Biofabrication of nanovesicles for brain diseases

Pasquale Picone, Domenico Nuzzo*

Background: Nanotechnologies promise to improve disease diagnosis and treatment, overcoming limitations of conventional treatments. In particular, extracellular vesicles (EVs) and artificial vesicles (AVs) are strongly emerging tools in nanomedicine (Leggio et al., 2020). Hybrid nanovesicles, AVs generated by coextrusion and sonication, also encapsulating nanoparticles’ cores with the membrane used in cell membranes, and extend circulation times to improve the stability and solubility of encapsulated molecules and drugs, promote transport across biological barriers, and increase the safety and efficacy of treatments (Leggio et al., 2020).

In this context, the biofabrication of cell-derived nanovesicles (C-DNv) has been recently proposed as a biological approach for the delivery of drugs by cells using different physical techniques including extrusion through micro-filters, microfluidic, sonication, or coextrusion method (Li et al., 2021). These methods require cellular destruction to obtain nanovesicles with characteristics similar to the cells membranes.

In literature, C-DNv generated are also called exosome-mimics (Vázquez-Rios et al., 2021) or artificial exosomes (Li et al., 2021). They are bioinspired cell-derived nanovesicles (Goh et al., 2017). This different terminology is due to the fact that C-DNv appear to be similar in several aspects to exosomes, but are produced through physical processes such as AVs. The C-DNv are great promise as drug delivery systems (DDS) due to their unique benefits of natural vesicles and synthetic vesicles. The biofabrication of C-DNv is a scalable and efficient alternative to EVs production to obtain a high yield of cell-derived nanovesicles (Ilahibaks et al., 2019). CNS disorders, such as neurodegenerative and tumors are among the most serious health problems (more than 600 diseases), degrading the quality of life and causing enormous economic costs. Today, the effectiveness of treatments represents an important and priority challenge for medicine. The presence of barriers, such as the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier are still an obstacle to delivering drugs to the brain (only small molecules can cross these barriers), reducing the efficacy of various therapies. Recently, it has been demonstrated that the extracellular vesicles and especially brain endothelial cells influence the formation of nanotubes involved in the construction of the BBB (Mentor and Fisher, 2021). In addition to crossing the BBB is necessary to target specific cell types such as neurons, astrocytes, oligodendrocytes, and microglia involved in specific brain diseases. Several DDSs, including nanovesicles, extracellular vesicles, and artificial vesicles have been studied for brain drug delivery (Dong et al., 2019; Nuzzo et al., 2021). Vesicles derived from cells and brain tissue have been biofabricated as DDS. In this review, we analyzed these new systems and their potential as DDS to contrast the brain disorders.

Cell-derived nanovesicles for brain disease: To date, few studies have focused on the biofabrication of cell-derived nanovesicles to counteract brain diseases. Dong et al. (2019) proposed neutrophil-derived extracellular vesicles and anti-tumor effects D2 (molecule derived from docosahexaenoic acid) to protect mouse brain injury from ischemic stroke. The authors, inspired by the binding of neutrophils to the endothelium during stroke, have generated nanovesicles derived from differentiated HL-60 cells (like neutrophils) by nitrogen cavitation. Nanovesicles specifically bind to inflamed brain endothelium to mitigate neuroinflammation after reperfusion therapy of ischemic stroke (Dong et al., 2019). Nanovesicles derived from macrophage membrane have nerve growth factor growth factor were generated by mixing the separated macrophage membrane with nerve growth factor solution by extrusion through the polycarbonate membrane (Xia et al., 2021). The results indicate that nanovesicles effectively delivered nerve growth factor to the spinal cord injury site to exert a neuroprotective effect (Wu et al., 2021). Wu and collaborators showed that nanovesicles derived from brain-derived endothelial cells, by serial extrusion, were good alternative nanocarrier to exosomes (Wu et al., 2021). In particular, they demonstrated that brain-derived endothelial cells and exosomes showed similar drug-loading capacity (Doxorubicin), BBB-crossing ability, and antitumor effects in various models, but the yield of brain-derived endothelial cells is substantially higher (500-fold) than exosomes (Figure 1A; Wu et al., 2021).

Biophysical strategies were used for the development of nanosystems by combining synthetic nanoparticles and cellular membranes. Hybrid nanovesicles is an emerging field where the properties of natural vesicles such as tropism, biological barrier penetration, long circulation, low immunogenicity, and high biocompatibility are combined with the advantages of synthetic materials including control of the process of manufacturing, high production, engineering, functionalization, drug loading, and stability. Numerous studies on hybrid nanovesicles have been developed (Li et al., 2021), but very few are present in the scientific landscape for brain applications. In this context, the cell membrane obtained by different cell types can be used to coat nanoparticles, enhancing the targeting capacity of these carriers. Different studies that use red blood cells or glioblastoma (GBM) membranes for GBM therapy were published (Mandarha et al., 2021). In addition, the extracted membrane can be modified to increase the capacity to cross BBB and to improve the drugs delivery in the tumor site. The aim is to coat the nanoparticles’ cores with the membrane used in coextrusion and sonication, also encapsulating several hydrophobic and hydrophilic drugs (Mandarha et al., 2021).

In 2017, the first work targeting GBM used membranes from red blood cells (cell membrane-coated nanoparticles) modified with the CDX2 (peptide derived from candoxin) which has a binding affinity toward the nicotinic acetylcholine receptors expressed on the surface of the brain endothelial cells (Chai et al., 2017). The results showed that the nanocarrier carrying doxorubicin presented a superior therapeutic efficacy with reduced toxicity effects (Chai et al., 2017). In a recent study, Gao et al. (2021) designed a miRNA-containg miRNA-containing miRNA with a PLGA peptide modification endows the M2-microglia and macrophage targeting ability and HA2 peptide promotes fusion of membranes of erythrocyte and endothelium. miRNA based nanovesicle targeting allows to design a drug delivery system to the tumor site and entered the cytoplasm of macrophages and microglia and shifted their pro-inflammatory M2 phenotype to anti-tumor M1 phenotype for GBM immunotherapy (Gao et al., 2021).

The mechanisms by which C-DNv is able to cross the BBB have yet to be fully elucidated. However, as several cell types are able to cross the BBB such as immune cells or cancer cells originating from cell membranes, could naturally have some cellular membrane surface proteins that could mediate the BBB crossing.

Brain tissue-derived nanovesicles: Recently, brain tissue has been used as a material for nanovesicles generation with potential biomedical applications. Synaptosomes, extracellular vesicles and fractions isolated from synaptic terminals that can be prepared by homogenization and gradient centrifugation of brain tissues contain specific vesicles and mitochondria and are considered a relevant model system for studying human synaptic dysfunction in neurodegenerative diseases. The synaptosome has been proposed as a mitochondria delivery system that could improve the mitochondrial transfer and cellular uptake in neuronal cells (Figure 1B; Picone et al., 2021). Synaptosomes derived from brain tissue can be used to replace or supplement damaged mitochondria in the cells with mitochondrial dysfunction is called mitochondrial transplantation. Mitochondrial transplantation has been considered as a potential therapy for several neurodegenerative diseases (Espino De la Fuente-Muñoz et al., 2020; Picone et al., 2021). Synaptosomes-mediated mitochondrial transplantation could be applicable for the treatment of many brain diseases characterized by mitochondrial dysfunction such as Alzheimer’s Disease, Parkinson’s disease, and multiple sclerosis.

Nanovesicles produced from the myelin brain, as a new potential carrier with an enhanced tropism for the brain tissues (from brain to brain), have been biofabricated (Picone et al., 2021a). In specific, myelin membranes are produced with an easy, efficient, cost-effective, and reproducible production protocol. They have high stability, and cytocompatibility, are able to load drugs and can carry a variety of cargos. Myelin nanovesicles to cross the BBB could be due to the presence of proteins (transferrin, apolipoprotein E, and glutathione) which are known to promote the crossing of nanovecesses through the BBB. In addition, are able to target specific brain regions, such as white matter, and preferentially interacting with microglial cells (Figure 1B; Picone et al., 2021a).

Nanovesicles tools for brain diseases, potentialities and limits: Articles on the biofabrication of nanostructures generated by cells or brain tissue represent great potential in the field of treatment of neuronal pathologies. The nanovesicles biofabricated could be used as brain drug delivery systems or could naturally transport molecules with therapeutic effects. Such systems could overcome the most important challenges related to the delivery of therapeutic agents to CNS. The main obstacle is the presence of BBB and the occurrence of side effects due to the dispersion of drugs that fail to enter the CNS. Furthermore, it could be an alternative for other therapeutic agents to CNS-specific regions, without affecting other CNS areas to avoid further damage. Therefore, formulations able to bypass physiological barriers selectively and
Perspective

Figure 1 | Schematic representation of the procedure used for cell-derived nanovesicles fabrication and their brain potential applications (A) and brain tissue-derived nanovesicles fabrication and their potential applications (B).

Created with BioRender.com.

targeted to specific regions and cells (neurons, microglia, or astrocytes) are essential factors for the development of effective therapy for the treatment of brain disorders. Furthermore, future problems are related to the solubility, stability, and consequently bioavailability of drugs.

Brain delivery systems can be an exciting and promising platform for overcoming the problems mentioned above. In this scenario, recently the cell or tissue derived-nanovesicles have shown great potential. In fact thanks to their nature and being similar to EVs they can present selective tropism, penetration of the biological barrier, long circulation, low immunogenicity, and high biocompatibility and being produced with physical means such as AVs they can be produced with high yields, engineered, functionalized and loaded with drugs of different nature (lipophilic and hydrophilic).

However, there is an urgent need to improve the extraction protocols, and the reproducibility and characterization of the processes. All this means such as AVs they can be produced with physical means such as AVs.

Passquale Picone, PhD, Pasquale.picone@irib.cnr.it.

Dong X (2018) Current strategies for brain drug delivery. Theranostics 8:1481-1493.

Dong X, Gao J, Zhang C, Hayworth C, Frank M, Wang Z (2019) Neutrophil membrane-derived nanovesicles alleviate inflammation to protect mouse brain injury from ischemic stroke. ACS Nano 13:1277-1283.

Espino De la Fuente-Muñoz C, Arias C (2020) The therapeutic potential of mitochondrial transplantation for the treatment of neurodegenerative disorders. Rev Neurosci 7:203-217.

Gao X, Li S, Ding F, Liu X, Wu Y, Li J, Feng J, Zhu X, Zhang C (2021) A virus-mimicking nucleic acid nanogel reprograms microglia and macrophages for glioblastoma therapy. Adv Mater 33:e2006116.

Goh WJ, Zou S, Ong WY, Torta F, Alexandra AF, Schifferers RM, Storm G, Wang JW, Czarny B, Pastorin G (2017) Bioinspired cell-derived nanovesicles versus exosomes as drug delivery systems: a cost-effective alternative. Sci Rep 30:14322.

Ilaibakis NF, Lei Z, Mol EA, Deshantri AK, Jiang L, Schifferers RM, Vater F, Sluijter JP (2019) Biofabrication of cell-derived nanovesicles: a potential alternative to extracellular vesicles for regenerative medicine. Cells 25:1509.

Kim YS, Kim JY, Cho R, Shin DM, Lee SW, Oh YM (2017) Adipose stem cell-derived nanovesicles inhibit emphysema primarily via an FGF2-dependent pathway. Exp Mol Med 13:e284.

Lai CP, Breakefield XO (2012) Role of exosomes/microvesicles in the nervous system and use in emerging therapies. Front Physiol 3:228.

Leggi L, Arrabito G, Ferrara V, Vivarelli S, Paternò G, Marchetti B, Pignatari B, Irazi N (2020) Mastering the tools: natural versus artificial vesicles in nanomedicine. Adv Healthc Mater 9:e2000731.

Li YJ, Wu JY, Liu J, Xu W, Qiu X, Huang S, Hu XB, Xiang DX (2021) Artificial exosomes for translational nanomedicine. J Nanobiotechnology 19:242.

Mendanda D, Vieira de Castro J, Ferreira H, Neves NM (2021) Biomimetic and cell-based nanocarriers - new strategies for brain tumor targeting. J Control Release 10:482-493.

Mentor S, Fisher D (2021) High-resolution insights into the in vitro developing blood-brain barrier: novel morphological features of endothelial nanotube function. Front Neuroradiol 15:661065.

Nuzzo D, Picone P (2021) Multiple sclerosis: focus on extracellular and artificial vesicles, nanoparticles as potential therapeutic approaches. Int J Mol Sci 18:8866.

Picone P, Palumbo FS, Federico S, Pitarresi G, Adamo G, Bongiovanni A, Chaves A, Cancemi P, Muccili V, Giglio V, Vetri V, Anselmo S, Sancataldo G, Di Liberto V, Nuzzo D (2021a) Nano-structured myelin: new nanovesicles for targeted delivery to white matter and microglia, from brain-to-brain. Mater Today Biol 7:100146.

Picone P, Porcelli G, Bavisotto CC, Nuzzo D, Galizzi G, Biagio PLS, Bulone D, Di Carlo M (2021b) Synaptosomes: new vesicles for neuronal mitochondrial transplantation. J Nanobiotechnology 19:6.

Picone P, and Nuzzo D (2022) Promising treatment for multiple sclerosis: mitochondrial transplantation. Adv Drug Deliv Rev 135:50-61.

Xia N, Gao Z, Hu H, Li D, Zhang C, Mei X, Wu C (2021) Nerve growth factor loaded macrophage-derived nanovesicles for inhibiting neuronal apoptosis after spinal cord injury. J Biomater Appl 36:276-288.

C-Editors: Zhao M, Liu W, Wang Lu; T-Editor: Jia Y