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Organ-on-a-Chip Microfluidic Systems for Tracking Exosomal Dynamic Communication

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ABSTRACT

Organ-on-a-chip microfluid systems (OCMS) are miniaturized three-dimension models of human tissue and organ, designed to recapitulate the crucial physiological and biological parameters of their corresponding *in vivo* parts. They have emerged as a powerful multifunctional tool for various applications such as personalized medicine, drug screening, due to its ability to show biomimetic composition, designs, and functions. Recently, OCMS have been employed to model and decode inter-organ communication via exosomes. Exosomes are biological nanovesicles with approximately 30-200 nm diameter, released from most of the cell types and participate in various cellular functions via intracellular communication and by carrying different cargoes including protein, and nucleic acids. Under pathological conditions such as cancer, the release of exosomes enhances tremendously, which are either fused or internalized by the recipient cells to elicit specific biological responses. The research pertaining to the exosomal communication has employed different methods for characterizing their release by the donor cells and uptake by the recipient cells, such as nano tracking analyzer, protein quantification, transmission electron microscopy (TEM), scanning EM (SEM), and immunogold-EM, exosome labeling kits, microbead-based flow cytometry. However, the research associated with the regulation of exosomal release and uptake has been impeded by the dearth of advanced techniques for capturing dynamics of exosomes. Here in, we discuss the advances in biosensing for tracking exosomal dynamic communication in OCMS, which will open new avenues of exosomal research using microfluidic engineering for modeling intracellular communication in OCMS.

1. Introduction

An organ-on-a-chip microfluid systems (OCMS) are three-dimensional (3D) microfluidic cell culture, composed of multi-channel systems in micrometre size range equipped with constantly perfused chambers, which can mimic the physiological roles of tissues and organs. The OCMS can efficiently achieve the minimal functional units for recapitulating tissue- or organ- level functionality [1]. Traditionally, the cell culture in the two-dimensional (2D) environment, which was initially found to effective due to the significant contribution in various aspects of biomedical research including *in vitro* drug screening, and for the study of pharmacokinetic, pharmacodynamics, and for deciphering novel pharmacological mechanisms [2–4]. However, the inability of 2D systems to sustain tissue-specific, distinguish functions of various cell types and precise evaluation of drug activities like that in native *in vivo* condition have been some of the major hurdles [5], which fetched the scientists' attention towards the potential of complex models encompassing multiple cell types with diverse cell pattern, and eventually caused a paradigm shift towards 3D cell culture models for

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depicting the *in situ* spatial complexity of living tissues as that in *in vivo* situation [6]. The requirements of optimal 3D culture vary depending on cell types and the distinctive features of cells in 3D cultures. Interestingly, 3D cultures systems have been found to serve as the excellent models for various studies including cytotoxicity, cell growth, survival, apoptosis, genotoxicity, drug discovery, and studies related to protein expressions, changes during differentiation, and developmental stages [7]. OCMS has been utilized for modelling of various human body organs including brain [8], lung [9], heart [10], and liver [11] by culturing the organ-specific cells either in the form of cell line or organoids, primary cell culture (Fig. 1A). The contribution of matrices and scaffolds in 3D cultures have been found to be exemplary, such as hydrogels [12], collagen [13], fibronectin [1], gelatin [14], laminin, agarose, and vitronectin [15]. Various forms can be achieved by the extracellular metrices (ECM) including random distribution of cells in the ECM or the formation of self-assembled aggregated microstructures, also referred as 3D organoids [16–18]. Despite numerous advantages of 3D culture systems over 2D culture systems, they also have drawbacks, for example variable dimension and morphology of organoids, and

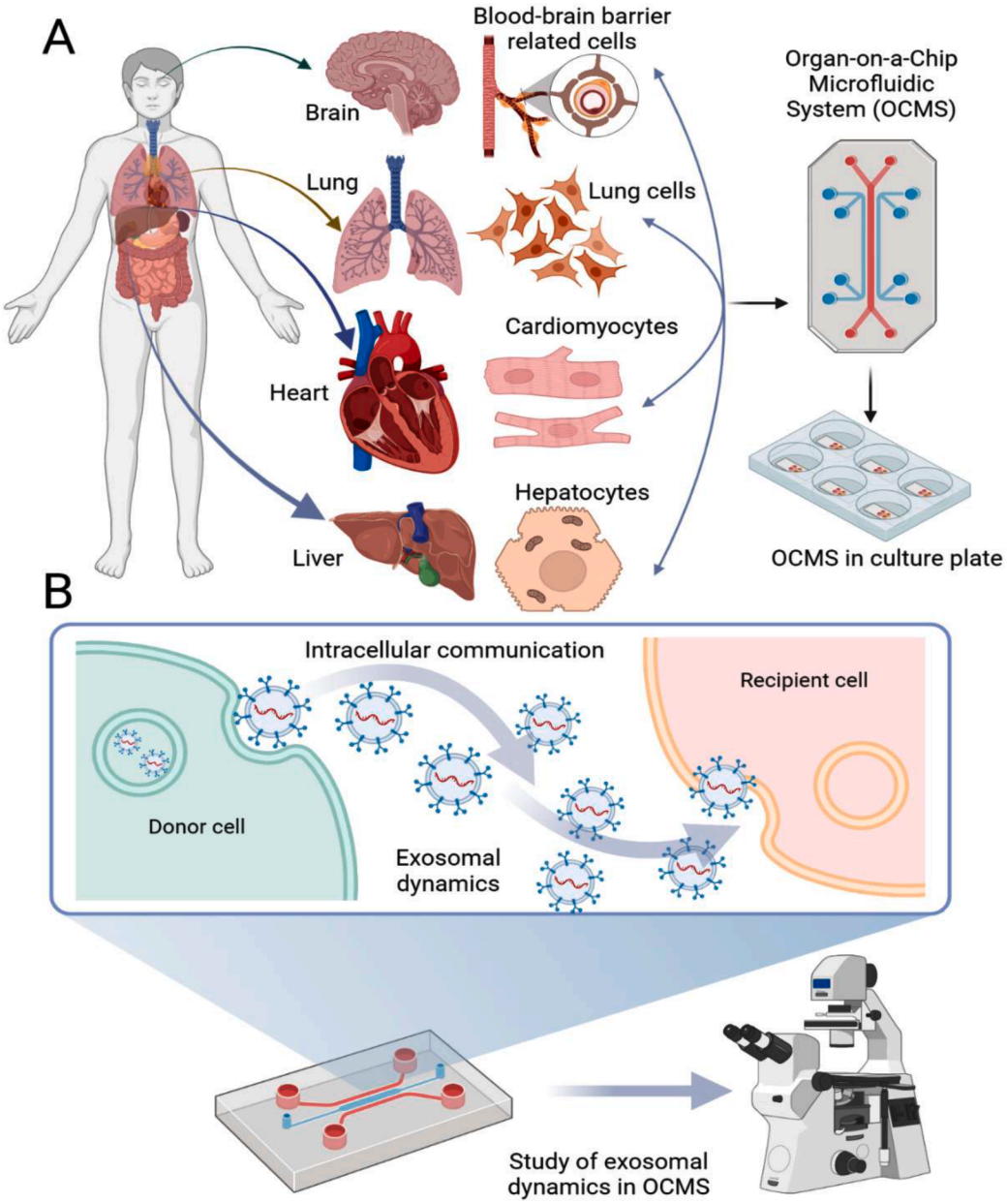

Fig. 1. Organ-on-a-chip systems (OCMS) for modelling various human body organ systems and exosomal dynamics for intracellular communication. (A) Modelling of various human body organs including brain, lung, heart, and liver into OCMS device by culturing the relevant cells specific or involved in the organ microenvironment. **(B)** Schematic representation showing the study of exosomal dynamics in OCMS during intracellular communication. (Created with BioRender.com).

difficulty in sustaining the stable position of cells in the 3D organoid microstructures for extended study. In addition, it is also troublesome to collect samples or harvest cellular constituents for the biochemical or genomic analysis. The difficulty to introduce tissue-tissue interfaces with multiscale design further push the system away from the real *in vivo* scenario, for example it is vital to include an interface between vascular endothelium and the neighbouring tissues including parenchymal cells for achieving an artificial system with function mimicking most of the organs. Cells are also deprived of the exposure to the physiological stimuli such as fluid shear stress, and compression, which plays direct or indirect role in the development and function of various organs in healthy and pathological condition [19,20]. The dearth of fluid flow also impedes the study of direct effect of interaction between circulating biofluids including blood and the stromal immune cells such as macrophage.

Interestingly, the paradigm shift in the multidimensional application of OCMS has opened a great avenue for latest biomedical studies and discoveries. This can be attributed to their numerous advantages, for example the two or more microchannels can be linked via permeable membranes, with different types of cells cultured on their either side. This can recreate the tissues demarcated by an interface such as the blood-brain barrier composed of endothelial cells, astrocytes, and pericytes [21,22]. Notably, these OCMS can integrate pertinent physiological stimuli, and allows the convenient assessment of organ-specific responses against cell-cell communication, and monitoring of pharmacodynamics of drug or toxins. In addition, microfluidic systems offer a number of advantages when compared to other in-vitro models. Firstly, they can model complex 3D microenvironments, like those found in vivo, enabling a greater degree of accuracy. Secondly, they can be used to precisely control the flow of fluids, allowing for a more accurate simulation of physiological conditions. Thirdly, they are highly miniaturized and can be used to screen a large number of samples in a much smaller space. Fourthly, the use of microfluidic systems can greatly reduce the cost of experiments compared to other in-vitro models. Finally, they can be used to monitor and analyse cellular behaviour in real-time, providing valuable insights into biological processes. Recently, OCMS have also been proven to be a powerful tool to track the release of exosomes in real-time, making them useful for various studies related to inter-organ communication via exosomes[23]. Exosomes are tiny nanovesicles with diameter around 30-200 nm, secreted in the extracellular space of most of the cell type [23,24]. Interestingly, the release of exosomes is dramatically increased under different pathological conditions such as cancer and neurodegenerative diseases and play significant role in intracellular communication leading to further disease pathogenesis by delivering various cargoes such as protein and nucleic acids to the recipient cells [25,26]. Several techniques have been utilized by researchers to investigate the potential role of exosomes under pathological conditions by tracking the release and uptake of exosomes [27,28]. Nevertheless, the scarcity of advanced techniques has led to the impediment of the precise monitoring of exosome dynamics. This article discusses the recent development in the OCMS for the biosensing of exosomal dynamics (Fig. 1B) and addresses possible pertinent questions in the field of exosome associated studies utilizing novel OCMS.

2. Methods for the fabrication of OCMS devices

Microfluidics have drawn a substantial interest of researchers in various fields of biomedical studies since their inception. This can be credited to the reduced size, weight, energy consumption, augmented sensitivity, and significantly low cost of batch production at the industrial scale. Interestingly, the process for developing organ-on-a-chip has extensively employed the fabrication of microfluids, yielding into an OCMS device [29]. In general, OCMS can be developed either by bottom-up or by top-down approach. In the *bottom-up approach*, the consideration for microstructures is focussed, followed by the seeding of cells into a microenvironment such as hydrogel for developing the vascular networks. In the *top-down approach*, first the microstructures are created, followed by seeding of cells. In addition, a hybrid approach is also employed, which include both bottom-up as well as top-down approaches [30]. Methods such as soft lithography, hot embossing, injection moulding, 3D printing have substantially popular for the fabrication of OCMS devices.

Soft lithography: In this technique, wide variety of materials are employed like elastomers such as polydimethylsiloxane (PDMS) [31]. Various approaches are utilized for the patterning of designs such as replica molding [32], capillary molding [33], solvent-assisted micro-molding [34], phase-shifting edge lithography [35], microcontact printing [36], and micro-transfer molding [33]. This technique offers an advantage of fast prototyping and incurs low cost. Sot lithography has been utilized for the fabrication of OCMS to mimic various organs including brain-on-chip [37], kidney-on-chip [38], and gut-and-liver-on-chip [39]. Soft lithography based microfluidic systems are advantageous for exosome studies due to their low cost, simple fabrication, high throughput, and precise control over the environment. These systems allow for the study of exosomes in a controllable and reproducible manner, while providing a rapid and efficient means to capture and analyse them. Additionally, they offer the possibility of integrating multiple assays, as well as the ability to manipulate the parameters of the environment to obtain more precise and accurate results. However, one potential disadvantage of such systems is their susceptibility to clogging, which can be difficult to clean and may lead to poor results. Furthermore, soft lithography-based systems are limited in their ability to accurately detect and quantify exosomes, as well as the degree of resolution that can be achieved [40,41].

Hot embossing: This is one of the most flexible and appropriate technique for the fabrication of polymer-based microchips by employing thermoplastic materials including polymethyl methacrylate (PMMA) [42]. In this method, initially a master is designed and constructed by photolithography, followed by utilizing an embossing machine, where the master is fitted. During this process, force, and isothermal heating or cooling are applied. This method also incurs low cost and can produce the polymer-based microstructures, yielding better aspect ratio and micro-pin lamellae with precise control of various guiding parameters and temperature [43,44]. Hot embossing based microfluid systems are being used increasingly for exosome studies due to its advantages. This method enables the fabrication of complex microfluidic systems with high precision and repeatability. It also allows for rapid prototyping, which makes it possible to quickly iterate and optimize designs. Additionally, the microfluidic channels can be designed to have a wide range of sizes and shapes, allowing for the study of various exosome characteristics. However, hot embossing also has some drawbacks. It is difficult to fabricate channels with larger dimensions, and the technique can be expensive, making it cost-prohibitive for certain applications.

Additionally, the technique is limited by the materials that can be used, so it may not be suitable for certain exosome studies [45,46].

Injection molding: This technique involves casting and injection molding procedure, which reduce the process of microfabrication. Several microdevices can manufactured at large scale by utilizing this method. However, the process of fabrication is intricate because of the necessity to control various parameters including temperature, injection rate, pressure for safeguarding the quality of production [47,48]. This method has been utilized for studying the angiogenic phenotype via patterning lung fibroblast and human umbilical endothelial cells by injected scaffold of 3D culture. This could create a vascularized microenvironment, equipped with high throughput ability. Injection molding based microfluid systems are advantageous for exosome study for several reasons. Firstly, these systems enable precise control over the microfluidic environment and can be used to create a range of different exosome structures. Additionally, they are scalable and can be used to produce a large number of exosomes in a short amount of time. However, they also have some disadvantages. Firstly, they require a high level of expertise and experience to operate and can be costly to maintain. Additionally, they can be prone to clogging and cross-contamination. Furthermore, they are not suitable for studies that require a large volume of samples. Overall, injection molding based microfluid systems offer great advantages for exosome study, but their drawbacks should be taken into consideration [41].

3D printing: The introduction of 3D printing has brough a major paradigm shift in the process for developing OCMS devices. This technique is competent to fabricate 3D microdevices with high-resolution, and much reduced cost of production [49–51]. A typical "layer-layer approach" is utilized where a material is layered from the bottom to top layer in additive production [52]. This method uses a computer to control the movement of a nozzle to create a 3D object. These processes can be used to create microfluid systems using a variety of materials including polymers, metals, and ceramics. The materials used depend on the application and the desired properties of the microfluid system. A few popular varieties of methods for 3D printing include stereolithography, focused-deposition modelling, and multi-jet modelling [53]. A major advantage of 3D printing is its ability perform rapid prototyping as it can directly fabricate various microstructures apart from the master molds produced via photolithography [54]. In addition, it allows the precise control and arrangement of the desired number of cells as a layer. This can be utilized for bioprinting of various artificial organs by performing different tasks such as designing, imaging, selection of materials, selection of cells, and bioprinting [55]. Despite several advantages, one of the limitations is that it has not been found to be apt with all kinds of materials [56]. Nevertheless, the application of 3D printing has been extensively utilized for fabrication of OCMS devices, for example modelling of 3D liver has been achieved by 3D

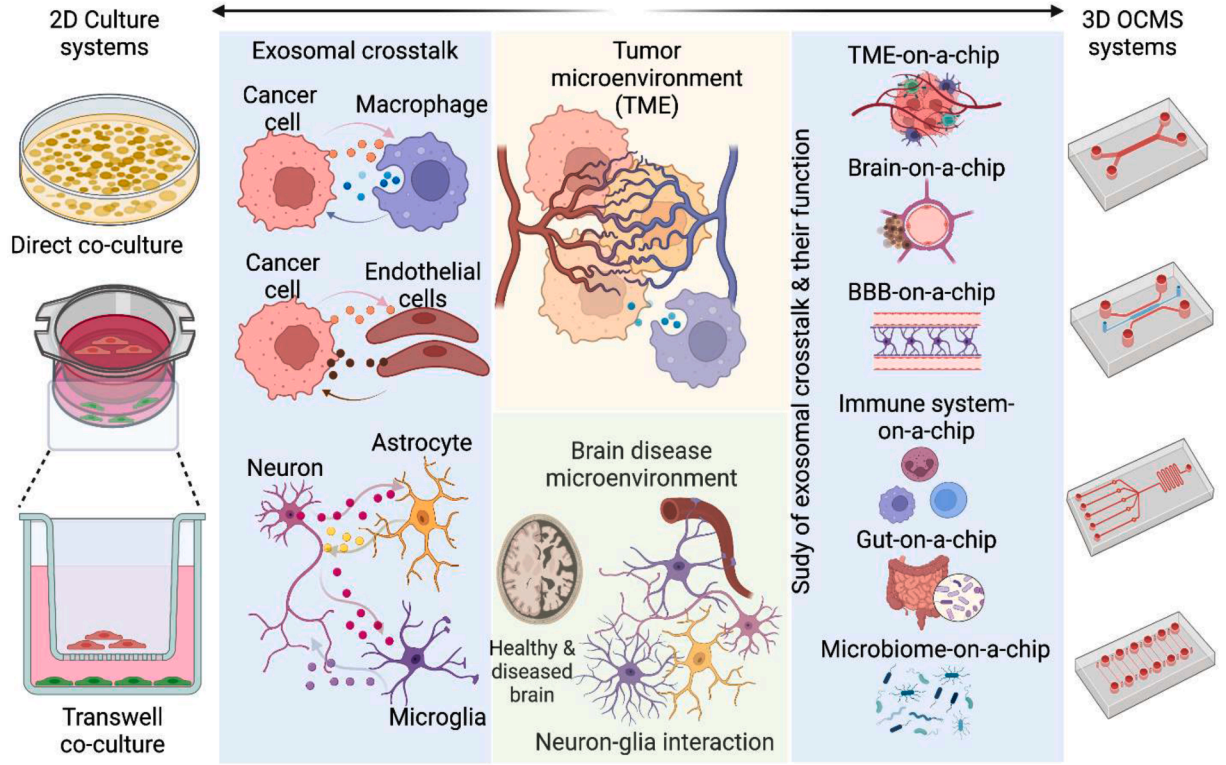

Fig. 2. Impersonation of tumor microenvironment (TME) and the brain disease microenvironment using microfluidic technology. Development of 2D culture systems and 3D organ-on-a-chip microfluidic systems (OCMS) for modelling physiological or pathological conditions including TME and brain diseases like neurodegenerative diseases such as Alzheimer's disease (AD), and Parkinson's diseases. For 2D culture systems, the cells involved in the TME such as cancer cells, macrophage, or endothelial cells can be cultured in a pair wise combination in Petri dish or Transwell co-culture insert for studying exosomal dynamics. In 3D OCMS, multiple cell types can be cultured in different types of microfluid devices using scaffolds like hydrogels or extracellular matrices (ECM) for studying exosomal dynamics. (Created with BioRender.com).

printing for the drug evaluation [57]. Organ level bioprinting has been demonstrated via 3D extrusion printing for studying the pseudorabies virus in the nervous system [58]. 3D printing based microfluid systems for exosome study offer many advantages. Firstly, they provide increased accuracy and decreased variability as compared to conventional methods. Secondly, they are cost effective and enable a wide range of studies to be conducted at a lower cost. Thirdly, they are easy to use and can be scaled up or down to fit specific needs. On the other hand, this technology also has some disadvantages. Firstly, it is still relatively new and therefore may not be as reliable as other technologies. Secondly, it may not be able to accurately detect some of the more complex exosome components. Finally, it can be difficult to scale up for large-scale studies [41,59].

Recently, the development of 3D-nanopatterned microfluidic chip has been gaining attention for various applications including the detection of exosomes [60], via biosensing of their cargoes such as protein, nucleic acids, and metabolites. In addition, as the exosome-based intracellular communication plays crucial role in the disease progression, it necessitates to develop appropriate OCMS to understand the dynamics of exosomes, which will facilitate the developing of novel therapeutics for various fatal diseases including tumour microenvironment (TME) and brain disease microenvironment (Fig. 2).

3. Understanding the dynamics of exosomes for modeling intracellular communication in OCMS

As noted above, exosomes are produced by endocytic pathway, and their biogenesis process starts with the formation of early endosomes which transform in late endosomes or multivesicular bodies (MVBs) that produce intraluminal vesicles (ILVs) by inward budding of the endosomal membrane. The production of these ILVs implies a reorganization of the endosomal membrane enriched in tetraspanins and the recruitment of endosomal sorting complexes required for transport (ESCRTs). After formation of ILVs, the endosomal membrane can fuse with lysosomes to degrade their content or fuse with the cellular membrane to release the ILVs as exosomes [61–63]. Several proteins have been suggested to regulate the release of exosomes such as Rab GTPases, SNAREs, and actin. Rab GTPases are molecules that modulate the traffic of MVBs between different cellular compartments and they play important roles. It has been demonstrated that Rab27a and Rab27b participate in exosome secretion in many types of cancer, controlling the MVBs size and their localization inside the cell to promote the docking of them at plasma membrane. Other proteins such as Rab11, Rab31 and Rab35 are implicated in the fusion between MVBs and the cellular membrane [64–68]. Similarly, SNARE proteins mediate the fusion of the MVBs with the plasma membrane through the interaction between specific vesicular SNAREs (v-SNARE) in the MVBs and target SNAREs (t-SNARE) in the cellular membrane. In addition, it has been suggested that this fusion process occurs in actin-enriched sites of the plasma membrane known as invadopodia, which are associated with degradation of extracellular matrix in cancer [65,69].

Despite of these proteins modulate the exosome release, some microenvironmental conditions can stimulate this process. For instance, it has been shown that hypoxia increases the secretion of exosomes produced by cancer cells to influence the behavior of stroma cells in the TME. Additionally, low levels of oxygen inside tumor can promote the production of increased levels of glycolysis, which is associated with elevated levels of exosome release. As a result, the lactate produced by glycolysis can induce the formation of an acidic environment increasing the secretion of exosomes [66]. Furthermore, it has been suggested that exosome release is influenced by intracellular levels of calcium that come from extracellular environment and enter into the cell through calcium channels or by calcium produced by the endoplasmic reticulum due cell activation, promoting tumorigenesis and metastasis [66,69]. Studies on breast cancer cells confirmed that the exosome secretion can be stimulated by calcium (Ca^{2+}) and regulated by Munc13-4, a Rab binding protein that depends on Ca^{2+} and controls the traffic pathway of MVBs mediated by Rab11 protein. Since cancer cells exhibit high levels of Ca^{2+} for the overexpression of calcium channels, they have an enhanced secretion of exosomes and induce the upregulation of Munc13-4 which plays a role as effector promoting the maturation of MVBs before their fusion with the cellular membrane and regulating the secretion of exosomes in metastatic cells [70].

Exosomes released into the extracellular medium can be taken up by the same cells that produced them or by other nearby and distant cells. It has been suggested that the exosome uptake might include three main processes such as the recognition of the target cell, the entry of the exosome into the cell and the release of the exosome contents into the cell [61,65,71]. Several mechanisms have been described to explain the uptake of the exosomes including the internalization through different types of endocytosis and the fusion of the exosome with cellular membrane. However, exosome uptake may also involve the interaction between specific surface proteins of exosomes and recipient cells including heparan sulfate proteoglycans (HSPG), lectins and adhesion molecules such as integrins, immunoglobulins, mucins, selectins and cadherins. Among the main types of endocytosis used by cells to internalize exosomes are phagocytosis, macropinocytosis, caveolin-dependent, clathrin-dependent and lipid raft-dependent endocytosis. Phagocytosis is the most common method of take up exosomes by immune cells through the formation of invaginations around the exosomes to internalize them and depends on specific receptors, dynamin2, actin cytoskeleton and phosphatidylinositol 3-kinase (PI3K). Macropinocytosis is one of the least mechanisms used for exosome uptake, involves the participation of invaginated membrane ruffles and is Na^{+} and PI3K-dependent. Caveolin-dependent endocytosis occurs through pits in the plasma membrane known as caveolae coated with caveolin protein, to internalize small materials. In contrast, clathrin-dependent endocytosis involves internalization of larger particles via pits coated with clathrin protein. Finally, lipid raft-dependent endocytosis is mediated by cellular membrane microdomains enriched in receptors, sphingolipids and sterols [68,72,73]. Alternatively, exosome membrane can fusion directly with the plasma membrane of the target cell leading to hemifusion, reorganization and complete fusion of both membranes. Several proteins as SNAREs, Rab, Sec1/Munc-18 and Syncytin-2/MFSD2a have been suggested to participate in this process [73,74]. Conclusively, the understanding of multifactorial pathways involved in the exosomal dynamics is crucial for developing an OCMS for the precise modelling of exosome-based intracellular communication.

Using microfluidic systems to monitor the dynamics of exosomes can present a variety of challenges. The specific equipment required to do so may require long-working distance objectives, that can be expensive and render difficulty to acquire. Additionally,

there is the challenge of using cells with different medium to ensure the accuracy of the observations. Additionally, the complexity of the microfluidic systems can be difficult to operate and maintain, requiring a skilled technician to ensure proper functioning. Finally, there can be issues with controlling the speed of the fluid flow, as well as ensuring that there is an adequate supply of exosomes for the system to monitor. All these challenges must be taken into consideration when using microfluidic systems to monitor the dynamics of exosomes [75].

4. On-chip characterization of exosomes and study of their release and uptake in OCMS

Microfluidic technology has emerged as an excellent choice for studying the exosome-based theranostic of various diseases including cancer and neurodegenerative diseases [76]. Owing to different advantages, it has gained a massive attention for clinical applications such as exosomes-based isolation and diagnostic device. For example, due to the large surface to volume ratio, and rapid mass diffusion in a short distance, the requirement of lower reagents' volume, and less time consumption [77], which is beneficial for the isolation of exosomes, its characterization, and detection. The exception ability to precisely control the fluid and incorporation of different functionalities in a single microfluidic chip can serve has an efficient and versatile platform for the analysis of exosomes, potentially applicable for disease diagnosis. Also, the microfluidic device can be customized to incorporate multiplexed detection ability for biomarker study. In addition, the multiplexed detection can offer augmented sensitivity and specificity for better biosensing with high throughput approach. Importantly, advanced techniques such as clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), atomic force microscopy (AFM), and other analytical techniques can be integrated with microfluidic device for the detection of exosomes, their cargoes, for developing potential biomarkers.

As mentioned above, monitoring of exosomes has been a major challenge in dynamic conditions, however recently various microfluidic devices have been developed to achieve on-chip labeling of exosomes, such as micromixers [78,79], nano flow cytometer (nanoFCM) integrated with on-chip microscopy[70], and microfluidic compartments with sensing microbeads for tracking the dynamics of exosome release from single cell [80]. Microfluid-based micromixer device consists of mechanical parts with size in micro range for mixing fluids, which is facilitated via external turbulence. In addition, the microstructure inside the channels of microfluid-based micromixer device provide a large surface-to-volume ratio and thereby enhances the efficiency of heat and mass transfer. In general, micromixers can be of either active or passive type. The active micromixers can be propelled via the application of various stimuli including pressure, electrical, sound, and magnetic fields, whereas the passive micromixer includes collisions, two-dimension barriers, and three-dimension lamination, convergence-divergence, and spiral structures [78,79]. Majority of the on-chip characterization of EVs employed the microfluid-based micromixers to augment the biochemical assays to facilitate the detection of EVs for example, the application of various lipophilic fluorescence dyes such as PKH26, PKH67, DiO, Dil. Notably, the combination of micromixers with biochemical sensors has gained a tremendous attention for the exosome detection.

Development of a nanoFCM has been another great advancement in the field of nano research, which has been shown to be integrated with on-chip microscopy for single lipid vesicle analysis. Interestingly, this device can precisely count the number of lipid vesicles with a limit of detection (LOD) of 120 fM by using a mere 20 μL of sample. It can visualise a single vesicle via fluorescence microscopy during their dynamic motion through numerous parallel microchannels in a pressure-induced flow. In addition, the imaging of vesicles can be accomplished through a standard epi-fluorescence microscope, which offers a convenient way for their detection. Importantly, the ability of nanoFCM to determine the size distribution of vesicles based on fluorescence intensity is an added advantage [81]. Interestingly, on-chip fluorescence-based detection has been widely used to make the device portable such as the application of random microlens diffuser [82], contact fluorescence microscopy [83], spectrally filtered passive Si photodiode array [84], and tapered fiber-optic faceplate [85]. Importantly, on-chip light sheet illumination method enabled precise monitoring of fluorescence labelled individual EVs via measuring their size and concentration in biofluids [86].

Another advancement in the context of analyzing exosomes in dynamics was demonstrated by Son *et al* group, which developed the microfluidic compartments, equipped with sensing microbeads for monitoring exosome release from single cells in a dynamic motion. The authors reported a reconfigurable microfluidic device for holding single cells along with antibody-conjugated sensing beads inside microcompartments with volume capacity of 20 pL for monitoring the release activity from HepG2 human hepatoma cells. Interestingly, the top portion of the microfluidic device possess an array of approximately 7000 microchambers, and the top portion can be moved up and down by the application of negative pressure. Micropatterning was performed in the bottom portion of microfluidic device for holding the cell attachment sites along with the microcompartments. This set-up was used for the detection of exosomes and inflammatory cytokines, released from single cancer cell and immune cells, respectively. Regarding the scheme for the detection, the cells introduced were captured on the inside surface of microfluidic device, followed by the introduction of sensing microbeads, which led to the confinement of single cells and microbeads when the microcompartments were moved down. The fluorescent conjugated secondary antibodies present in the liquid bathing the microbeads and the cells would bind the cells secreted molecules; captured on the microbeads, which causes the fluorescence in microbeads. The time-course change in fluorescence intensity of microbeads facilitated the study of dynamics of release activity from the single cell [80].

Other advanced techniques for studying the dynamics of exosomes include real-time imaging of multivesicular body–plasma membrane fusion for measuring the release of exosomes from single cells [87], utilizing a live cell reporter of exosome release and uptake for studying direction finding phenotype of migrating cells [88]. Recently, SERS based monitoring the trajectory of SERS-active hybrid exosomes uptake has provided insight into the possible role of metal nanoparticles [89]. Imaging flow cytometry has also been utilized for tracking the uptake of EVs cargo [90]. In the recent COVID19 pandemic scenario, research about sensing of pathological condition or virus infection via exosomal cargoes has also been demonstrated [91]. Nevertheless, the modeling of intracellular

exosomal communication in organ specific OCMS device is need of the hour.

5. Emerging OCMS devices involving exosomal dynamics

The development of micro-devices mimicking the physiological systems has evolved through blended innovations in tissue engineering, microstructure engineering, and stem cell biology. Consequently, numerous culture system designs at microscale level have been established that reconstitute the functions at tissue and organ levels. Recently, several OCMS devices have been established for the study of exosomal dynamics between donor and recipient cells, and further contributing to different biological responses. In a research by Kang *et. al.*, a novel microfluidic device has been proposed for the study of on-chip biogenesis of natural killer (NK) cell-derived exosomes in non-small cell lung cancer, demonstrating the antitumor activity. Notably, it was found that the NK-graphene oxide chip showed cytotoxic effect on circulating tumor cells (CTCs). This device could be potentially applicable for patient-specific NK-based immunotherapies. In addition, the CTCs could be used for prospective diagnostic or prognostic purposes [92]. Kim *et. al.* developed a 3D human liver-on-a-chip mimicking the creation of premetastatic niche by breast cancer-derived EVs [93]. Th authors demonstrated that breast cancer-derived EVs activate liver sinusoidal endothelial cells (LSECs) in the liver-on-a-chip, causing endothelial to mesenchymal transition and damage of vessel barriers. Moreover, it was found that transforming growth factor β1 (TGFβ1) in breast cancer derived EVs increases the fibronectin, an adhesive extracellular matrix protein, on LSECs, which enables the adhesion of breast cancer cells to the liver microenvironment. Similar observation was found with EVs isolated triple-negative breast cancer (TNBC) patients with liver metastasis carrying augmented TGFβ1 levels. Categorically, this study established an efficient OCMS as the liver-on-a-chip, which could be utilized for the study of dynamics and function of exosomes in a liver environment [93]. In a study conducted by Wu et al., a digital organ-on-a-chip was developed to evaluate hepatotoxicity and EVs-based immunotherapy against liver cancer. This device featured an array of microwells combined with cellular microspheres, resulting in substantially increased parallelism compared to traditional organ-on-a-chip for drug development. This platform demonstrated anti-cancer capability of sorafenib at 10 μM concentration. Additionally, the platform also showed the efficacy of natural killer cell derived EVs (at 50 μg/mL) for liver cancer. This OCMS provides a high-parallelism, low-variability analytical device for toxicity testing, and the exploration of new anticancer treatments, enabling the collective effort to combat cancer. [94]. Fig. 3 depicts the different applications of OCMS for the exosome-based study.

6. Multi-organ on a chip platform for tracking intracellular exosome communication

Due to their complex structure and minuscule size, it can be difficult to determine the exact role EVs play in various signalling pathways [95,96]. To gain a better understanding of the potential of EVs, it is vital that we can observe and track their movements in real-time. For instance, Morad et al. were able to identify transcytosis as the mechanism by which breast-cancer-derived EVs breached an intact BBB, using fluorescent protein-labelled EVs in combination with in vivo and in vitro models, including a BBB-on-a-chip [97]. The lipophilic dye method is often used in OOC-based EV research; however, this method is only suitable for observing single-organ-level interaction and is not suitable for studying cross-organ interaction via live cell-produced EVs [98]. To address this, Oh et al. developed a method where cells are transfected with a cytomegalovirus-driven GFP-tagged CD63 vector in order to produce GFP-labelled exosomes [99]. By designing a cell-culturing microfluidic chip, they were able to track single exosomes in real-time during cell-to-cell exosome delivery. Furthermore, in a follow-up study, a graphene-oxide quenching-based molecular beacon

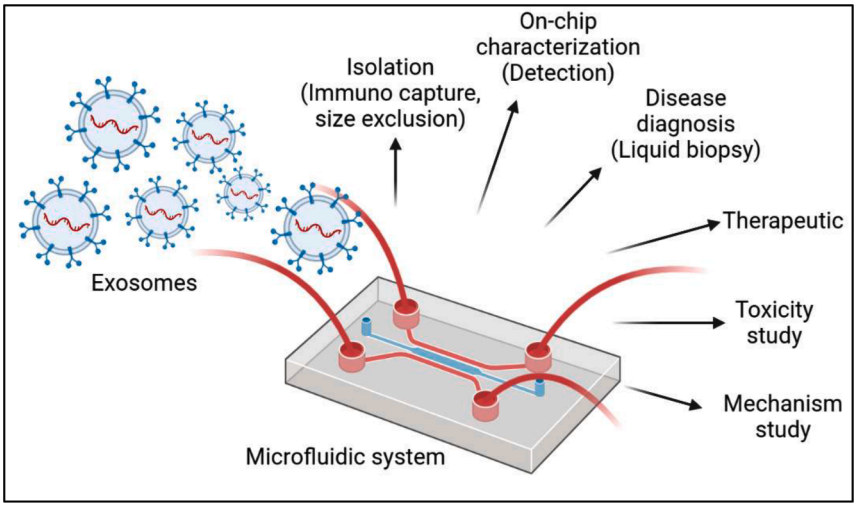

Fig. 3. Different applications of microfluidic system for exosome-based studies. Representative diagram showing major uses of microfluidic systems for the exosomal studies including their isolation, on-chip characterization, liquid biopsy for the diagnosis of diseases, therapeutics, toxicity study, and exosomal crosstalk-based mechanism study. (Created with BioRender.com).

imaging technique was employed for direct and real-time tracking of single exosomes, which contained the specific miRNA of interest, during cell-to-cell exosome delivery [100]. This technique allowed them to label specific exosomal miRNA inside of cells before they were released through exosomes.

7. Integration of biosensors to organ on a chip for detecting exosome or other analytes on their surface

The incorporation of biosensing techniques into OCMS devices allows for the analysis of microenvironments (such as pH, dissolved oxygen, and temperature), cell metabolism and function (like metabolic parameters, secreted biomarkers, organ activity, and barrier integrity), and responses to external stimuli (such as electrical, mechanical, and drugs). Through the integration of analytical biosensors with OCMS platforms, the necessary information is obtained to maintain a reproducible and controllable cell culture. These in-line biosensors have four major advantages when integrated with OCMS: compatibility with microfluidics, high specificity, and sensitivity, they are label-free, and they are minimally invasive [101].

Several advancements have been accomplished in the context of integrated biosensors with microfluidics for exosome profiling such as fluorescence integrated microfluidics methods, integration SERS-based methods, integration with electrochemical methods, and integration with field-effect transistor (FET) methods [102]. The fluorescence integrated microfluidics methods include 3D-nano-patterned microfluidic chip, Sandwich immunoassays, graphene oxide/polydopamine (GO/PDA) nano-interface, microfluidic herringbone-grooved chip based on affinity, and thermophoretic concentration. SERS-based methods include SERS-active 3D nano-bowls, beehive-inspire macroporous SERS Probe, MB@SiO2@Au@aptamer substrates, based on polydopamine-encapsulated antibody-reporter Ag(shell)–Au(core) multilayer (PEARL), sandwich immune complex based on antibody labelled magnetic beads and AuNRs. Electrochemical methods include electrochemical biosensor based on Zr based metal−organic Frameworks, aptamer recognition induced multi-DNA release and cyclic enzymatic amplification. FET methods include chemically functionalized graphene FET, reduced graphene oxide FET, and RGO-FET strategy based on a membrane biotinylating [102].

8. Challenges in the application OCMS for biomedical research and exosome study

Even though, OCMS device has a lot to offer, yet there exist various obstacles, which are required to be conquered. One of the major challenges in the application of OCMS include the sourcing of human cells with high standards, however with the advent of induced pluripotent stem cells (iPSCs) and organoids, this issue has been resolved to a large extent [103,104]. Low throughput of many OCMS devices is another crucial obstacle, which requires attention for further improvement. The high throughput OCMS devices can offer the facility to run several replicates of experiments, which is useful for conducting statistically sound studies, which can also fulfil the requirement of pharmaceutical industry standard validation systems [105]. Nevertheless, the employment of automation technique can tackle this hurdle by culturing and analysing many low throughputs OCMS devices at the same time.

The development of human OCMS is typically accomplished using commercially available human-derived primary cells, cell lines, organoids, or iPSCs. While cell lines are convenient for applications such as improving data reproducibility and drug development during the early stages, they are not ideal for recreating the *in vivo* scenario. As a result, primary cell culture or patient-derived cells are often used instead. iPSCs have become increasingly popular due to their capacity to produce various cell types that may not be available commercially. Nevertheless, the application of iPSCs for OCMS development can be difficult because they do not always show a fully matured and differentiated phenotype [106]. Additionally, the use of PDMS for fabricating OCMS devices is not suitable for drug studies due to drug absorption. This is especially true for hydrophobic drugs, whose absorption by PDMS is significant [107, 108].

9. Conclusion and future outlook

The advancements in the development of OCMS are evident that human organ-on-a-chip can be utilized for addressing varieties of biomedical problems, which could not be delivered by depending on conventional culture systems. Importantly, OCMS lined by cells from patient organoid present efficient in vitro pre-clinical models. Additionally, the ability of OCMS to mimic human pathophysiology and sense the clinical responses against therapeutic intervention has enhanced their reliability compared to the drug testing in animals, which can also be conveniently extended to toxicity evaluation. Over the decades, OCMS devices have emerged as an effective and useful alternative when the human studies are found to be difficult to conduct for example in case of tests related to radiation exposure. Even due to the dearth of a reliable model for genetic disease, the genetically engineered 3D cell cultured OCMS devices can be a boon for the biomedical research, which can also be helpful for the study of rare genetic diseases. For the past several decades, scientists have been successful to develop OCMS to mimic various human body organs including the brain-, lung-, heart-, and liver- on-a-chip. Nevertheless, the dramatic surge in the exosome related studies including intracellular communication with multifaced roles including disease pathogenesis, and progression, has created an important space for developing advanced OCMS devices for modelling of exosome-based intracellular communication, which will be beneficial for drug screening to identify novel therapeutic targets via blocking of exosome release.

In recent times, various research teams have been employing OCMS to examine exosome dynamics and its capabilities. This is only the beginning in their application of OCMS devices in studying exosome dynamics in both healthy and unhealthy conditions. Such devices have the potential of being utilized for personalized and precise medicine and have the capability of reducing the time and cost of drug efficacy testing as an alternative to human clinical trials. The development of OCMS has had a significant impact on biomedical studies, and has opened a great opportunity to study exosome dynamics and intracellular communication via more advanced OCMS

models.

The future outlook of OCMS is extremely promising. This technology has the potential to revolutionize the way we study exosomal communication. Through this technology, scientists can better understand how exosomes interact with cells and other molecules in the extracellular environment. This could lead to a better understanding of how exosomes are involved in the communication between cells and the extracellular environment. Furthermore, this OCMS technology can be employed to study exosomal communication to gain a better understanding of the mechanisms by which certain drugs affect intracellular exosomal. This could lead to more effective and safe drugs for treating diseases. In addition, this technology could be used for personalized medicine. By studying exosomal dynamics in OCMS, scientists can gain a better understanding of how a person's individual physiology and genetic makeup affect exosomal cargoes. This knowledge could be used to develop personalized treatments and therapies tailored to each individual.

CRediT authorship contribution statement

Abhimanyu Thakur: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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