel, building biological scaffolds, and using regulatory factors. It creates an environment like the natural body, helps cells self-assemble and differentiate, and finally forms organoids with specific organ properties and structures.

Active matrix gels simulate the ECM to help cells grow and differentiate, simulating the matrix environment for normal cell growth. Select or optimize them 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.[19] Tune the matrix gel to adjust the physical properties so as to simulate the microenvironment of a specific organ and promote cell growth and functional expression.

Bioactive scaffolds are essential in tissue engineering for providing mechanical support and spatial characteristics that mimic natural organs, offering cells a 3D structural framework that replicates tissue mechanical properties and spatial features, with construction involving material selection from natural options such as collagen and gelatin or synthetic ones like polylactic acid-hydroxyacetic acid copolymers, and design tailored to organ-specific needs allowing soft scaffolds for soft tissue organoids like liver and kidney or stiff compositions for hard tissue organoids like bone.[10] Recently, progress has been made in the field of biomedical engineering, promoting the innovation of composite bioactive scaffold materials; these materials can better mimic the microstructures of natural organs and enhance functions in medical applications such as tissue engineering.

Growth, regulatory factors such as cytokines are important for 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 BMP pathway is crucial for the development of skeletal organoids.[20,21] Researchers adjust the concentration and duration of action of signaling molecules, guide cells to develop in specific lineage directions, and culture organoids in the laboratory. The organoids simulate the structure and function of real organs.

2.3 Applications of organoids

Organoids are a key cell culture method in biomedicine, used to simulate tissue-like structures and can be long-term cultured and construct 3D structures. These characteristics make them have many biological and clinical applications, and can be used as miniature human organ models to carry out mechanism research. The importance of organoids in biobanks lies in being able to preserve complex cellular structures, thus providing support for future research or treatment. They have great potential in the field of precision medicine and can formulate personalized treatment plans for individual patients. Organoids are also of great significance in regenerative medicine, being able to have new ways to repair or regenerate damaged tissues, bringing hope for the progress of medical treatment (Figure 1).

Organoids are used in applications such as modeling genetic diseases, drug screening, personalized cancer treatment, gene repair and transplantation, biobank construction, and regenerative medicine, like the aspects in modeling genetic dis, drug screen, personalized cancer treat, gene repair, transpl, biobank build, and regener med. Since 2009, research has focused on rectal organoids with cystic fibrosis transmembrane conductance regulator (CFTR) mutations, using Crispr/Abe to reproduce pathological phenotypes in models of cystic fibrosis, polycystic kidney disease,fabry disease, retinitis pigmentosa, microcephaly, and down syndrome.[22] The "mutation-repair-recovery" closed-loop process is completed within 7–14 days, providing a basis for setting dose and delivery parameters.[23] Liver, heart-lung-liver multi-organ chips, and 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 hours, significantly reducing the risk of failure in later stages.[24] In the field of cancer, relying

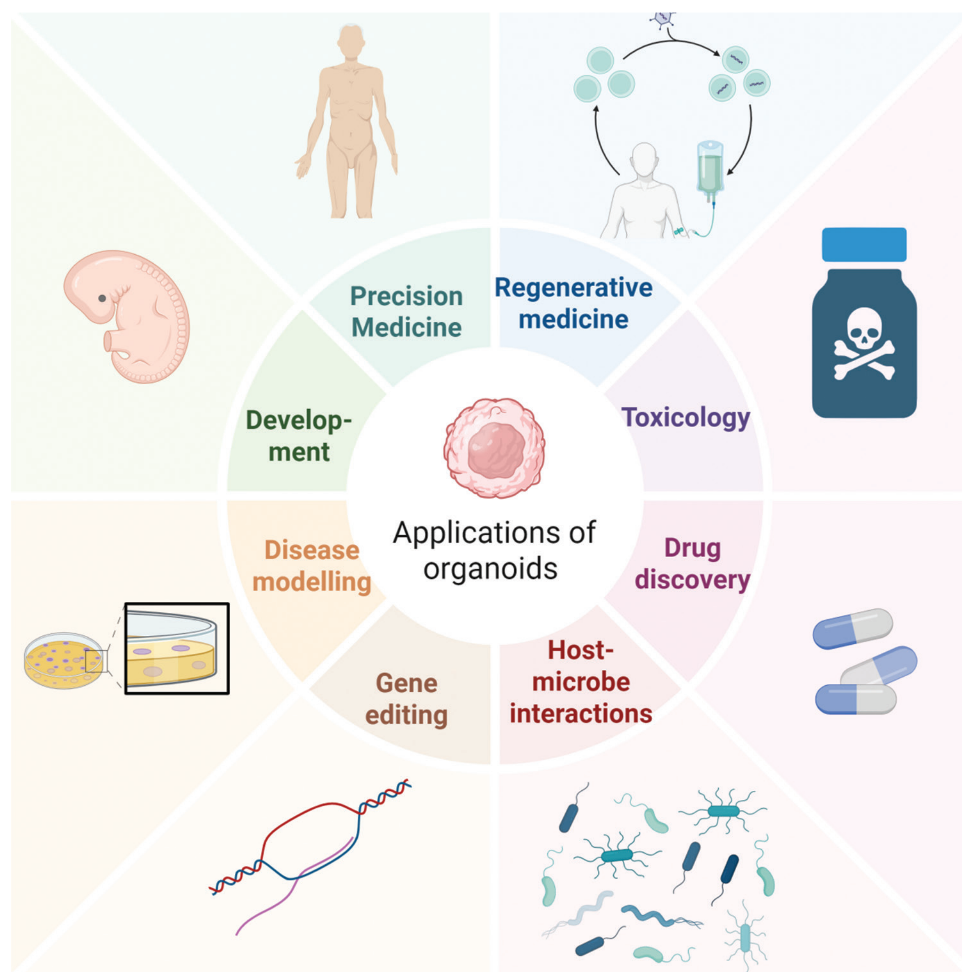

Figure 1. Applications of Organoid Technology. A chart presents the status of organoid technology in multiple fields such as the growth field of developmental biology, the continuous research status field of disease models, the customized treatment field of precision medicine, the repair or replacement tissue field of regenerative medicine, the field of evaluating the effect of substances in toxicology, the field of identifying new therapies in drug discovery, the field of exploring health impacts in host-microbiome interactions, the field of modifying gene maturation in gene editing. Created with BioRender.com.

on a biobank of 500 patient-derived organoids (PDOs) that retain 95% of the primary mutation load, organoid-tumor-infiltrating lymphocyte (TIL) co-culture or single-cellomics can be used to accurately predict the efficacy of PD-1 inhibitors, PRMT5 inhibitors, enzalutamide, EGFR-TKIs, and ADCs, with an 85% consistency rate and has entered prospective trials.[25] In terms of gene repair, Crispr/Cas9 or Abe achieves >90% homologous repair in intestinal, retinal, lung, and brain organoids. Combined with vascular endothelial co-culture or immune-modulating hydrogels, this approach maintains function for over six months and reduces infarct volume by 40%. Patient-derived PDOs serve as a living biological sample bank, spanning ten types of tumors and non-tumor diseases.[26,27] They maintain genetic fidelity and tumor heterogeneity even after continuous passage, enabling new target discovery, drug combination optimization, and prediction of sensitivity to radiotherapy and chemotherapy. Regenerative medicine views organoids as scalable autologous transplants. Intestinal, liver, bile duct, and neural organoids reconstruct functional crypts-villi, alveoli, or neural synapses in animal models, alleviating short bowel syndrome, acute liver failure, and neurodegenerative diseases.[28] Crispr-mediated gene correction expands applications to complex conditions like 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 nanometers to 1 micrometer and are encased in a phospholipid bilayer membrane.[29] EVs are primarily classified into three principal types based on their size and the pathways through which they are formed: EVs, which measure between 30 and 150 nanometers in

diameter and arise from the intricate process of fusion between multivesicular bodies and the plasma membrane, microvesicles, with diameters ranging from approximately 100 to 1,000 nanometers, which are produced by the direct budding off of the plasma membrane, a process that allows them to carry a variety of biological signals, and apoptotic bodies, which are notably larger than 1,000 nanometers and are formed through the fragmentation that occurs during the later phases of apoptosis, serving as a means for the cellular debris to be cleared away in a controlled manner.[30] EVs carry cell-specific components, such as proteins, lipids, mRNA, microRNA (miRNA), DNA fragments, and metabolites, forming a stable molecular fingerprint. Integrins, glycoproteins, and ligands on the surface help with tissue targeting and also participate in communication between cells, organs, and the host microenvironment, regulating biological processes such as immune response, inflammation, angiogenesis, tumor metastasis, and tissue regeneration.[31-33] EVs are regarded as promising new cell-free therapy and precise delivery systems. It has low immunogenicity, minimizing adverse immune risks to the greatest extent, good compatibility with biological systems, able to achieve body integration and function, and can be engineered for targeted drug or nucleic acid delivery, 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 nanometers to 1 micrometer, and the thickness of the membrane is 4 to 6 nanometers.[34,35] There are cholesterol, sphingomyelin and phosphatidylserine, forming a hard and negatively charged shell. Transmembrane proteins such as CD63, CD81, CD9, integrins and ligands are embedded in the membrane surface, making the cell have target recognition and adhesion.[36-38] There are specific proteins, lipids, mRNAs, miRNAs, DNA fragments and metabolites of mother cells in the lumen, becoming a relatively stable "cargo" storage place.[39] EVs subpopulations are derived from multivesicular bodies, in which there are many small vesicles. The structure of microvesicles is more uniform. This structure enables EVs to resist enzymatic degradation, pass through physical barriers, and deliver active molecules to targets, which is important in cell communication and biology (Figure 2).[6,40,41]

3.3. Classification of EVs

EVs are divided into 3 major subclasses based on biogenesis, size, and biochemical properties. EVs are a distinct type, with a diameter of 30-150 nanometers, which are released into the extracellular space after the fusion of multivesicular bodies with the plasma membrane, carrying substances such as transmembrane proteins (such as CD63, CD81, CD9) and nucleic acids (such as mRNA, miRNA), and are used for intercellular communication and have therapeutic potential.[42,43] Microbubbles (with a diameter of 100-1000 nanometers) bud from the plasma membrane and have markers such as integrins on their surface for cell signaling. Apoptotic bodies (with a diameter greater than 1000 nanometers) are derived from the fragmentation of the cell membrane during apoptosis and contain nucleosomes, histones, and DNA fragments of the dead cells.[44,45] EV's produced by bacteria (BEVs) can be divided into different subtypes according to the type of cell wall. Outer membrane vesicles (OMVs) of Gram-negative bacteria contain lipopolysaccharides, which are very important for the interaction with host cells and the

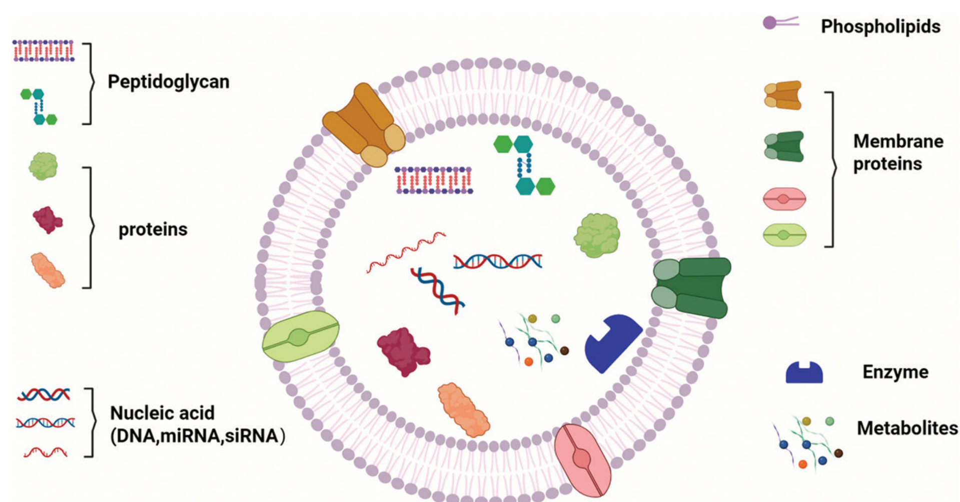

Figure 2. Structure of EVs. Nanospheres with a diameter of 30 - 1 micrometer are EVs. They have a phospholipid bilayer membrane with a membrane thickness of about 4 - 6 nanometers and transmembrane proteins on the surface. There are stable cargoes such as specific proteins, lipids, miRNAs, DNA fragments, metabolites inside. Created with BioRender.com.

immune response. Cell membrane vesicles (CMVs) of Gram-positive bacteria do not contain lipopolysaccharides and will affect their functions. BEV subtypes are different in size, membrane composition, cargo and targeting, etc. This diversity can bring opportunities for the diagnosis and treatment of diseases.[46]

4. Overview of OEVs

OEVs secreted by organoids are roughly the same as EVs from 2D cell culture. OEVs help the cells in organoids to exchange information and transport substances, and are key intercellular communication media. But because organoids have a unique 3D structure and diverse cell types, OEVs are different from traditional EVs. In fact, OEVs bear a closer resemblance to 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 (100–1000 nm) are directly pinched off via local outward budding of the plasma membrane, dependent on actin remodeling and the small GTPase ARF6, and rapidly released into the culture medium. Second, EVs (30–150 nm) are formed from early endosomes via double invagination to create intraluminal vesicles (ILVs). The ESCRT-0/I/II/III complexes recognize ubiquitinated cargo and drive membrane curvature, followed by maturation of early endosomes into multivesicular bodies (MVBs). The fate of MVB is regulated by small GTPases such as Rab27a/b, Rab35, and RalA, as well as fusion molecules such as cortactin and synaptotagmin-7, if MVB migrate along microtubules and dock with the plasma membrane, SNARE complexes mediate exosome release, achieving paracrine/autocrine signaling, if MVB fuse with lysosomes or autophagosomes, their contents are degraded, and signaling is terminated. The third source is apoptotic bodies (50–5000 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 integrates four major steps: endocytosis, sorting, transport, and membrane fusion. The proportion of subtypes and release efficiency depend on the microenvironment of the organoid, metabolic state, and genetic background, laying the foundation for subsequent functional studies.[4]

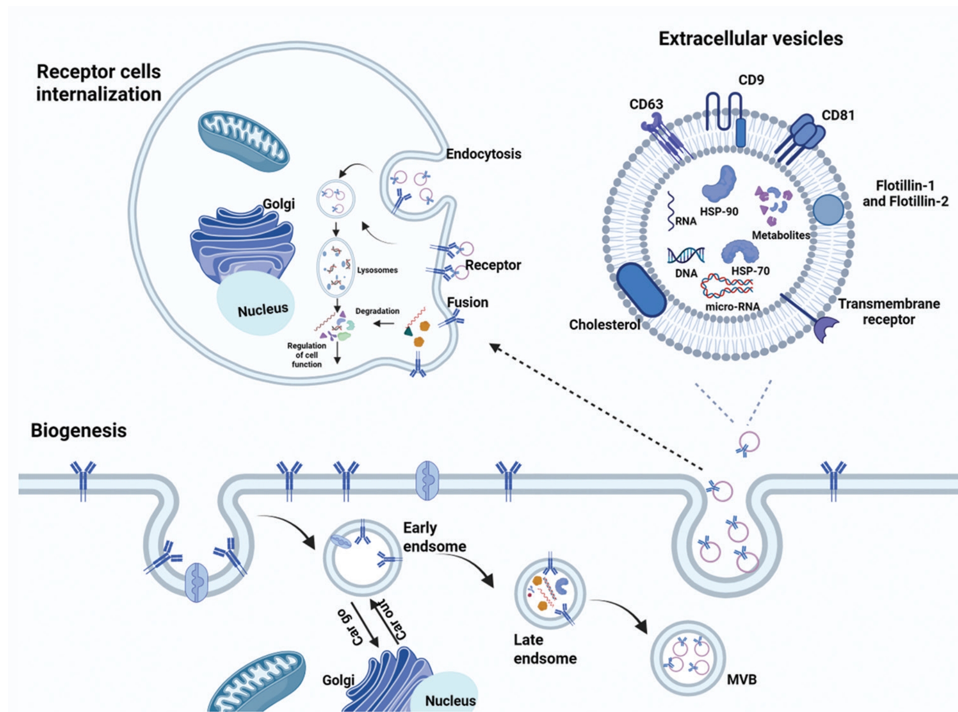

Figure 3. The Biogenesis Process of OEVs. The biogenesis of OEVs is complex, starting with plasma membrane invagination to form early endosomes, which mature into late endosomes through cargo exchange with the Golgi. Late endosomes accumulate intraluminal vesicles and can either fuse with lysosomes for degradation or merge with the plasma membrane to release EVs. OEVs are phospholipid bilayer vesicles that transport RNA, DNA, and proteins, and recipient cells internalize them via membrane fusion, endocytosis, or receptor-mediated pathways. Created with BioRender.com.
Abbreviations: CD63: Cluster of Differentiation 63; CD9: Cluster of Differentiation 9; CD81: Cluster of Differentiation 81; MVB: Mammalian extracellular vesicles.

4.1.2. Structure of OEVs

OEVs have a typical lipid bilayer vesicle structure; the membrane components are highly similar to the donor cell membrane and are more specialized.[47] Exosomal subpopulation membranes have ceramides, gangliosides, diunsaturated lipids, and the ratio of PC and diacylglycerol is relatively low.[5] The outer leaflet has more cholesterol and phosphatidylserine. This asymmetric distribution makes the vesicle harder and speeds up the endocytosis process of the target cell with the help of the "eat me" signal. EVs are formed in the multivesicular bodies of endosomes. There are differences from microvesicles from the plasma membrane. There is less polyunsaturated glycerophosphoserine in their membranes, which indicates that the biogenesis pathway determines the characteristics of the membrane. Due to the different lipid components, the Laurdan fluorescence anisotropy of EV membranes is much higher than that of cell membranes, which shows that EV membranes are more ordered and harder. But when the pH of the microenvironment decreases, the rigidity of the membrane decreases reversibly and the fluidity increases, which is beneficial for fusion with the target cell membrane.The activity of the transmembrane flipase in the EV membrane is higher than that in the cell membrane, so that the lipids between the two layers can be quickly exchanged.[9] Sphingomyelin is closely arranged with cholesterol, and it carries out corresponding activities to resist phospholipase and oxidative stress in the circulation.[9,48] This structure - function coupling enables OEVs to maintain morphological - functional integrity over long distances and for long periods in the 3D culture media, and also provides predictable physical parameters for downstream engineering modifications.

4.1.3. Composition of OEVs

There are substances in the OEVs, which are divided into three categories: lipids, nucleic acids, proteins, and have the characteristics of organoids, and need to refer to the context.[2] In addition to structural phospholipids, the 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.[5,49] At the nucleic acid level, the intestinal virus RNA mainly includes small RNA (less than 200 nucleotides), miRNA, and transfer RNA (tRNAs), accounting for about 15%. Long-chain coding and non-coding RNAs as well as repetitive sequences (such as endogenous retrovirus HERV, Alu sequence, L1 sequence) will be selectively packaged, and sorting is mediated by the specific motif (CUGCC) in the 3' untranslated region (3' UTR) or the hnRNPA2B1 recognition element.[49] EV-DNA is a double-stranded DNA fragment of the genome with a length of 100 to 2.5 kilobases, which exists in vesicles. Its mutation spectrum is consistent with the gDNA of parental organoids, and it may be able to be used as a biomarker for liquid biopsy.[2] Protein complexes reflect biological pathways. EVs have transmembrane proteins (CD63/CD81/CD82), MHC - II, endosomal sorting complex (ESCRT comp) (Alix, TSG101) and molecular chaperones (Hsc70/Hsp90). Microvesicles have plasma membrane proteins such as integrins, P - selectin, ARF6, etc. Apoptotic bodies contain histones but no glycoproteins.[1] In terms of functional proteins, PI3K, MAPK, ErbB family kinases, and oncogenic or matrix remodeling factors such as EGFRvIII, HIF-1α, and MMP/ADAM10 can be delivered by EVs to recipient cells, activating MAPK/Akt, downregulating E-cadherin, or degrading the ECM, thereby driving the spread of invasive phenotypes. Overall, the molecular composition of OEVs not only precisely reflects the physiological-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 OEVs

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 α6β1/β4 directs OEVs toward lung fibroblasts and epithelial cells, αvβ5 targets liver Kupffer cells, and the α4β1-Tspan8 complex precisely targets pancreatic cells[50], 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 via heparan sulfate proteoglycans in microvascular endothelium, Phosphatidylserine (PS) is recognized by the TIM4/4 receptor, mediating macrophage phagocytosis, glycans such as free oligosaccharides synergize with CCL18 to attract OEVs to CCR8-positive glioblastoma cells.[51] Subsequently, OEVs enters cells via four main pathways: clathrin-mediated endocytosis (CME) dominates in bone marrow mesenchymal stem cells, caveolin-dependent endocytosis promotes uptake in some cases and may also negatively regulate it, macropinocytosis depends on actin polymerization and extensively takes up OEVs from the culture medium, and macrophages efficiently internalize OEVs via phagocytosis. A few reports indicate that OEVs can directly form a 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, determining whether their contents are functional or degraded. 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

Replace the normal culture medium with the conditioned medium within 24 to 48 hours before collection to reduce interference, and then collect the culture medium and complete the extraction of EVs within 24 hours.[52] The process is to remove cells by low - speed centrifugation, filter through a 0.22 μm filter membrane, then perform ultrafiltration through a 100 kDa filter membrane, and then separate OEVs through two rounds of ultra - high - speed centrifugation. Operations such as fluorescence detection of surface markers are carried out. To obtain accurate results, the source of exosome cells needs to be considered. This is the development of super - traditional separation methods (Figure 4).[53]

4.3. Differences between OEVs and EVs

Organoids and traditional cell cultures are different in structural organization. Organoids have a 3D structure, while traditional cell cultures are in a 2D state. The interactions between cells in the 3D environment and those in the 2D environment are more complex and have more abundant characteristics. Many studies support this situation. For instance, human mesenchymal stem cells (hMSCs) grown in a 3D environment produce EVs (3D-hMSC-EVs) that not only are produced in larger quantities but also have higher levels of CD63 expression.[54] A study demonstrated that 3D mesenchymal stem cells released EVs at 28 times higher concentration than traditional methods, correlating with increased CD81 and CD9 expression levels, and these vesicles enhanced neurite formation and branching to support brain regeneration.[55] Compared with 2D-EVs, 3D-MSC-EVs have enhanced biological functions and therapeutic potential for AD, and there are differences in miRNA and protein levels.[56] Research on EVs from gastric cancer cell lines cultured in 2D and 3D environments demonstrated downregulated ADP-ribosylating protein 6 and elevated miRNA expression, with 3D cultures yielding more smaller EVs, studies on 3D-MSC-EVs for cardiac repair showed superior cardioprotective effects compared to 2D-EVs, indicating enhanced EVs efficacy through 3D systems, investigations into bone marrow MSC secretome revealed corneal wound healing benefits, suggesting 3D culture augments secretome effectiveness and optimizes culture conditions.[57] To monitor the recovery of traumatic brain injury (TBI) after injection, mice used MSC derivatives cultured in 2D and 3D. It was found that mice

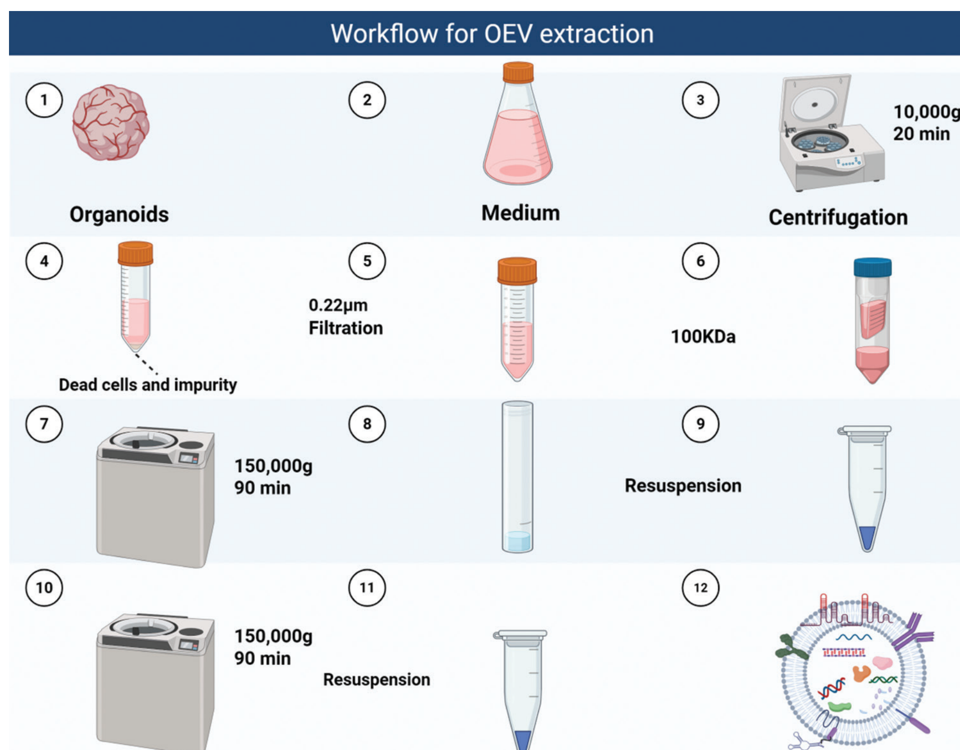

Figure 4. OEVs Extraction Workflow. Harvest organoid culture medium, centrifuge at 10,000 g for 20 minutes at 4°C to remove debris, filter the supernatant through a 0.22 μm filter membrane, use a 100 kDa ultrafiltration membrane, and then ultracentrifuge at 150,000 g for 90 minutes at 4°C to harvest exosome-like OEVs. Created with BioRender.com.

treated with MSC cultured in advanced 3D organoids were in better condition, especially in terms of angiogenesis and neural recovery. The cell structure cultured in 3D is close to the physiological state, and can promote the secretion of EVs as in the body, which is different from the situation in 2D culture. What we studied is EVs cultured in 3D, not OEVs of complex multicellular organoids. It is considered that EVs are more superior in biological function and yield.[58] This view is tenable. 3D-EVs are more powerful than 2D-EVs in cell contact communication. Organoids have more cell subtypes and a larger contact area, making cell - to - cell communication more complex. Therefore, it is foreseeable that EVs are more complex and precise than 3D-EVs.

New evidence shows that organoids produce more OEVs than conventional EVs from the same cells. OEVs have enhanced biological functions. This function may come from the origin of organoid stem cells, and key characteristics are inherited by their EVs. Then it is possible to mass-produce OEVs based on the characteristics of the original organoids and customize them for application (Table 1).

5. Layer-specific EVs release and signalling - structural complexity of organoids encourages intralayer EVs exchange and "secondary" EVs secretion

Organoid structures simulate the spatial arrangement of natural tissues and determine the physiological functional characteristics of cells. The layered structure leads to heterogeneity, and different cell populations exist in different 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.

EVs in the organization often release from the inner layer and mainly transmit signals to adjacent or upper layers. This means that EVs from inner layers can influence neighbouring cells, contributing to localised 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

When interpreting EVs produced by organoids, care is needed. For example, the release of specific miRNAs in EVs into the extracellular space may not reflect the expression of their source, but instead trigger systemic responses in adjacent cells or tissues. In addition, it is very important to figure out the dynamic changes of EV signals between the buried layer and the outer layer for clinical research or diagnosis.

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.[59,60] Zhou and a group of people said that the EVs of human retinal organoids carry small miRs that can change the gene expression of recipient cells, which indicates that there is a connection between the function of EVs and the structure of organoids.[59] When evaluating the impact of EVs from

Table 1. Comparison of differences among 2D-EVs, 3D-EVs, 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 3D environment	Significantly amplifies intercellular communication by increasing cell subtype variety and contact area
EV Production	Lower quantity of EVs produced	Higher quantity, with 28 times more concentration than 2D methods[58]	Significantly larger quantities compared to conventional EVs from the same number of cells[58]
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. CD9 Expression	Lower levels[54,55]	Higher CD63, CD81, CD9 expression levels	Anticipated to exhibit even higher expression levels due to stem cell origin[55]
MicroRNA and Protein Levels	Less variation in microRNA and protein levels[56]	Significant variations compared to 2D-EVs[56,57]	Inherits key stem cell-derived properties, leading to enhanced functionalities
Application Potential	Limited scalability and application specificity[56]	Potential for tailored applications based on organoid characteristics[56]	Opens possibilities for manufacturing OEVs at scale for specific applications

cellular organoids in regenerative medicine and disease models, it is necessary to describe the structure of cellular organoids. This view places emphasis on this point.

5.2. Influence of necrotic core on EVs composition

When organoids grow to over 400-500 micrometers, because nutrient and oxygen diffusion is not very good, there will be a necrotic core. The internal cells lack nutrient and oxygen and will necrose, thus forming an area of stress-dead cells.[61] Gradient guides organoid differentiation and maturation; there are viable cells around the injury in the necrotic core.[62] It is possible to trigger the release of EVs, which may carry damaged or inflammatory contents. These EVs can reach the upper tissues, carrying damaged contents such as proteins and RNA that show metabolic abnormalities, and can interfere with the functions of cells in the viable tissue layer.[63] Stress signals from EVs may damage neighboring healthy cells, may trigger inflammation, or may affect metabolism.[64] EVs produced by necrosis or EVs contaminating the extracellular space can change the characteristics and reduce the integrity of the vesicles. This should be noted. When evaluating EVs for downstream use, the pathological state of the internal organoids needs to be considered (Figure 5).

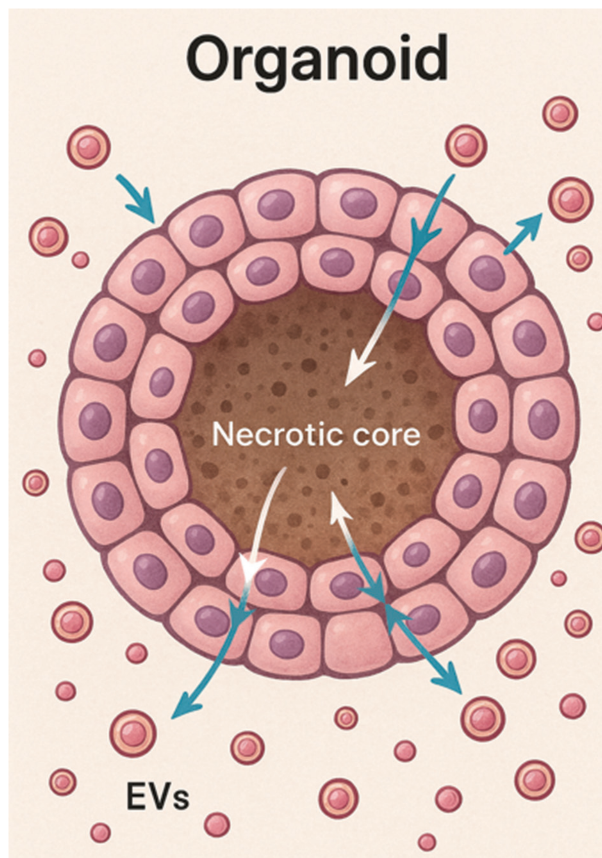

Figure 5. When the diameter of organoids exceeds 400–500 μm, the diffusion of nutrients and oxygen will be restricted, and there will be a necrotic core. Cells in the core will undergo necrosis due to starvation, hypoxia, genomic stress, or death signals. Although signals may guide differentiation and maturation, the necrotic core will damage living cells and trigger the release of EVs carrying 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 produced by necrosis will contaminate the extracellular vesicle pool, disrupt the molecular profile, and reduce the integration of vesicle function, so the pathological state in organoids needs to be considered before downstream applications.

5.3. Autophagy in organoids and its effects on EVs signalling

Autophagy is a key cellular stress response that regulates the degradation and recycling of components. In organoids, autophagy affects the composition of exosome biogenesis, thereby affecting intercellular signal transduction and possibly altering cellular homeostasis.[65] Organoids have unique physiological microenvironments with various cell - type health states, and the autophagy - EV dynamic interaction is very important.

Stress factors such as nutrient deficiency, hypoxia, organelle accumulation, etc. trigger autophagy. Autophagy helps cells survive by not only clearing problems but also providing substrates for metabolism during stress. Recent studies have shown that autophagy regulates the secretion of EVs in various ways. Schuster *et al.*[65] found that autophagy affects the EVs composition of glioblastoma tumor organoids, and then autophagy-related pathways regulate the characteristics of immunomodulatory signaling biomolecules.There are changes in the association between autophagic activity and EVs RNA packaging, and the cargo profile changes, which can reflect the cellular stress state.[66]

Autophagy-related EVs are different from EVs derived from healthy cells. When analyzing the exosome profile, these two populations need to be distinguished. Autophagy-related EVs may cause relatively large interference to the research on EVs produced by organoids.

6. Application of OEVs in organoid research

EVs are cell-free platforms for transporting bioactive substances and enabling intercellular communication. They are very good therapeutic agents and delivery vehicles and have great potential in modern medicine. OEVs are promising in treating diseases such as cancer and genetics, and can also deliver genetic material to target cells to improve therapeutic efficiency. Moreover, OEVs can reflect the growth conditions of organoids. Combined with organoid technology, they can improve drug screening methods and be helpful for disease and cancer research. 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 pathway of inflammasomes, which is the core mechanism. Research indicates that cells can package ASC specks, NLRP3, activated Caspase-1, and mature IL-1β 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 IL-1β/TNF-α stimulation induces epithelial and immune cells to synergistically release ASC speck-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 luminal and basal surfaces, exhibiting a "leaping expansion" phenomenon difficult to explain solely by soluble factors.

EVs from different origins have been shown in numerous studies to have immune-regulating properties. For instance, intestinal epithelial cell-derived EVs have the ability to control Treg cells.[67] 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 (LPS). 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. In particular, one important molecular regulator of inflammatory management is Let 7.

6.1.2. Salivary gland OEVs for epithelial repair

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

Chansaenroj *et al*. developed a magnetic 3D bioassembly platform for creating functional salivary gland organoids and non-invasively culturing hDPSCs with magnetic nanoparticles.[68] The success of the M3 DB platform in SGo building was demonstrated by the created SGo's broad expression of SG-specific markers (AQP 1, MUC 7, KRT 5, KRT 14, CHRM 3, TUBB 3).[68] After that, 3D-hDPSC-EV and SGo-OEV were created by extracting the EVs secreted by 3D-hDPSC and SGo, and an epithelial damage model was used to evaluate their capacity for repair. Growth of epithelial cells and effects of neural healing were among the evaluation criteria.[68] Direct SGo transplantation had a 25% therapeutic efficacy, 3D-hDPSC-EV had a 15% therapeutic efficacy, and SGo-OEV had a 60% therapeutic efficacy. 99 distinct proteins, many of which are intimately linked to adhesion, bio-regulation, biogenesis, development, and cell proliferation, were shown to express abnormally in SGo-OEV by proteomics analysis. This finding is particularly noteworthy, as it distinctly illustrates the disparities between 3D-EV and OEVs, with OEVs displaying markedly enhanced biological functions in comparison to 3D-EV.[69] The treatment effect of OEVs surpasses the traditional SGo transplantation. But it is currently unclear whether this is a unique phenomenon or a more widespread 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 with high expression in EVs have related effects. The miRNA-rich vesicles produced by EVs play a role through these pathways: promoting epithelial cell differentiation via the PTEN/miR-146a-5p axis, enhancing cell migration via the FAK/Rac1/miR-31-5p axis, and promoting inflammation resolution via the NF-κB/miR-21-3p axis. The experimental result is that applying EVs derived from local ovarian cancer to full-thickness skin defect mice can accelerate wound healing. Histological analysis shows that the granulation tissue in the wound of the experimental group mice is denser, the epithelial regeneration layer is thicker, and the vascular density of VEGF, FGF2, and CD31+ is all increased. Masson trichrome staining shows that the collagen fibers are arranged in order and the deposition is increased, which indicates that EVs derived from ovarian cancer plays a key role in vascular reconstruction and ECM repair *in vivo*. Furthermore, OEVs induce keratinocytes to undergo an epithelial-mesenchymal transition-like phenotype shift, thereby promoting re-epithelialisation. Specifically, during the early wound healing phase, OEVs activate TGF-β/Smad

and Wnt/β-catenin signalling 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 OEVs activation gradually diminish, allowing cells to regain their epithelial phenotype and reconstruct the epithelial basement membrane barrier function.

In the investigations conducted by the Lee research team, iPSCs were effectively employed to create epidermal organoids (iEpiO). The organoid characteristics declined when epidermal cells were in 2D culture. EVs from iEpiO in 2D and 3D were isolated for study. Nanoparticle tracking analysis showed comparable extracellular vesicle particle sizes between 3D and 2D cultures but double the EVs quantity in 3D, with reduced Alix and Tsg101 protein expression and increased CD9 levels, VEGF was abundant in 3D-derived EVs, elevating VEGF mRNA in human umbilical vein endothelial cells and promoting angiogenesis through PCR and tube formation assays, small RNA sequencing indicated higher expression of 16 specific miRNAs in 3D-EVs, highlighting regulatory roles in cellular communication.[70] The mouse skin wound healing experiment confirmed the therapeutic effect. The healing rates of mice treated with 3D-EVs were 1.6 times higher than those of the control group on days 3, 5, and 7. Through immunohistochemistry observation, it was found that 3D-EVs enhanced the expression of VEGF in the treatment site, and there was a strong biological response. This discovery is of great significance to the field of regenerative medicine. It reveals the challenges faced by organoid technology. Many key organoid achievements, especially their complex 3D structures, often fail when transferred to 2D culture, which restricts their application in treatment. There are differences in the expression profiles of two states under multiple markers, resulting in significant changes in the biological activity of EVs. OEVs have great medical application potential and can solve the problem of low extraction efficiency of traditional EVs. OEVs may have more obvious physiological effects than the parent organoids, and the progress of organoid technology may promote the research of OEVs. Complex organoid models, such as vascularized and neuralized organoids, are likely to produce OEVs with specific medical functions, which are helpful for treating neurodegenerative diseases and improving angiogenesis in ischemic tissues. Integrating 3D bioprinting with CRISPR/Cas9 gene editing technology will enhance OEVs, so as to develop customized treatment models for various diseases.

If the organoid technology is mature, it will promote the research related to OEVs. Many small vesicles may have a greater impact on 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 ability, and can also be used as new biomarkers for drug toxicity assessment. In drug 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 become ideal "biological delivery carriers".[71] EVs can pass through physiological barriers such as the blood-brain barrier (BBB) 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 by means of electroporation, transfection, or co-culture, avoiding problems such as low drug solubility, short half-life, and non-target toxicity. For example, encapsulating doxorubicin into EVs derived from macrophages can precisely target the tumor microenvironment in organoids and greatly reduce nephrotoxicity.[72] 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, PD-1 negative EVs secreted by CAR-T cells can avoid cytokine storm. Surface engineering operations are carried out through fluorescent peptides, dendrimers, polyethylene glycol or albumin binding domains to extend the circulating half-life of EVs in organoid perfusion fluid, and local release is also achieved through continuous methods of external fields such as magnetic fields or photosensitivity.[73] 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, the drug loading efficiency, and the functional indicators of organoids, so as 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 attracted attention in the biomedical field because of their characteristics and applications. Compared with traditional EVs, they have advantages: low immunogenicity and lower immune risk; perform well in cell-free environments. Also, the delivery mechanism of organoid extracellular vesicle-like vesicles is relatively efficient because they are rich in

bioactive molecules (including proteins, lipids, and nucleic acids), can affect the gene expression of target cells, and create new treatment approaches for various diseases.[74] The yield and physiological activity of EVs are stronger than those of traditional EVs, and they can also be improved by genetic or chemical methods so as to be more suitable for research or treatment.[75] The development of OEVs is restricted. Natural OEVs have inherent limitations, the therapeutic mechanism is unclear, the protein and RNA molecular pathways for enhancing functions have not been found, and the production is also limited due to the size of organoids and technical problems.[76] Organoid and OEVs technologies are still in the early stage. The lack of standard protocols is an obstacle to the wide application of OEVs in research and clinical practice, making it difficult to realize the transformation of 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 need to be standardized and kept consistent. When isolating EVs, standards need to be determined according to size, density, or surface markers, and minimum requirements for exosome vesicle concentration, purity, and key functional molecules need to be set. 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 compliant with Good Manufacturing Practice (GMP) standards, thereby addressing the current shortcomings in technical standardisation (Figure 6).

8. Summary and outlook

OEVs are natural nanocarriers that have been re-recognized with the rise of organoid technology, combining the 3D

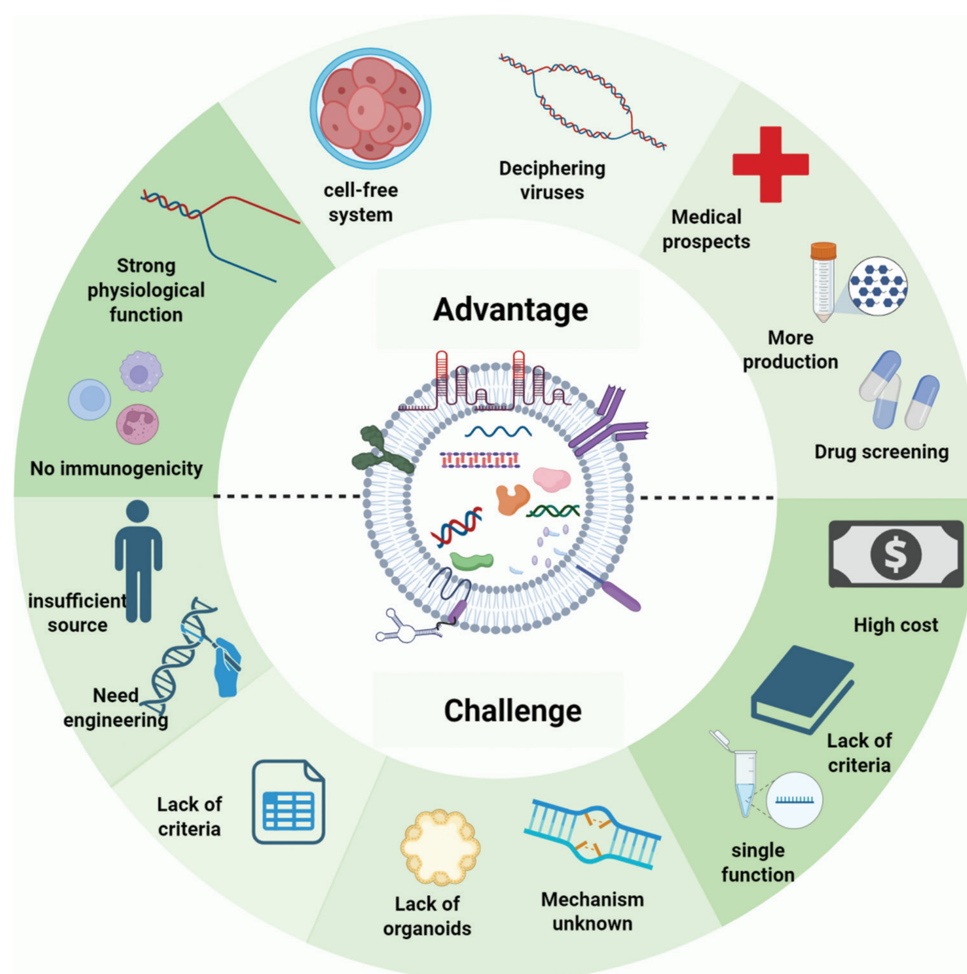

Figure 6. Advantages and Challenges of Organoids and OEVs. Organoids offer benefits like cell-free system and applications in drug screening but face challenges such as insufficient source and high costs. OEVs provide advantages like more production and better drug delivery but struggle with unclear mechanisms and needing engineering. Created with BioRender.com.

physiological complexity of organoids with the intercellular communication capabilities of EVs.[77] Over the past five 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 LPS-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 via fatty acid metabolism-related proteins, and epidermal OEVs accelerate wound vascularization via VEGF-rich cargo. Compared to 2D/3D-EV, OEVs have high yield, strong physiological activity, and low immunogenicity. It has been applied in genetic disease models such as cystic fibrosis, Alzheimer's disease, and 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 from the laboratory to the clinic, and the preparation of organoids is still in the small-scale stage. Key bottlenecks include: poor scalability and 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 standardised production workflows and GMP compliance. These limitations result in insufficient exosome yields to meet systemic administration requirements, confining applications to localised delivery or *in vitro* model construction.

Looking ahead, OEVs research and translation will focus on three key directions: "scalability, intelligence, and standardization." First, leveraging perfusion bioreactors, 3D bioprinting, and synthetic hydrogel scaffolds to establish scalable and quality-controlled organoid production platforms, and achieving a significant increase in OEVs yield by activating Rab27a/b or inhibiting TSG101 through Crispr at secretory nodes. Second, using multi-omics-AI joint analysis as the core, we will establish a "molecular fingerprint-functional map" database for OEVs from different tissue sources.[3] By combining organ-on-a-chip and microphysiological systems, we will track the spatiotemporal distribution and transcriptional/post-translational dynamics of OEVs in target tissues in real time, thereby identifying the "key cargo" determining therapeutic efficacy and its functional networks. Third, develop modular engineering strategies to introduce light-sensitive, magnetically responsive, or enzyme-cleavable release elements at the organoid stage, enabling targeted and controlled release of OEVs. Utilize cell membrane biomimetic coating and immune-modulating hydrogel microcapsules to reduce reticuloendothelial system clearance and extend circulation half-life. Additionally, there is an urgent need for an international consensus organization to lead the development of specialized guidelines for OEVs, standardizing culture substrates, separation processes, characterization metrics, and efficacy evaluation systems, and establishing a traceable GMP-grade production chain.[78] 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 bring a new "*in situ* regen" model to regenerative medicine, providing affordable, accessible and sustainable solutions for organ shortage and chronic disease burden during the global aging period.

In conclusion, 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 needs to focus on improving organoid culture technology, enhancing the functional diversity, and using innovative methods to construct functionally integrated organoids that can meet the needs of clinical transplantation treatment. At the same time, by 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 improve treatment. The continuous progress of organoid technology will directly promote the development of EVs of organoids, enabling the improvement of yield and quality and the enhancement of functions. This will further broaden the potential applications in the biomedical field. We expect this review to highlight the great potential of these cutting-edge technologies and stimulate the enthusiasm for further research and development in this developing field.

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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 authors declare they have no competing interests.

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Ethics approval and consent to participate

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