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Efficacy of Extracellular Vesicles in Organ-On-Chip Models: A Comprehensive Systematic Meta-analysis for Degenerative Diseases

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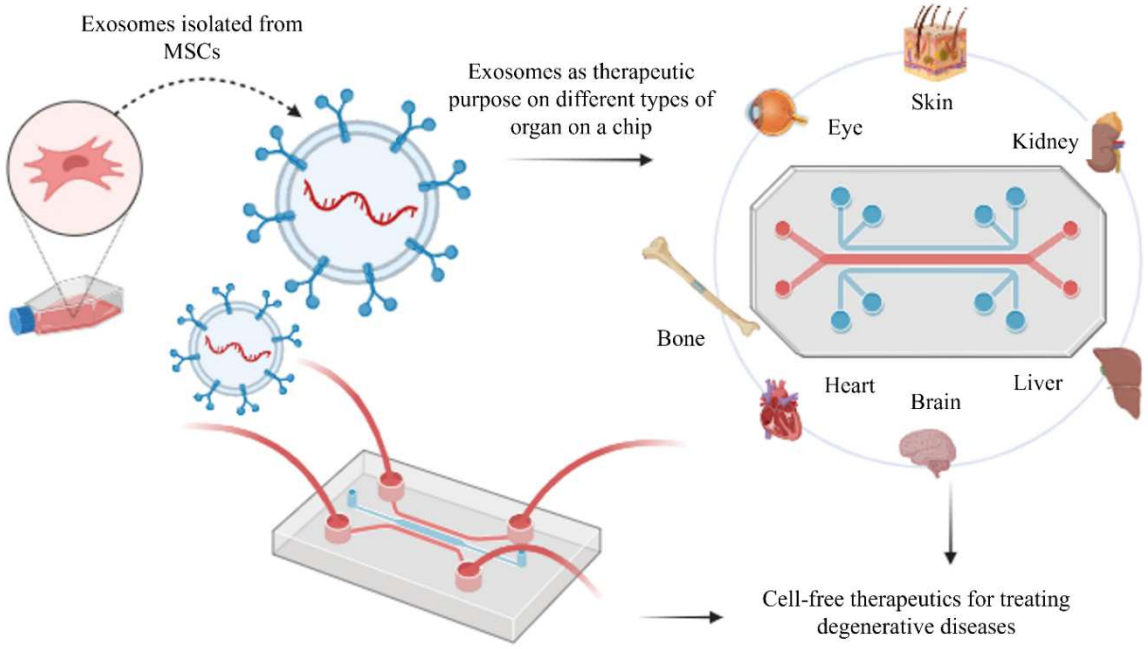
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Efficacy of Extracellular Vesicles in Organ-On-Chip Models: A Comprehensive Systematic Meta-analysis for Degenerative Diseases

Graphical Abstract



This research elucidates trends in extracellular vesicles within organ-on-chip models for degenerative diseases. Employing various analytical representations such as Sankey plots, Chord diagrams, and Network mapping, we demonstrate the organ-on-chip model's efficiency in mimicking human and animal tissues and emphasize extracellular vesicles crucial role in enhancing regenerative medicine by providing cell-free therapeutic strategies within these advanced models.

Abstract

Extracellular vesicles (EVs), particularly exosomes (EXOs), have emerged as a potential therapeutic agent owing to their ability to induce tissue repair, modulate immune responses and attenuate inflammation. Concurrently, Organ-on-a-chip (OoC) technology has recently emerged as a sophisticated pre-clinical platform to recapitulate the structure, function and physiological characteristics of human tissues. The integration of EV-based therapies with OoC models provides a promising approach to study the mechanisms of diseases and assess the therapeutic outcomes in degenerative diseases under physiologically conditions.

This meta-analysis evaluated the therapeutic potential of EVs in OoC models for degenerative diseases by analyzing 12 studies published between 2016 and 2024. The included studies encompassed diverse degenerative disease models involving the kidney, skin, bone, brain, eye, liver, and heart. The findings indicate that EVs derived from multiple cell sources consistently promoted tissue regeneration, reduce inflammation, and improve tissue repair across different OoC disease models. Network-based analyses further revealed significant relationships among disease models, EV sources, OoC platforms, and chip complexity. The studies also demonstrate that OoC technology has progressed from simple microfluidic devices to sophisticated systems capable of reproducing key structural, functional, and pathological features of human tissues more accurately than conventional two-dimensional (2D) and three-dimensional (3D) cell culture models, while also addressing several limitations associated with animal models.

Based on these findings, the combination of EV-based therapies and OoC technology represent a promising strategy for advancing regenerative medicine and the treatment of degenerative diseases. By providing more reliable models for studying disease mechanisms and therapeutic responses, these platforms have the potential to accelerate preclinical studies, accelerate translational research, and facilitate the development of personalized therapeutic approaches, and decrease reliance on animal models.

Keywords: Organ-On-Chip, Extracellular Vesicles, Degenerative Diseases, Regenerative Medicine, Therapeutics.

1. Introduction

Degenerative diseases encompass a broad spectrum of disorders characterized by the progressive loss of tissue or organ structure and function, often because of aging and chronic pathological processes. These diseases are associated with cumulative cellular and molecular damage that compromises tissue integrity, disrupts physiological homeostasis, and ultimately results in irreversible functional deterioration [1]. Most degenerative diseases progress with ageing, and the factors that drive their pathophysiology range from environmental influences to molecular impacts, challenging our understanding of their mechanisms of origin. This category includes renal impairments like chronic kidney disease [2]; dermatological conditions like chronic wounds [3]; musculoskeletal disorders such as osteoarthritis [4]; neurological disorders such as Alzheimer's disease (AD) [5], amyotrophic lateral sclerosis (ALS) [6], neuroinflammation [7], and Parkinson's disease [8]; cardiovascular conditions like cardiac fibrosis (CF); ophthalmic issues such as corneal epithelial wounds [9]; and hepatic issues such as liver fibrosis [10]. These diseases present considerable challenges in both diagnosis and treatment.

To overcome these limitations, extracellular vesicles (EVs) have been recently emerged as a rapidly advancing therapeutic platform in regenerative medicine and biomedical research owing to their ability to promote tissue repair, modulate immune responses, and facilitate intercellular communication. Exosomes (EXOs) originate in endosomes and then interact with various intracellular vesicles and organelles to form their ultimate composition. Their components, including nucleic acids, proteins, lipids, amino acids, and metabolites, can differ depending on the cell type. EXO in bodily fluids can provide a multidimensional diagnostic readout for numerous degenerative diseases by revealing changed cellular or tissue states. Its ability to efficiently exchange biological components can inform the development of EXOs-based therapies. It can also transport cargo from source to target cells [11, 12] and has been recognized as mediator of a recently identified intercellular communication mechanism, potentially improving the therapeutic efficacy of EV transplantation.

The pathophysiology and physiological behaviour of cells in vivo can be better understood through the use of animal models of degenerative disorders. However, Previous researches has highlighted the limitations of using animal models to investigate human and veterinary diseases and develop effective therapies [13]. Preclinical animal models often fail to accurately predict therapeutic

efficacy and safety because they do not fully replicate human or target animal physiology and disease characteristics [14–16]. Moreover, significant physiological differences exist between commonly used laboratory animals and humans or other animal species, limiting the translational value of these models. These differences occurs at multiple levels, including genetic makeup, metabolic pathways, immune system function, and organ structure and function. For example, variations in the expression and activity of drug-metabolizing enzymes can result in species-specific differences in pharmacokinetics, leading to inaccurate predictions of drug efficacy and toxicity. Such differences are particularly important in complex organ systems, including the kidney, skin, musculoskeletal system, heart, liver, and brain, where even subtle physiological variations can result in markedly different disease progression and therapeutic responses.

This variability has led researchers to question the limitations and ethical implications of using animals in research. However, ethical concerns arise from the potential for pain and distress experienced by animals, as well as debates about the moral status of animals and their utility in advancing scientific knowledge. Scientifically, the predictive accuracy of animal models for human and veterinary conditions is often questioned, prompting a shift towards alternative methods. which mainly includes- in vitro systems, computer modeling, and advanced technologies like OoC [17], which combines biology with microtechnology to replicate crucial elements of human or target animal physiology. To guide and manipulate minute volumes of fluid (ranging from picolitres to millilitres), the chip is designed as a microfluidic device with networks of microscale channels [18][19]. OoC technology aims to replicate human/animal physiology more closely and reduce reliance on animal models, enhancing both ethical standards and scientific outcomes. Consequently, the limitations of conventional animal models raise concerns about the reliability of preclinical data for identifying therapeutic compounds that warrant further evaluation in human or veterinary studies..

This meta-analysis aims to comprehensively evaluate the applications of OoC models in the study and treatment of degenerative diseases, with a special emphasis on the role of EV. By evaluating the effectiveness of these advanced models in replicating disease pathology and assessing therapeutic interventions, this study seeks to provide a robust understanding of their potential in preclinical research. Additionally, the analysis will identify existing gaps in current research,

offering critical insights and suggesting future directions to enhance the development and application of OoC models in degenerative disease studies.

2. Materials and methods

2.1. Study design and search strategy

The present meta-analysis was carried out in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria [20]. A comprehensive literature search was performed across multiple databases, including PubMed, Google Scholar, Web of Science, and Embase, to identify studies evaluating the efficacy of EVs in organ-on-chip (OoC) models for degenerative diseases. The latest search update was performed on November 02, 2023. The search method included multiple combinations of search terms and medical subject headings (MeSH) terms such as ("extracellular vesicle" OR "EV" OR "EXOs " OR EXO OR "microvesicle" OR "MV" OR "shedding vesicle") AND ("organ-on-chip" OR "organoid" OR "organ chip" OR "tissue chip") AND ("degenerative diseases" OR "microfluidics" OR "kidney diseases" OR "renal diseases" OR "wound healing" OR "kidney disease" OR "kidney dysfunction" OR "chronic kidney disease" OR "renal fibrosis" OR "chronic renal failure" OR "chronic renal insufficiency" OR "CKD" OR "renal dysfunction" OR "skin" OR "wound healing" OR "arterial restenosis wound" OR "bone" OR "osteoarthritis" OR "brain" OR "Alzheimer's disease" OR "neuroinflammation" OR "amyotrophic lateral sclerosis" OR "ALS" OR "eye" OR "corneal epithelial wound healing" OR "liver" OR "liver disease" OR "heart" OR "cardiac fibrosis"). Additionally, the bibliographies of included research and pertinent reviews were carefully searched to find any additional eligible studies.

2.2. Inclusion Criteria

After the duplicates had been removed, the articles were screened using the specified inclusion and exclusion criteria. Studies were included if they met the following criteria: (1) were original research articles; (2) investigated EVs as a therapeutic intervention; (3) reported outcomes evaluating the efficacy of EVs in treating or ameliorating degenerative diseases, such as improvements in cell viability, tissue regeneration, or functional performance; and (4) employed OoC models.

2.3. Exclusion Criteria

To ensure the methodological quality and relevance of the included studies, the following exclusion criteria were applied to finalise the articles: (1) non-English-language publications; (2) studies lacking an appropriate control group or employing inappropriate comparator groups; (3) studies that did not utilize OoC models; (4) review articles, editorials, conference abstracts, commentaries, and opinion papers; and (5) studies that did not report sufficient quantitative outcome data for analysis. Exclusion criteria involved: (1) Non-English-language studies; (2) Studies lacking a control group or involving inappropriate comparisons. (3) Studies that did not use OoC models. (4) Reviews, editorials, and opinion pieces. (5) Studies without adequate quantitative outcome data.

2.4.Data Extraction and Synthesis

Using a standardised extraction form, three authors independently extracted the data. Each of the included studies provided the following information: (1) Characteristics of the Study: Publication year, author(s), country, and funding sources, (2) OoC model details: Type of OoC model, species origin (human or animal-derived), and specific degenerative disease model, (3) EV characteristics: Source of EV, isolation and characterization methods, and dosage, (4) Outcomes: Primary and secondary efficacy outcomes, including measures of cell viability, tissue regeneration, and functional improvements. Extracted data were synthesized qualitatively in a descriptive summary and quantitatively using meta-analytic techniques.

2.5. Statistical analysis

In this meta-analysis, data from all included studies were summarised and analysed using R software (version 4.3.3). To visualize the relationships and flow between different variables, a Sankey diagram was created utilizing the high charter, html tools, and dplyr packages. The diagram was then rendered for presentation using the html widgets package. To visualize the relationships between OoC models, disease models, EXOs sources, and the complexity of chip designs, a network diagram was created employing various packages such as igraph, ggraph, tidygraph, ggplot2, dplyr. Additionally, a Chord diagram was generated to illustrate the connections and interactions among variables, employing the circlize package. These visualizations provided comprehensive insights into the data, enhancing the interpretability and presentation of the meta-analysis results.

2.6. Quality assessment

Assessing publication bias ensures the integrity and credibility of the meta-analysis focusing on the impact of EXO on various elements of the use of OoC for degenerative illnesses. The methodological quality of the 12 eligible studies was reviewed, including an analysis of the risk of bias, examined using the visualization (robvis) tool for Bias Risk Assessment [21] which provides a standardized graphical representation of bias across predefined methodological domains. A major method was visually assessing a bias risk graph for asymmetry, which could indicate the presence of publishing bias. The assessment enabled the identification of potential sources of bias related to study design, participant selection, outcome assessment, and reporting, thereby facilitating a comprehensive evaluation of the internal validity of the included studies. Our goal in using these comprehensive methodologies was to comprehensively address any potential bias and ensure that our meta-analysis provided an unbiased synthesis of the present evidence on the favourable effects of EXO in the context of OoC for degenerative diseases.

3. Result

3.1. Study selection and characteristics

Using Medline databases, 529 papers published between 2016 and 2024 were first located and categorized. After the removal of 106 duplicate records, 423 studies remained for initial screening based on their titles and abstracts423 studies were left for initial screening based on their titles and abstracts after 106 duplicate data were removed. After Following this initial screening, 115 irrelevant studies were excluded, leaving 308 articles for further assessment308 studies remained for additional analysis after 115 studies were deemed irrelevant. Of these, 67 articles could not be retrieved, resulting in 241 full-text articles being assessed for eligibility241 of them were evaluated for eligibility after 67 studies could not be retrieved. During the full text review, 229 articles were eliminated excluded during this evaluation for following a variety of reasons: There were a total of 73 were the review papers, 11 were book chapters, 8 were inaccessible publications, 2 were articles written in languages other than English, 37 employed unsuitable study designs study designs that were deemed unsuitable, 45 articles that excluded that did not employed EVs as a therapy option, and 53 articles that did not make use of utilised OoC models. Consequently, 12 studies [21–32] fulfilled met the predefined eligibility criteria and were included in the requirements and were added to the meta-analysis. The study selection process was conducted in

accordance with the adhered to the PRISMA guidelines and is illustrated in the flow diagram (Figure 1).

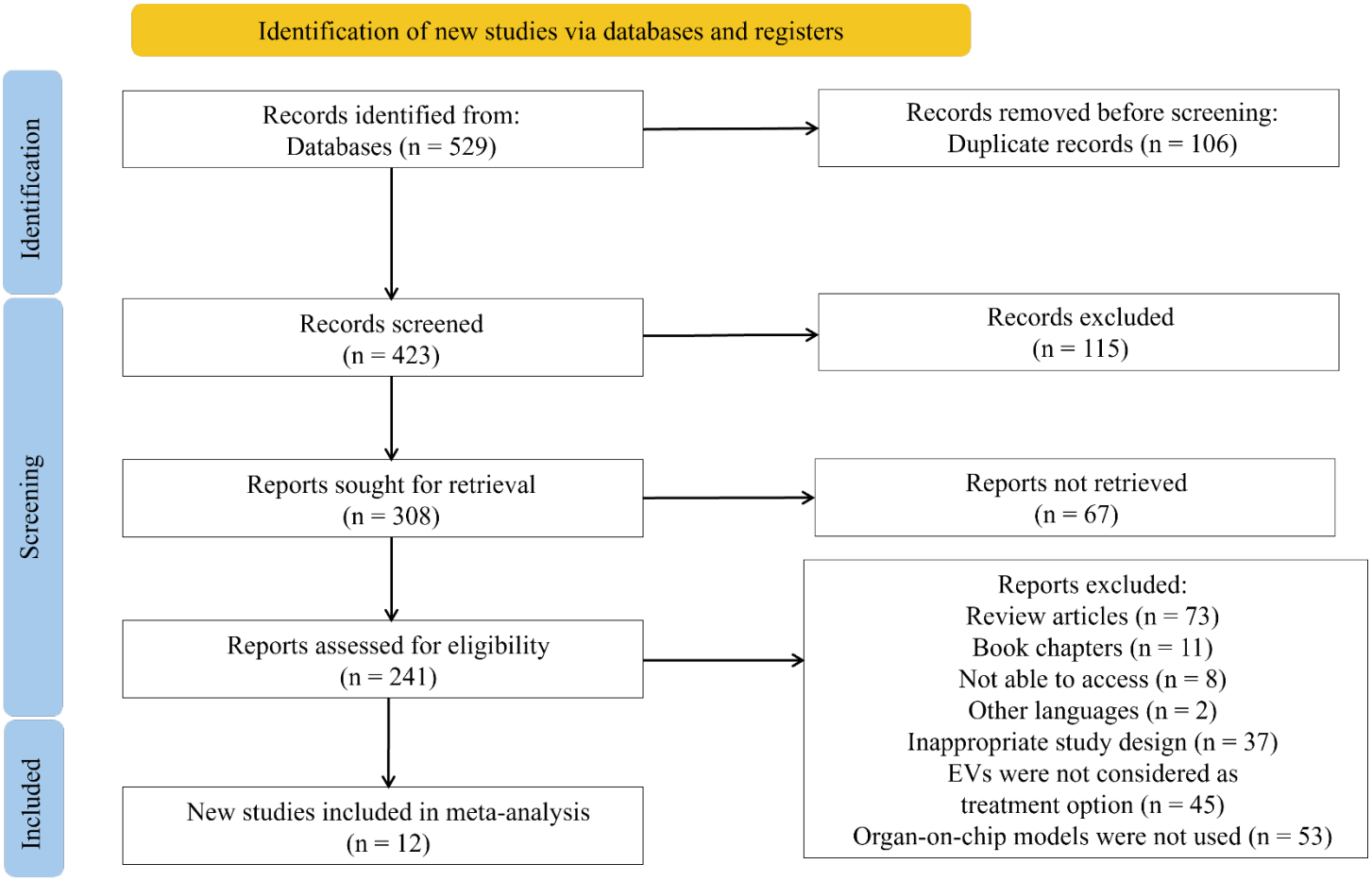


Figure 1. Representation of the Study Selection Process using PRISMA Guidelines.

3.2.Risk of bias

Figure 2 presents a risk of bias assessment for 12 studies [21–32], evaluated across eight predefined methodological domains (D1-D8). Each domain is color-coded to indicate the level of bias: green (+) for low risk, yellow (-) for unclear risk, red (X) for high risk, and blue (?) indicated insufficient or unavailable information. The eight domains assessed included study design (D1), selection criteria (D2 and D3), baseline characteristics (D4), outcome measures (D5), chip design (D6), and device fabrication (D7). However, several studies display high risk in the application

domain (D8), indicating concerns regarding the validation of therapeutic efficacy and biodistribution studies.

Despite the overall favourable quality, several methodological limitations were identified. High risk of bias was most frequently observed in the application validity domain (D8), reflecting concerns regarding the validation of therapeutic efficacy and biodistribution analyses. Specifically, the study by D. Petta et al. 2024 exhibited high risk in domains in both D1 (study design) and D8 (application validity), suggesting bias concerns. Additionally, the study by NGUYEN et al. 2022, Chatterjee et al. 2023, J. Kim et al. 2023, and He et al. 2022 show high risk in D4 (baseline characteristics) indicating potential imbalances or insufficient reporting of baseline conditions.

Incomplete reporting was also observed in a small number of studies. SM et al. 2024 did not provide sufficient information regarding domains chip design (D6) and device fabrication (D7). Similarly, Westergard et al. 2016 lacked adequate information regarding device fabrication (D7). In contrast, studies conducted by R. Hao et al. 2023 and Yixuan Shang et al. 2024 demonstrated a consistently low risk of bias across all evaluated domains, reflecting high methodological rigor and comprehensive reporting.

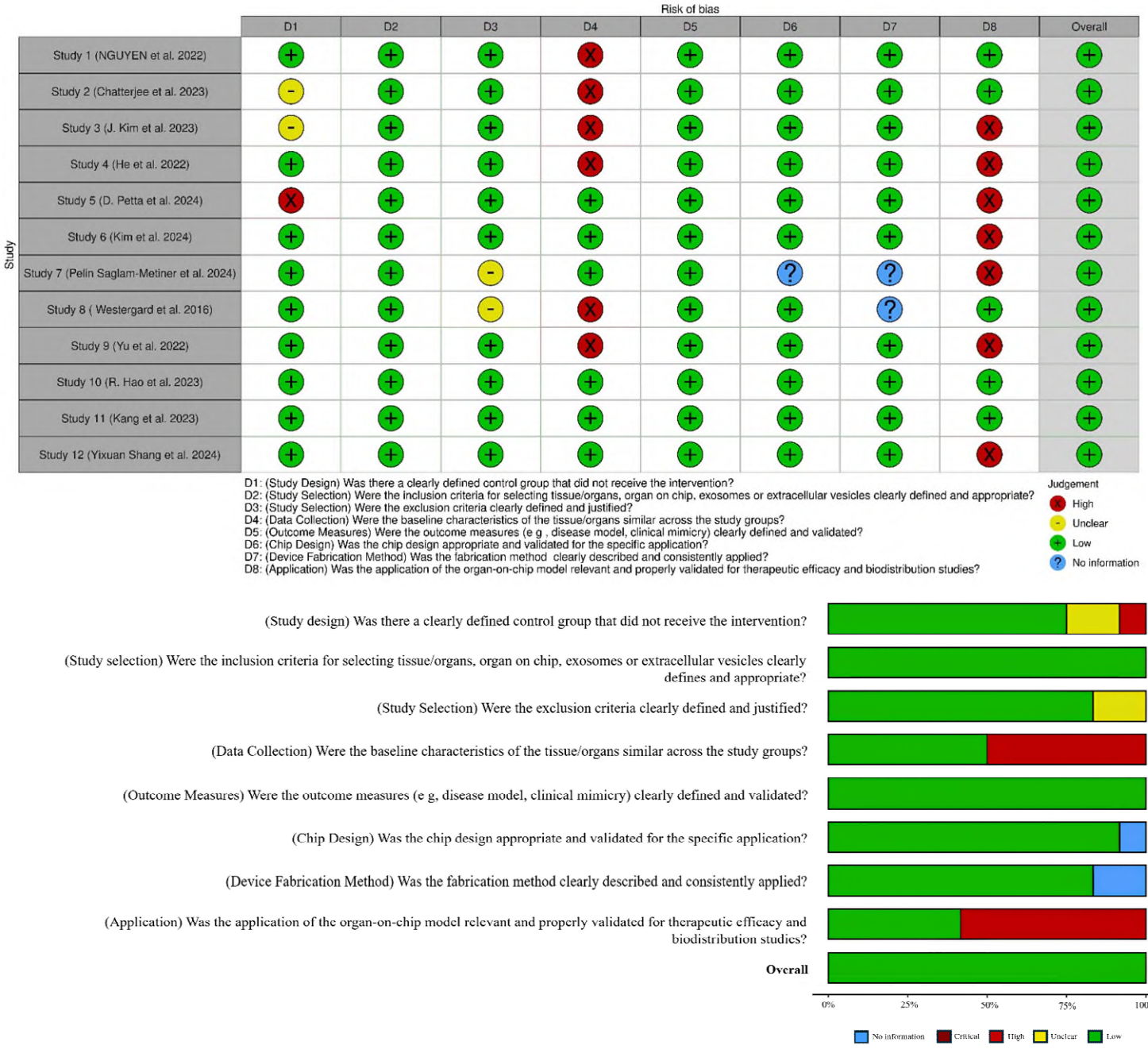


Figure 2. Risk of bias in individual studies.

3.3. Comparative Analysis of Conventional and OoC Models

Drug discovery and biomedical research have benefited greatly from the use of conventional models, for example, animals and cell culture systems [33]. In the fields of drug discovery, disease research, and risk assessment, OoC systems outperform traditional 2D as well as 3D cell culture approaches. In 2D cultures, cells grow as monolayers on a flat surface, whereas in 3D cultures, cells typically grow within a scaffold or gel-like matrix, better mimicking the native tissue environment [34]. While 2D models provide valuable data at relatively low cost, they fail to fully recapitulate the complex pathophysiology observed in patients, necessitating the application of computer modelling, systems biology, and advanced computational approaches to predict in vivo drug responses. Although 3D culture models better reproduce biomimetic tissue architecture and physiological barriers than conventional 2D monolayer cultures, they remain limited in their ability to replicate the dynamic mechanical, biochemical, and multicellular interactions present in living organs Nonetheless, OoCs can overcome the limitations of 3D culture models in mimicking clinically pertinent condition presentations that include various tissue types and intricate physiological activity at the organ's level [35, 36].

OoCs also enable for the modulation of fluid movements, simulating a wide spectrum of dynamic mechanical stimuli, including those induced by physiological circulation (e.g., interstitial flow and blood circulation), which cannot be which cannot be readily reproduced using conventional 3D culture models. This capability enables more accurate and physiologically relevant assessments of complex drug responses in biological systems. In addition, OoCs have distinct advantages over conventional animal studies. Although animal studies can reproduce physiological complexity at the whole-organism level, their translational relevance to humans remains limited due to significant interspecies differences in important disease pathways and gene expression patterns [37]. Furthermore, recent systematic reviews have revealed limited concordance between findings from animal studies and human clinical outcomes, highlighting the need for novel methods that more accurately simulate human-relevant physiological conditions [38–40].

3.4. Meta-analysis

3.1.1 Qualitative analysis

I. *Characteristics of Included Studies*

The meta-analysis provides a thorough overview of the included studies, summarizing papers that were included, including information on the different OoC models, disease models, authorship, publication year, geographic location, exosome EXOs sources, and isolation techniques used. The kidney-on-chip (KOC) models were employed to investigated renal injury, with two key studies conducted by NGUYEN et al. [31] from Germany and Chatterjee et al. [29] from the USA examining the therapeutic potential of EVs in biomimetic renal microenvironments. NGUYEN et al. utilized human bone marrow-derived mesenchymal stem cells (hBMSCs) and employed differential ultracentrifugation for EXOs isolation. Chatterjee et al. focused on human renal proximal tubule epithelial cells (hRPTEpCs) and utilized continuous density gradient ultracentrifugation combined with size exclusion chromatography for EXOs isolation. Skin-on-chip models explored wound healing and arterial restenosis wounds. J. Kim et al. [21] from the Republic of Korea used MSCs with a combination of centrifugation and filtration for EXOs isolation. He et al. [26] from China investigated the use of human induced pluripotent stem cells (hiPSCs) for arterial restenosis wound healing, using ultracentrifugation for EXOs isolation. In the bone-on-chip models focusing on osteoarthritis, D. Petta et al. [32] from Switzerland used both hBMSCs and human adipose stem cells (hASCs), although the specific isolation methods were not available. Brain-on-chip models included studies on AD and neuroinflammation, with Kim et al. [24] from Korea using hiPSCs and a combination of centrifugation and filtration for EXOs isolation. SM et al. [22] from Turkey utilized bone marrow-derived stem cells (BMSCs) isolated using a kit. For ALS, Westergard et al. [23] from the USA employed neuron cells (NSC34 cells) with EXOs isolation facilitated by a kit. Eye-on-chip models for corneal epithelial wound healing included studies by Yu et al. [27] and R. Hao et al. [25] from China. Yu et al. used BMSCs isolated via differential ultracentrifugation, while R. Hao et al. utilized human fetal bone marrow (hFeBMSCs) with ultracentrifugation for EXOs isolation. Liver-on-chip models investigated liver disease, with Kang et al. [30] from the Republic of Korea focusing on *L. paracasei* HY7014 and *L. casei* HY7207, employing sequential centrifugation and filtration for EXO isolation. Heart-on-chip models studying CF included research by Shang et al. [28] from China, which utilized rat cardiac cells and ultracentrifugation for EXOs isolation.

Table 1: Characteristics of Included Studies in the Meta-Analysis.

Organ-on-chip model	Disease model	Author	Year of publication	Geographical area	EXOs Source	Isolation methods	Reference
Kidney	Renal injury	NGUYEN et al.	2022	Germany	Human Bone Marrow-derived Mesenchymal stem cells	Differential ultracentrifugation	[31]
	Renal injury	Chatterjee et al.	2023	USA	Human Renal Proximal Tubule Epithelial Cells	Continuous Density Gradient Ultracentrifugation and Size Exclusion Chromatography	[29]
Skin	Wound Healing	J. Kim et al.	2023	Republic of Korea	Mesenchymal Stem Cells	Centrifugation+ filtration	[21]
	Arterial Restenosis Wound	He et al.	2022	China	Human-induced Pluripotent Stem Cells	Ultracentrifugation	[26]
Bone	Osteoarthritis	D. Petta et al.	2024	Switzerland	Human Bone Marrow Stem Cells & Human Adipose Stem Cells	Ultracentrifugation	[32]
Brain	Alzheimer's Disease	Kim et al.	2024	Republic of Korea	Human induced pluripotent stem cells	Centrifugation+ filtration	[24]
	Neuroinflammation model	SM et al	2024	Turkey	Bone marrow-derived Stem Cells	Kit	[22]
	Amyotrophic lateral sclerosis	Westergard et al	2016	USA	Neuron Cells	Kit	[23]
Eye	Corneal epithelial wound healing	Yu et al	2022	China	Bone marrow-derived Stem Cells	Differential ultracentrifugation	[27]
	Corneal epithelial	R. Hao et al.	2023	China	Human fetal bone marrow	Ultracentrifuged	[25]

	wound healing						
Liver	Liver Disease	Kang et al.	2023	Republic of Korea	*L. paracasei* HY7014 & *L. casei* HY7207	Sequential centrifugation + filtration	[30]
Heart	Cardiac Fibrosis	Shang et al.	2024	China	Rat Cardiac Cells	Ultracentrifuged	[28]

II. Characteristics of EXOs Treatment Studies in OoC *Models*

Conventional 2D cell culture techniques have several disadvantages, including their inability to recapitulate the complex replicate the in vivo tissue microenvironment, relatively high operational costs, time-intensive protocols, and high experimental failure rates. These shortcomings have driven the development of advanced 3D cell culture systems Among these, microfluidic OoC technology has emerged as a promising approach, offering physiologically relevant models with broad applications in drug discovery, disease modelling, tissue engineering, and biomedical research [41].

In our study, 12 articles [21–32] met the predefined inclusion criteria and were included in the meta-analysis. The meta-analysis focuses on the application of various OoC systems for treating degenerative diseases with EVs. The included disease models comprised the kidney, skin, bone, brain, eye, liver, and heart. Precise regulation of both the external and internal cellular microenvironment is essential for OoC systems. When combined with micromachining and cell biology, have the ability to perfectly imitate normal human or veterinary physiological properties. When designing an OoC, several factors must be considered, including dynamic mechanical stress, fluid shear stress, level gradients, and cell patterning [42]. Depending on their design and application, OoC systems can be classified as either single-organ or multi-organ platforms (Tables 1 and 2 summarize the various chip models).

A KOC model was used to study renal disease. Nguyen et al. [31] combined human renal and liver organoids in an Multi-organ on chip (MoC) platform to evaluate therapeutic effects and biodistribution, using 2D-cultured renal tubuloids and human liver organoids co-cultured with Matrigel as ECM. Chatterjee et al. [29] used a microfluidic KOC model to investigate the impact of circulating EVs from heart failure and cardiorenal syndrome patients on renal injury, focusing on the short RNA cargo of EVs and its effects gene expression and renal injury pathways. They

used a single OoC with two microchannels, co-culturing human renal microvascular endothelial cells and hRPTEpCs on Matrigel as the extracellular matrix (ECM), with Polydimethylsiloxane (PDMS) as the chip material.

Skin-on-chip models have been employed to study wound healing and arterial restenosis. J. Kim et al. [21] developed photodegradable hydrogel microparticles (PHMPs) for the on-demand administration of therapeutic EVs. Using a droplet microfluidic device, PHMPs were created to encapsulate EVs, enabling controlled and sustained release following ultraviolet irradiation. The authors used a single-organ OoC platform with two microchannels, incorporating monodispersed photodegradable hydrogels with gelatin as the ECM and silicon and PDMS as the chip materials. He et al. [26] reported that EVs derived from hiPSC-derived endothelial cells (hiPSC-ECs) can prevent arterial restenosis by promoting autophagy in endothelial cells. This therapeutic effect is largely mediated by miR-126, which enhances endothelial cell function and survival. These findings support the development of EV-based therapies for vascular diseases by exploiting the regenerative potential of hiPSC-ECs while avoiding the risks of direct stem cell transplantation. The authors used a single-OoC platform with one microchannel, culturing HUVECs with hiPSC-EC-derived EVs, using Matrigel as the ECM and glass, silicon, and PDMS as the chip materials.

For Osteoarthritis (OA), a joint-on-chip model was employed. D. Petta et al. [32] developed a microfluidic OA joint-on-a-chip model, to assess the efficacy of Ortho biologics in a personalized human setting. This model includes cartilage and synovial compartments, enabling the creation of an OA microenvironment to assess the therapeutic effects of MSC-derived EVs using patient-derived cells and synovial fluid. The authors used a single-organ OoC platform with two microchannels, co-culturing articular chondrocytes and synovial fibroblasts within a fibrin-, collagen-, and hyaluronic acid-based hydrogel, using synovial fluid (SynFluid) as the ECM and glass, resin photopolymer, and PDMS as the chip materials.

For AD, Neuroinflammation, and ALS, a neuro-on-chip (NOC) model has been employed. Kim et al. [24] demonstrated that gut microbiota-derived metabolites and EVs significantly influence neural differentiation, growth, and synaptic plasticity. The gut-brain axis chip acts as a great platform for investigating these interactions and highlights the potential of microbial products as therapeutic agents for neurodegenerative diseases. The authors used a multi-OoC with two microchannels, in which Human intestinal epithelial cells (Caco-2) co-cultured with minimum

essential medium and *Lactobacillus casei* and *Lactobacillus paracasei,* using geltrex and polyethylene glycol (PEG) as the ECM and silicon and PDMS material for generation of chip. SM et al. [22] develop a NOC model using hiPSCs differentiated into neurons, astrocytes, oligodendrocytes, and microglia to evaluate the therapeutic potential of MSC-derived EVs for neuroinflammation. The model replicated TNF-α-induced neuroinflammation and assessed the effects of EVs on reducing inflammatory markers and improving cell survival and function. The authors used a single-organ OoC platform with two microchannels, co-culturing human cerebral microvascular endothelial cells with collagen type I, using Matrigel as the ECM and PDMS as the chip material. Westergard et al. [23] investigate the cell-to-cell transmission of dipeptide repeat proteins (DPRs) associated with C9orf72 gene mutation, the most common genetic cause of ALS and frontotemporal dementia (FTD). Using multiple cell culture systems, particularly patient-derived motor cells, the researchers demonstrate that DPRs can spread between cells via EXOs-dependent or independent pathways, potentially contributing to the advancement of these neurodegenerative disorders. They used a single-organ OoC platform with three microchannels, culturing primary rat cortical neurons in neuronal growth medium with poly-D-lysine as the ECM.

For corneal epithelial wound healing, a cornea-on-chip (CoC) model was employed. Yu et al. [27] and R. Hao et al. [25] developed a human CoC model that mimics the structural organisation and biological characteristics of the human corneal membrane. The chip model includes human corneal cells and a porous outer membrane, providing a dynamic, physiologically relevant platform for studying corneal diseases and testing therapeutics. Yu et al. [27] used a single-organ OoC platform with two microchannels containing human corneal cells, fabricated using glass, silicon, and PDMS. R. Hao et al. [25] also used a single-organ OoC platform with two microchannels, incorporating hFeBMSC by using ORICell medium as ECM, and glass, silicon, and PDMS as the chip materials.

For Liver function metastasis, a liver-on-chip model was employed. Kang et al. [30] explored a gut-liver axis chip to investigate the effects of gut microbiome-derived compounds and EVs on hepatocyte function. The chip models the interface between the gut and the liver, allowing researchers to investigate microbiota-host interactions and their implications for liver function. The authors used a multi OoC platform with two microchannels, co-culturing Caco-2 cells with HepG2 cells, using an elastomer and PEG as the ECM, with silicon and PDMS as the chip

materials. Applied multi-OoC with two channellings, Caco-2 co-cultured with Hepg2 cells by using Elastomer, PEG as ECM, and Silicon and PDMS material for generation of chip.

For CF, a heart-on-chip model was used. Shang et al. [28] developed a biomimetic cardiac-fibrosis-on-chip model employing Janus structural colour film (SCF) to investigate the therapeutic effects of mesenchymal stem cell-derived EXOs s (MSC-EXOs) on CF. The authors used a single-organ OoC platform with two microchannels, co-culturing cardiomyocytes (CMs) and cardiac fibroblasts (CFBs) within a GelMA-based ECM, with PDMS and poly(methyl methacrylate) (PMMA) serving as the chip materials (Table 2).

Table 2: Summary of different degenerative organ-on-chips models used for biomedicine.

Types of Tissue/ organs	Disease model	Classification	Channels	Tissue/organ sources	Co-cultured cells	Extracellular matrix (ECM)	Material used	Clinical mimicry	Chip design	Device fabrication method	Application	Reference
Kidney	Renal injury	Multi-organ	2 channels	2D-cultured renal tubuloids	Human liver organoid	Matrigel	NA	Biodistribution and therapeutic efficacy and identification of potential side effects	Tissue HUMIMIC Chip2 microfluidic model	NA	Therapeutic efficacy and biodistribution	[31]
	Renal injury	Single	2 channels	Human Renal Microvascular Endothelial Cell	Human Renal Proximal Tubule Epithelial Cell	Matrigel	PDMS	Cardiorenal syndrome (CRS) elicits adverse transcriptional and phenotypic responses	Each chip includes epithelial cells in the apical channel and endothelial cells in the basal channel.	NA	Extracellular vesicles from heart failure patients with cardiorenal syndrome cause renal injury by regulating TGF-β signaling and other pathways	[29]
Skin	Wound healing	Single	2 channels	NA	Monodispersed photodegradable hydrogel	Gelatin	Silicon and PDMS	EVs release kinetics	Droplet-based microfluidic chip, SU8-3035 spin-coated onto a silicon wafer exposed to UV light using a photomask	Photolithographic and soft lithographic	Enhanced retention and controlled release.	[21]
	Arterial restenosis wound	Single	1 channel	HUVECS or HiPSCS-EV	Mir-126	Max gel	Glass, Silicon and PDMS	EC migration and tube formation	PDMS layer and a glass substrate spin-coating SU8-2035 negative photoresist onto a glass wafer.	Photolithography.	Arterial stenosis is prevented by autophagy regulation.	[26]
Bone	Osteoarthritis	Single	2 channels	Articular chondrocyte and synovial fibroblast	Hydrogel with Fibrin, Collagen, and Hyaluronic acid	Synovial fluid (synflu)	Glass, resin photopolymer and PDMS	Screening	The central channel holds synovial fluid (SYNFLU), while the flanking hydrogel compartments are secured by trapezoidal posts to prevent leakage, host articular	3D printed using a digital light processing (DLP) printer	A personalized osteoarthritic joint-on-a-chip model to screen the efficacy of mesenchymal stem cells in treating osteoarthritis by replicating the	[32]

									chondrocytes and synovial fibroblasts. Two external channels are for the culture medium.		joint microenvironment and monitoring individual responses to treatment.	
Brain	Alzheimer's Disease (AD)	Multi-organ	2 channels	Human intestinal epithelial cells (Caco-2)	Minimum Essential Medium and *Lactobacillus casei* and *Lactobacillus plantarum*	Geltrex and polyethylene glycol (PEG)	Silicon and PDMS	Studying the interaction of microbial-derived metabolites and EXOs s	The first layer of SU-8 5 (5 μm in thickness) was patterned on a 4-inch silicon wafer to create bridge channels and a second layer of SU-8 100 (150 μm in thickness) was patterned to align the first layer to create two main channels.	Two-step lithography	Gut microbiota-derived metabolites and extracellular vesicles enhance neural differentiation and maturation and protect against Alzheimer's disease, suggesting potential therapeutic strategies for neurodegenerative disorders.	[24]
	Neuroinflammation model	Single	2 channels	Human cerebral microvascular endothelial cells	Collagen Type I	Matrigel	Polydimethylsiloxane (PDMS)	Therapeutic properties against neuroinflammation for humanized biomimetic neural-tissue construction	NA	NA	TNF-α induced inflammation in humanized neural tissue-on-chip formed by differentiated neural and glial cells can be alleviated by BMSC-EVS.	[22]
	Amyotrophic lateral sclerosis (ALS)	Single	3 channels	Primary rat cortical neurons	NA	Neuronal growth medium, poly D lysine	NA	Toxicity and cell-to-cell transmission.	Two-chamber microfluidic devices	NA	Dipeptide repeat proteins (DPRS) linked to c9orf72-ALS/FTD are transmitted between cells via EXOs -dependent and EXOs -	[23]

											independent pathways.	
Eye	Corneal Epithelial wound healing	Single	2 channels	Human corneal epitheliu m	NA	NA	Glass, Silicon and PDMS	Therapeutic	Silicon wafers and fused silica glass wafers (Ø4 in., 500 µm in thickness)	Soft lithography	Significantly accelerate corneal epithelial wound healing by promoting cell migration and reducing inflammation.	[27]
	Corneal Epithelial wound healing	Single	2 channels	Human fetal bone marrow	NA	ORIC ell medium	Glass, silicon and PDMS	Secretion, yield	Reusable SU-8 photoresist master was fabricated on a silicon wafer	Soft lithography	Accelerate corneal epithelial wound healing by promoting cell migration and proliferation.	[25]
Liver	Liver function	Multi-organ	2 channels	Human intestinal epithelial cells (Caco-2)	Hepg2 cells	Elastomer , polyethyle ne glycol (PEG)	Silicon and PDMS	Interactions of microbiota-derived small molecules and recipient cells.	Gut-liver axis chip by AutoCAD	Two-step lithography	Enhance hepatocyte function	[30]
Heart	Cardiac Fibrosis	Single	2 channels	Coculturi ng cardiomy ocytes (CMS) and cardiac fibroblas ts (FBS)	Cardiac Fibroblast	GelMA	PDMS monomer and poly (methyl methacryl ate) (PMMA)	Micro physiological visuals	Janus structural color film (SCF)	NA	1. It captures physiological intercellular crosstalk and enabling precise disease modeling and drug screening. 2. Superior perceptual capabilities for capturing and responding to a weak cardiac force, demonstrating synchronized structural color shifts	[28]

3.2.1 Quantitative analysis

I. Mapping Study Characteristics: EXOs Treatment in OoC Systems

The Sankey diagram illustrates a comprehensive visualization of the interconnectedness and trends of EVs within OOC research. It highlights the trends and relationships within this field by showing how various categories, such as OOC models, publication years, geographical locations, EV sources, and extraction methods, are interconnected. Each chord starts from one category and ends at another, showing the relationship and transitions between them. Additionally, the width of the chords in the Sankey plot represents the volume or frequency of the connections between different categories. A wider chord indicates a stronger or more frequent relationship between the categories it connects, whereas a narrower chord signifies a weaker or less frequent connection.

Furthermore, the rectangular nodes, represent specific entities within each category, including OoC models (bone, brain, eye, heart, kidney, liver, skin), publication years (2016, 2019, 2022, 2023, 2024), geographical locations (China, Germany, the Republic of Korea, Switzerland, Turkey, and the USA), EVs sources (BMSC: Bone marrow-derived mesenchymal stem cells; MSC: Mesenchymal stem cells; NSC34 Cells: Neuron Cells; PB: Probiotic bacteria's; RCC: Rat Cardiac Cells; hBMSCs: Human bone-marrow-derived mesenchymal stem cells; hRPTEpCs: Human Renal Proximal Tubule Epithelial Cells; hiPSCs: Human induced pluripotent stem cells; hBMSCs & hASCs: Human Bone Marrow Stem Cells & Human Adipose Stem Cells; hFBMSCs), and extraction methods (CDGU+SEC: Continuous Density Gradient Ultracentrifugation and Size Exclusion Chromatography; CF: Centrifugation+ filteration; DU: Differential Ultracentrifugation; U: Ultracentrifugation; K: Kits). The coloured links connecting these nodes visually represent the relationships and flow between categories, with their width indicating the frequency or strength of these connections. The diagram's vertical arrangement, termed steps, organizes the nodes into groups reflecting their respective categories, showcasing the sequential progression of data from OoC models to extraction methods (illustrated in **Figure 3**).

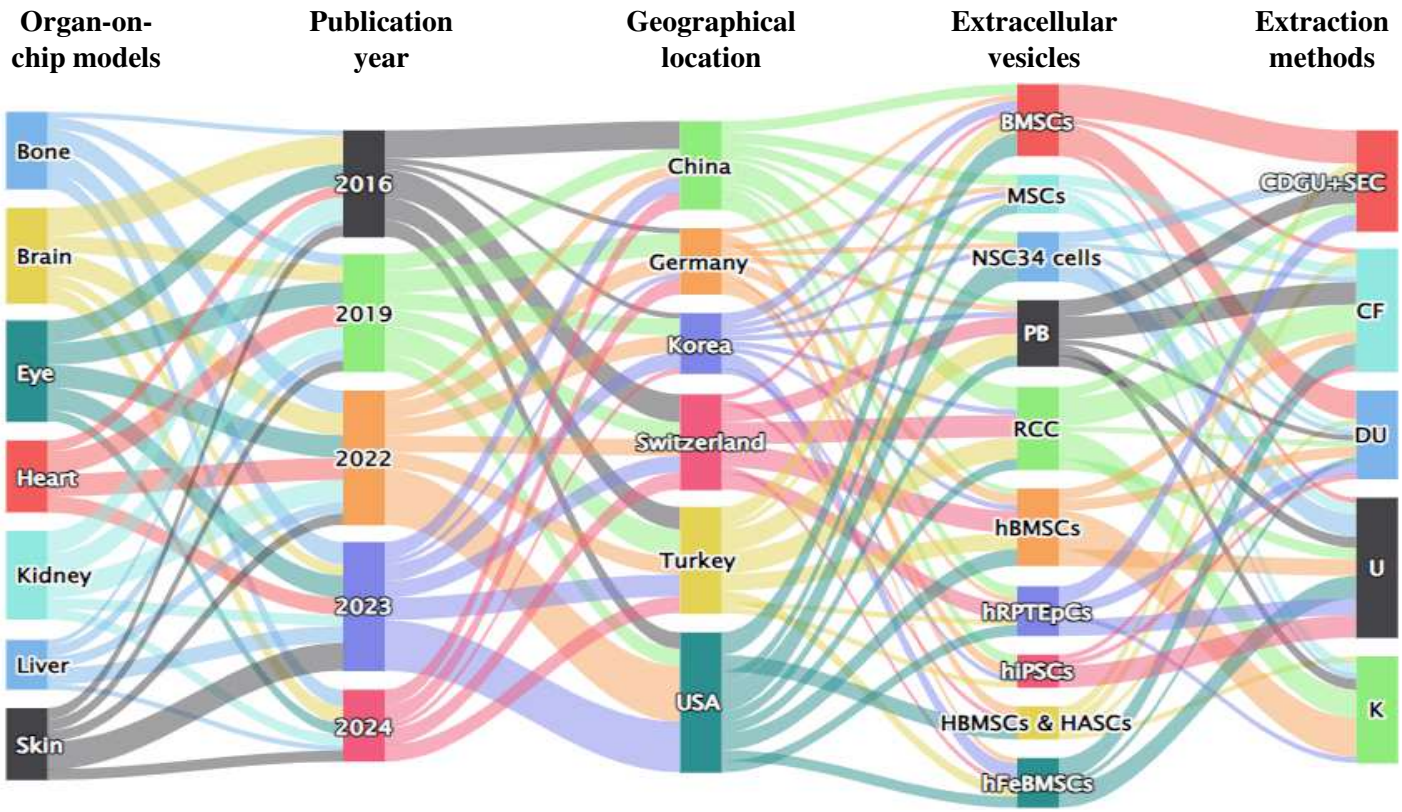


Figure 3. Sankey plot of raw extracted data to visualize interconnectedness and trends of EVs within OoC models. **Abbreviations: BMSC:** Bone marrow derived mesenchymal stem cells; **MSC:** Mesenchymal stem cells; **NSC34 Cells:** Neuron Cells; **PB:** Probiotic bacteria's; **RCC:** Rat Cardiac Cells; **hBMSCs:** Human bone-marrow derived mesenchymal stem cells; **hRPTEpCs:** Human Renal Proximal Tubule Epithelial Cells; **hiPSCs:** Human induced pluripotent stem cells; **HBMSCs & HASCs:** Human Bone Marrow Stem Cells & Human Adipose Stem Cells; **hFeBMSCs:** Human fetal bone marrow; **CDGU+SEC:** Continuous Density Gradient Ultracentrifugation and Size Exclusion Chromatography; **CF:** Centrifugation+ filteration; **DU:** Differential Ultracentrifugation; **U:** Ultracentrifugation; **K:** Kits

II. Comparative Analysis of Core Components in different OoC Systems

To further elucidate the comprehensive comparison of various OoC models and their core components, data from the included studies were organized and analyzed. Based on the core components in different OoC systems, a chord plot was genrated (as illustrated in Figure 4). The y-axis lists different OoC models, including kidney, skin, bone, brain, eye, liver, and heart, while the x-axis displays normalized values ranging from 0 to 0.7 representing the relationships between the OoC models and their core components. The core components are represented with abbreviations: single organ (SO), multi-organ (MO), one channel (OC), two channels (TWC), three channels (THC), polydimethylsiloxane (PDMS), silicon and polydimethylsiloxane (SPDMS), glass, silicon and polydimethylsiloxane, glass resin photopolymer and polydimethylsiloxane, polydimethylsiloxane and polymethyl methacrylate, soft lithography (SL), bio-3D printing (B3DP), co-cultured cells (CCC), and ECM. The chord plot (**Figure 2**) illustrates the interconnections among these components. For instance, PDMS, SPDMS, and GSPDMS exhibit strong associations with multiple OoC models, indicating their prevalent use in OoC systems. The plot also highlights the diversity of materials and fabrication techniques employed, as seen in the varied chords connecting different segments. The width of each chord is proportional to the number of studies reporting a particular combination of core components; therefore, wider chords indicate more frequently reported combinations, whereas narrower chords represent less frequently reported combinations (as depicted in **Figure 4**).

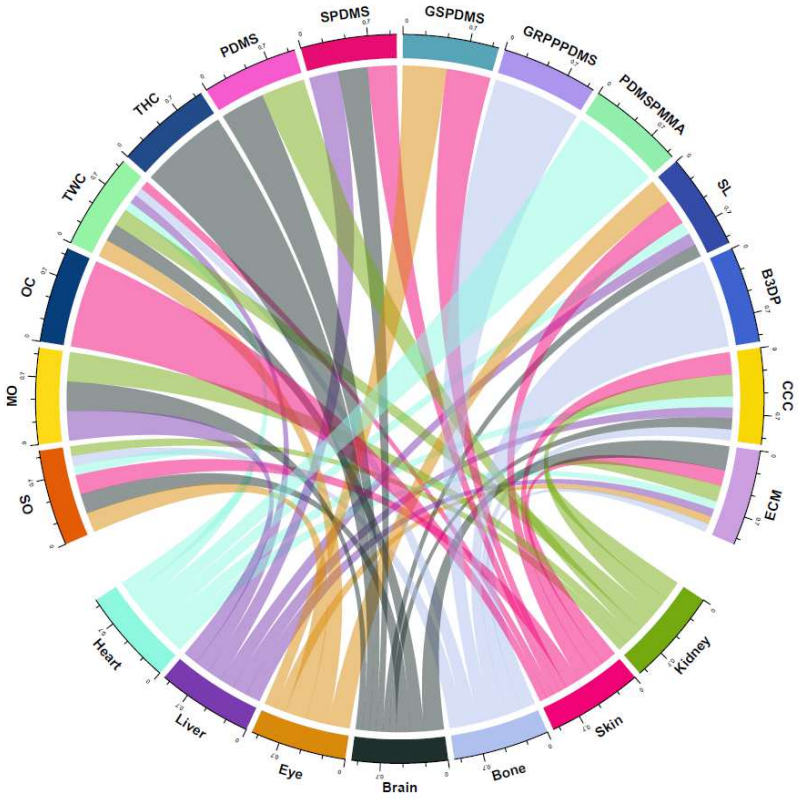


Figure 4. Chord Diagram of different characteristics of degenerative organ-on-chips models used for biomedicine. **Abbreviations: SO:** Single organ**; MO:** Multi-organ; **OC:** One channel; **TWC:** Two channel; **THC:** Three Channel; **PDMS:** Polydimethylsiloxane; **SPDMS:** Silicon + Polydimethylsiloxane; **GSPDMS:** Glass + Silicon + Polydimethylsiloxane; **GRPPDMS:** Glass+ resin photopolymer + Polydimethylsiloxane, **SL:** Soft lithography; **B3DP:** Bio 3D printing; **CCC:** Co-cultured cells; **ECM:** Extracellular matrix.

III. *Mapping Study Characteristics of different OoC Models in association with Disease Models and EXOs Sources*

The microfluidic devices provide a physiologically relevant and precise platform for investigating disease mechanisms and evaluating potential therapeutic strategies by replicating the complex structure and functions of both human and animal organs. In renal injury KOC systems [43] replicate the kidney's structure and functionality using renal tubuloids and endothelial cells, providing detailed insights into pathophysiology and therapeutic responses. In OA models, joint-on-chip technology replicates the cartilage and synovial tissue microenvironment, facilitating the investigation of MSC-EXO therapies in cartilage regeneration [32]. Moreover, the application of

OoC systems extends to neurodegenerative diseases. Models simulating the gut-brain axis or neural tissue allow researchers to investigate the effects of microbial metabolites or exosomal proteins on diseases such as AD and ALS [23]. These models substantially improve our understanding of disease pathophysiology and the evaluation of therapeutic approaches because they can include specific cell types, ECM, and multi-organ interactions. Consequently, OoC technology reduces reliance on conventional animal models, providing a more ethical and effective platform for preclinical testing.

The selection of EV sources is critical for developing effective therapies using OoC models. EVs, particularly EXOs, derived from diverse cell types such as hBMSCs, hRPTEpCs, and hiPSCs, each of which offers distinct therapeutic properties. For instance, hBMSC-derived EVs are widely used in OA models because of their regenerative potential in cartilage repair and anti-inflammatory effects. Similarly, hRPTEpC-derived EVs are utilized in renal injury models to investigate their role in renal repair and mitigation of cardiorenal syndrome. In neurodegenerative disease models, hiPSC-derived EVs are studied for their neuroprotective effects and ability to promote neural differentiation, offering potential therapeutic strategies for conditions such as AD and ALS. These EVs sources are isolated using advanced techniques such as ultracentrifugation and filtration, to ensure high purity and functionality (**Table 1**). The strategic use of specific EV sources in OoC models enables precise disease modeling and therapeutic evaluation, paving the way for personalized and effective treatment strategies for degenerative diseases.

The connections between the OoC model nodes and organoids are depicted with solid red edges, whereas green dotted edges represent the association between the OoC models and their corresponding disease models. Blue dotted edges indicates the EV sources used for the study. This visual representing highlights the diverse applications of OoC, while illustrating the integration of EV-based therapeutic strategies. Notably, multi-organ-on-a-chip (MoC) platforms provide a more physiologically relevant environment by recapitulating inter-organ communication, thereby enabling a more comprehensive understanding of disease pathogenesis and therapeutic responses. In addition, the network diagram demonstrates the versatility of OoC technologies and their potential to reduce reliance on conventional animal models, offering a more ethical, reproducible, and efficient platform for preclinical drug evaluation while accelerating the development of precision and personalized medicine (**Figure 5**).

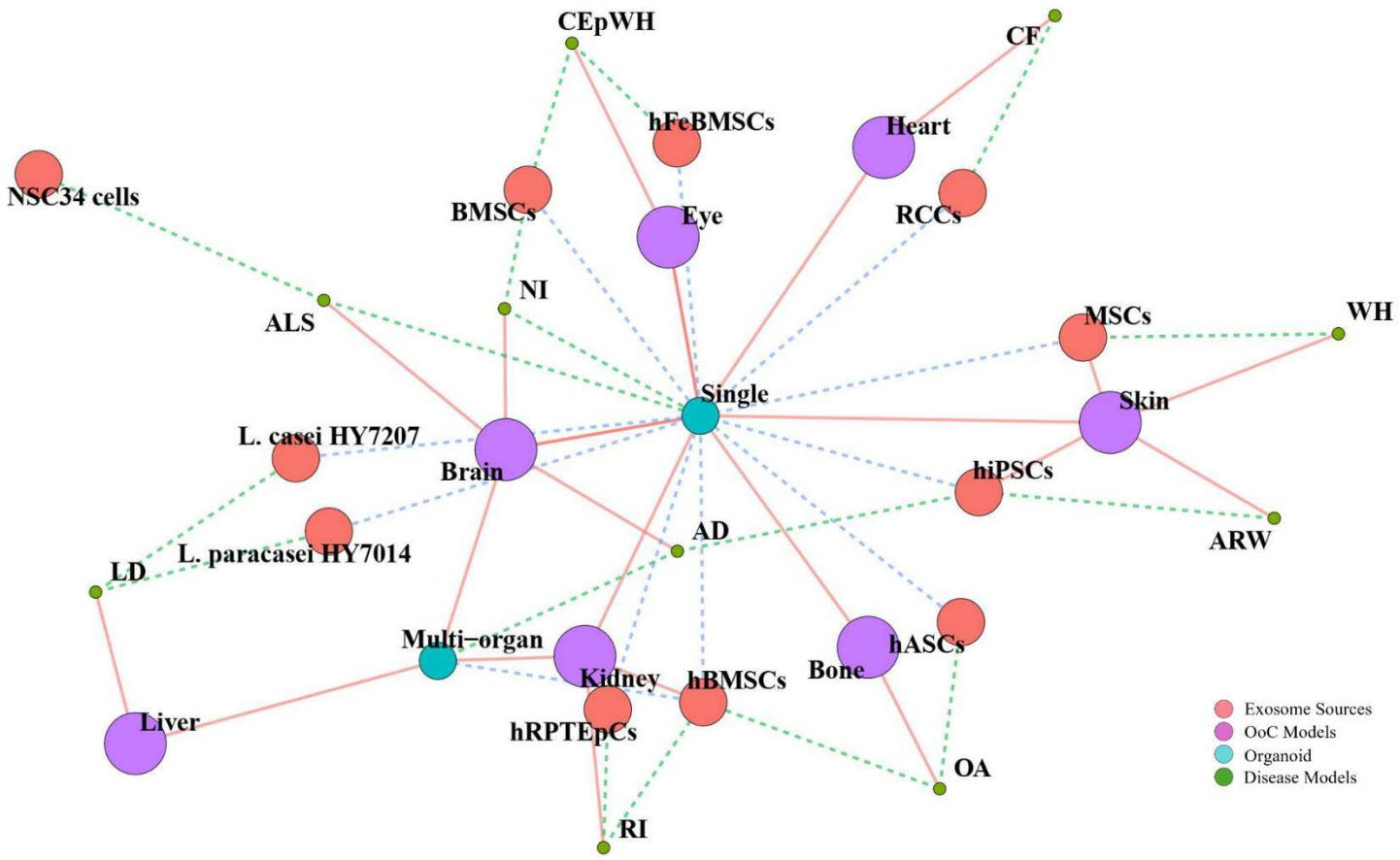


Figure 5. Network Mapping of Organ-on-Chip Models, Disease Models, and EXOs Sources. **Abbreviations: RI**: Renal Injury; **WH**: Wound Healing; **ARW**: Arterial Restenosis Wound; **OA**: Osteoarthritis; **AD**: Alzheimer's Disease; **NI**: Neuroinflammation; **ALS**: Amyotrophic Lateral Sclerosis; **CEpWH**: Corneal Epithelial Wound Healing; **LD**: Liver Disease; **CF**: Cardiac Fibrosis; **hBMSCs**: Human Bone Marrow-derived Mesenchymal Stem Cells; **hRPTEpCs**: Human Renal Proximal Tubule Epithelial Cells; **MSCs**: Mesenchymal Stem Cells; **hiPSCs**: Human Induced Pluripotent Stem Cells; **hASCs**: Human Adipose-derived Stem Cells; **BMSCs**: Bone Marrow-derived Mesenchymal Stem Cells; **NSC34 cells**: Neuron Cells; **hFeBMSCs**: Human Fetal Bone Marrow-derived Mesenchymal Stem Cells; Probiotic Bacteria: ***L. paracasei* HY7014**, ***L. casei* HY7207**; **RCCs**: Rat Cardiac Cells.

4. Discussion

The integration of EVs with OoC technology represents a transformative advancement in biomedical research [44], particularly for understanding and treating degenerative diseases. This

meta-analysis consolidates evidence from multiple of studies, underscoring the effectiveness of EVs in various OoC models. These models accurately simulate diverse disease contexts, including renal injury, OA, neurodegenerative diseases, wound healing, and CF, thereby providing a more physiologically relevant representation of human tissue environments and disease pathologies compared to traditional methods. OoC systems are engineered to replicate the microenvironment of human tissues and organs with exceptional precision, thereby overcoming the inherent limitations associated with conventional 2D and 3D cell cultures and animal models (**Figure 6**). While traditional 2D cultures are beneficial for basic research, they fail to mimic the intricate, 3D architecture and functionality of human tissues [45]. Although advanced 3D cultures provide improved physiological relevance, they still cannot fully capture the dynamic interactions and complex functions characteristic of human organs. Similarly, although animal models reproduce physiological complexity at the whole-organism level, their translational relevance is often limited because of species-specific differences in disease pathways, gene expression profiles, and overall physiology [46]. OoC models bridge these gaps by incorporating human-derived cells and more accurately simulating physiological conditions. For instance, KOC models replicate the renal microenvironment using renal tubuloids and endothelial cells, providing detailed insights into disease mechanisms and therapeutic responses that are more predictive of human outcomes. Similarly, joint-on-chip models enable precise evaluation of the regenerative potential of MSC-EXOs in cartilage and synovial tissue repair for osteoarthritis. OoC technology offers numerous advantages that enhance the physiological relevance of experimental model systems. By providing a more realistic representation of the human tissue microenvironment and disease pathophysiology, OoC models help bridgethe gap between in vitro research and clinical applications [47]. This enhanced physiological relevance is essential for understanding disease mechanisms and developing effective therapeutic strategies. In drug development, OoC models offer improved predictive power for drug efficacy and toxicity, potentially reducing the reliance on animal models [48]. By accurately reproducing human tissue responses, these systems facilitate the identification of promising therapeutic candidates, reduce the likelihood of failure during clinical trials, decrease the costs associated with late-stage drug attrition, and accelerate the drug development process.

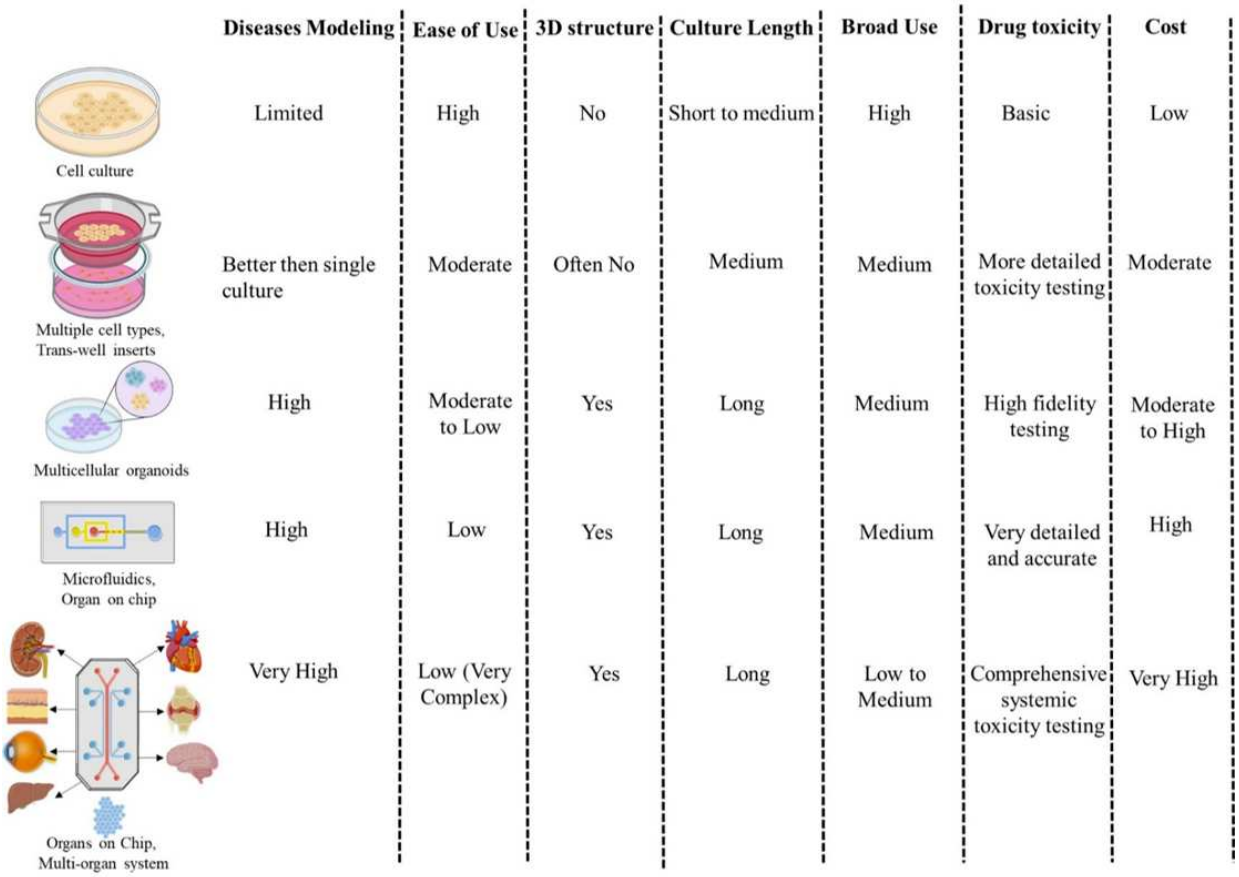


Figure 6. Overview of in-vitro model system and current uses.

EXOs have attracted significant attention because of their roles in intercellular communication and their potential as therapeutic agents. They can be derived from a variety of cell types, including hBMSCs, hRPTEpCs, MSCs, hASCs, probiotic bacteria, hFeBMSCs, NSC34 neuronal cells, RCCs, and hiPSCs. Each source provides unique therapeutic properties. For example, hBMSC-derived EXOs promote cartilage repair and reduce inflammation in osteoarthritis models, whereas hRPTEpCs derived EVs support renal repair, and attenuate cardiorenal syndrome. The studies included in this meta-analysis demonstrate the versatility and therapeutic potential of EXOs -based treatments in OoC models. For example, hBMSC-derived EVs significantly enhances cartilage regeneration in joint-on-chip models, while hiPSC-derived EVs exhibit neuroprotective effects and promote neural differentiation in neuro-on-chip models. Collectively, these findings highlight the potential of EVs as a therapeutic approach in regenerative medicine.

Additionally, the risk of bias assessment indicates that although most studies exhibit low risk of bias in domains such as study design, selection criteria, and outcome assessment, concerns

remained regarding the validation of therapeutic efficacy and biodistribution domain (D8).These findings underscores the need for standardized and rigorous methodologies to evaluate these critical aspects in OoC models. Establishing robust validation frameworks will improve the credibility,reproducibility and translational potential of OoC studies, thereby facilitating the progression of promising therapies from preclinical research to clinical application. This meta-analysis highlights the potential of integrating EVs with OoC technology to advance the development of regenerative therapies for degenerative diseases. Future studies should focus on evaluating the efficacy and safety of MSC-derived EVs in more sophisticated OoC platforms, including multi-organ systems that better replicate human physiology. Such advances will further improve our understanding of disease mechanisms and therapeutic responses while strengthening the role of OoC technology as a viable alternative to conventional animal models in preclinical research.

This systematic review and meta-analysis have certain limitations. The small number of studies, combined with heterogeneity in disease models, EV sources, isolation and characterization protocols, dosing regimens, treatment durations, and OoC platform designs, hampered direct quantitative comparisons and may have influenced the generalizability of the pooled results. Second, most of the included research used preclinical OoC models with insufficient validation versus clinical outcomes, limiting the results' direct translational usefulness. Third, inconsistencies in EV characterisation and reporting, such as particle concentration, cargo composition, administration routes, and therapeutic endpoints, could have introduced methodological variability despite the use of predetermined inclusion criteria. Furthermore, the lack of defined fabrication processes and performance indicators for OoC platforms makes cross-study comparisons difficult and emphasizes the need for better methodological harmonization. Finally, the literature search was limited to English-language publications indexed in the MEDLINE database, potentially excluding relevant studies and introducing language and database selection bias. Future research including larger datasets, standardized EV and OoC protocols, multi-organ chip platforms, and clinical validation studies will be critical for strengthening the evidence basis and facilitating the implementation of EV-based treatments into clinical practice.

5. Future perspective

One of the most important prospects in this discipline is the use of OoC technology to diminish and eventually eliminate the inclusion of animal models in biomedical research. OoC systems are a desirable substitute for animal experiments due to the ethical issues and translational constraints they raise. With their increased ability to replicate human organ functioning and disease states, these advanced models offer a more applicable platform for assessing the effectiveness of EXOs - based treatments. The use of patient-specific cells in OoC models will enable customized treatment. Researchers can construct customized disease models that accurately reflect the distinct genetic and environmental elements driving the evolution of disease by utilizing cells taken from specific patients. This approach allows for the testing of personalized therapeutic strategies, improving treatment efficacy and reducing adverse effects.

Additionally, the future direction of OoC technology could significantly impact veterinary medicine by providing more accurate models for animal health, enabling the development of advanced treatments for various veterinary diseases. Promoting collaborative research and data sharing among academic, industrial, and regulatory stakeholders will accelerate the development and adoption of OoC technology. Establishing open-access databases and repositories for OoC model data will facilitate the dissemination of knowledge and best practices, driving innovation in the field.

6. Conclusion

This meta-analysis illustrates whether OoC technology and EVs can significantly change the fields of regenerative medicine and animal testing. Compared to traditional animal models, OoC models provide a more efficient and ethical means of researching disease processes and testing treatment approaches. The analysis's conclusions are in favour of OoC systems ongoing development and use in preclinical research, especially in the context of degenerative disorders.

Availability of data and material: The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of Interest: The authors declare no conflict of interest.

Authors' contributions: Conceptualization, H., G. and C.-M.C.; methodology, H., G., and R.M; software, H., G., and R.M; validation, C.-M.C.; formal analysis, H. and G.; investigation, C.-M.C. resources, C.-M.C.; data curation, H. and G., writing—original draft preparation H., G., and R.M.; writing—review and editing, H., G., and R.M.; visualization, C.-M.C.; supervision, C.-M.C.; project administration, C.-M.C.

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