Full Artificial Exosomes: Towards New Theranostic Biomaterials

Pablo García-Manrique[1,2], Gemma Gutiérrez[1], M. Carmen Blanco-López[2,*]

[1]*Department of Chemical and Environmental Technology, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain*

[2]*Department of Physical and Analytical Chemistry, University of Oviedo, Julián Clavería 8, 33006 Oviedo, Spain*

*Correspondence: cblanco@uniovi.es (M. Carmen Blanco-López)

Abstract:

Bio-nanotechnology routes have been recently developed to produce Full Artificial Exosomes: biomimetic particles aiming to overcome certain limitations regarding Extracellular Vesicles biology and manipulation. These particles could become true therapeutic biomaterials in the near future. Here, we outline the current preparation techniques, their explored and future possibilities and their present limits.

Keywords:

Extracellular Vesicles, Artificial Exosomes, Biomimetic Material, Bio-nanotechnology, Nanomedicine

Extracellular Vesicles: The revolution of cell biology and physiology (365)

The last decade represent a revolution in our knowledge about human body homeostasis based on cell communication. Advances in our comprehension of the development and expansion of several pathologies are greatly due to the understanding of Extracellular Vesicles (EVs) biological behaviour, with special focus on Exosomes [1].

The increasing amounts of data about composition, biogenesis, and roles of exosomes in physiological and several pathologies have opened new possibilities in diagnosis and therapy [2]. Exosomes have unique characteristics that emerge from their cellular origin. These confer them a high value as new biomarkers for diagnosis, stratification

and plannification/evaluation of treatment efficacy. They represent the access to a set of molecules with information about their origin cell and its status through all the lipids, proteins and nucleic acids travelling in both, the membrane and cargo. The opportunity to get all these valuable cellular info and the fact that it comes also from hard-to reach tissues, has promoted the term *liquid-biopsy*, a new frontier in the clinical field.

On the other hand, exosomes combine the advantages of both nanocarriers, (particles for the efficient delivery of molecules), and therapy agents. Nowadays, they are considered as the most promising drug delivery systems, especially for gene therapy in different disorders (such as genetic deficiencies or anti-tumour progression) [3]. This attention is noticed in several papers published during the last 5 years, regarding the modification of targeting moieties and/or the encapsulation of endogenous and exogenous material during exosomes pre- or post-isolation from cell culturesThese represent the development of so-called Exosome-based Semi-Synthetic Nanovesicles, a subtype of artificial exosomes, which englobe all exosomes with modification for intended purposes. The have been even tested for autologous therapy.

Besides the great explosion of techniques for semi-synthetic exosomes development, the main drawbacks concerning their clinical applications are the production, isolation, modification, and purification at large-scale clinical grade. This need is the driving force at the search for a new perspective: the design and manufacture of full synthetic exosomes mimetic particles based on Bio-nanotechnology. The following sections are an overview of these techniques, presented as the two trends in nanofabrication: the Top-Down and Bottom-Up approaches (see **figure 1**).

Top-Down methodologies: Bioengineering Cells as membrane fragments precursors (378)

Top-Down methodologies relies on the production of nanosized material by fragmentation of bigger and more complex units to smaller products. In this case, production of artificial exosomes starts from cultured cells. Different methodologies based on this approach have been developed, mainly for drug delivery, but also for cell

proliferation enhancement and for the generation of exosome mimetic models applied to bio-distribution analysis (**table 1**).

Extrusion over polycarbonate membrane filters is a common practice to reduce and homogenize size distribution of colloidal systems. Applying this technique to cultured cells, NVs for the treatment of tumours by targeted encapsulated chemotherapeutics have been developed with a simple commercial liposome extruder and diminishing pore size filters [4,5]. Moreover, a scaled-up version was developed with a device designed to be used with conventional lab centrifuges [6]. Mass production of NVs with this device was applied in a study for cell proliferation enhancement [7].

The use of microfluidics devices is also reported for artificial exosomes preparation. A simple pressurization over and array of parallel hydrophilic microchannels-based device was described for the production of NVs aimed to endogenous RNA delivery to targeted cell cultures [8]. The fabrication of microchannels on micro-blades (fabricated in silicon nitride) resulted on a device able to slice living cells during their flowing through the channels [9]. The incorporation of exogenous material to the cells suspension enhanced their encapsulation by plasma membrane fragments during re-assembling, and described a method for *in vitro* exogenous material delivery.

All these methods are suitable for the production of higher amounts of effective particles in comparison to natural exosome release yield (over 250 fold times larger). Sizing and biochemical profiling of NVs had high similarities with exosomes. Their ability to exhibit receptors and co-receptors, essential to target and produce effective interactions with receptor cells populations, is of special relevance. And, of course, their natural origin from cells confers them immunotolerance.

Beside these positive characteristics, these methods have some drawbacks. Cargo sorting lacks of selectivity due to passive encapsulation of surrounding medium during self-assembly of membrane fragments. Another relevant issue regarding the production of these mimetic particles is the need of final purifications steps identical to those used for exosome isolation, which requires trained personal and are time-consuming.

Bottom-Up techniques: mimicking plasma membrane through the preparation of artificial bilayers (410)

Bottom-Up techniques, on the other hand, create complex structures of higher order by the manipulation of physical and chemical properties of some molecules (supramolecular chemistry). These methods are well known in the cosmetic and pharmaceutic industry by the preparation of liposomes, particles formed by a bilayered structure of lipids that resemble the plasma membrane. This connection is the starting point to design and create artificial exosomes: the preparation of a synthetic bilayer that is then functionalized with selected proteins to mimic desired exosomal functions.

From the diverse methods for the preparation of liposomes, one of the more employed in the development of mimetic NVs is the Thin Film Hydration Method (TFHM). TFHM is a two-steps process where a dried film of lipids is hydrated by an aqueous media with the compounds to be encapsulated. Artificial exosomes have been produced with a classical liposome formulation [10] and a lipid composition simulating the exosomal one [11]. By chemistry-based bio-conjugation procedures, MHC Class I/peptide complexes and ligand involved in T-Cell receptor interactions and activation have been attached to vesicles for immunotherapy (*ex vivo* and *in vivo* cell expansion). Also the incorporation of APO2L/TRIAL for induction of apoptosis and down-regulation of T-Cell activation in autoimmune diseases, such as antigen-induced arthritis, was reported [11]. These NVs were evaluated in the treatment of hematologic tumour cells [12].

More recently, a different method based on micro-emulsification and micelle assembling was described for the encapsulation of BSA as a model to simulate artificial antigen-presentation to Dendritic Cells [13]. To specifically target DCs, a monoclonal antibody against DEC205 (a highly expressed receptor on the surface of DCs that facilitate endocytosis) was selected.

The main advantage of this strategy relies on the production of a high pharmaceutical grade product, since the final composition is fixed by the selected formulation. However, publications on this topic are still scarce. Besides, since these are methods

adapted from the conventional routes of liposome production of liposomes, expensive high purity lipids are required (especially if functionalization with proteins is going to be carried out). The attachment of multiple molecules to the NVs is also challenging, since conjugation procedures requires stable and specific conditions [12].

On the other hand, encapsulation of nucleic acids is a challenge process that deals with a very unstable type of molecules, and seems that the best encapsulation efficiencies are obtained with cationic lipids, more immunogenic than regular counterparts [13].

Concluding remarks and future perspectives (259)

The incipient recent success in basic and clinical studies by using full artificial exosomes has created the basis of the future nanomedicine. The multidisciplinary approach with contributions from molecular biology, engineering, biotechnology and chemistry will be essential to overcome the limits present in up-today methods. Further research work is necessary to improve the methods of production, but the solution seems to pass through the combination of techniques designed with both approaches: semi- and full synthetic artificial exosomes.

Techniques for the modification of cells prior to exosome isolation, could be perfectly coupled to any Top-Down method in order to tailor artificial exosomes with complementary elements to those natural presented in selected donor cells. Possibly, these could also be used to enhance the physical stability of generated products, a not fully studied property.

Regarding Bottom-Up techniques, microfluidics, a really versatile platform for particle production and drugs encapsulation, represent a very promising approach. This enables rapid testing of multiples formulation thanks to the quickness in product generation, but also with the consuming of significantly less amount of chemicals. In that direction, alternatives to lipids may be explored. Currently, our research group is testing the possibilities of niosomes (vesicles formulated with Non-ionic surfactants) as an alternative to lipid-based particles in the development of artificial exosomes. The main advantages of these vesicles are their wide spectra of starting compounds, more economical, better physical and chemical stability, and high grade of biocompatibility . As new productions routes are improved, novel nanovesicles closer to real exosomes

will be available, making possible personal nanomedicine and theranostics agents adapted to particular needs.

References

[1] Yáñez-Mó, M. *et al.* (2015) Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* 4, 1-60.

[2] Fuhrmann, G. et al. (2015) Cell-derived vesicles for drug therapy and diagnostics: opportunities and challenges. *Nano Today* 10, 397-409.

[3] Van der Meel, R. *et al.* (2014) Extracellular vesicles as drug delivery systems: lessons from the liposome field. *J. Control. Release* 195, 72-85.

[4] Jang, S.C. *et al.* (2013) Bioinspired exosome-mimetic nanovesicles for targeted delivery of chemotherapeutics to malignant tumors. *ACS Nano* 7, 7698-7710.

[5] Hwang, D. W. *et al.* (2015) Noninvasive imaging of radiolabelled exosome-mimetic nanovesicles using ^{99m}Tc-HMPAO. *Sci. Rep.* 5, 15636.

[6] Jo, W. *et al.* (2014) Large-scale generation of cell-derived nanovesicles. *Nanoscale* 6, 12056-12064.

[7] Jeong, D. *et al.* (2014) Nanovesicles engineered from ES cells for enhanced cell proliferation. *Biomaterials* 35, 9302-9310.

[8] Jo, W. *et al.* (2014) Microfluidic fabrication of cell-derived nanovesicles as endogenous RNA carriers. *Lab Chip* 14, 1261-1269.

[9] Yoon, J. *et al.* (2015) Generation of nanovesicles with sliced cellular membrane fragments for exogenous material delivery. *Biomaterials* 59, 12-20.

[10] De La Peña, H. *et al.* (2009) Artificial exosomes as tools for basic and clinical immunology. *J. Immunol. Methods* 344, 121-132.

[11] Martinez-Lostao, L. *et al.* (2010) Liposome-bound APO2L/TRAIL is an effective treatment in a rabbit model of rheumatoid arthritis. *Arthritis Rheum.* 62, 2272-2282.

[12] De Miguel, D. et al. (2013) Liposomes decorated with APO2L/TRAIL overcome chemoresistance of human hematologic tumor cells. *Mol. Pharmaceutics* 10, 893-904.

[13] Li, K. *et al.* (2015) A novel micro-emulsion and micelle assembling method to prepare DEC205 monoclonal antibody coupled cationic nanoliposomes for simulating exosomes to target dendritic cells. *Int. J. Pharm.* 491, 105-112.

Table 1. Full artificial exosomes published works based on Top-Down and Bottom-Up Bio-nanotecnology.

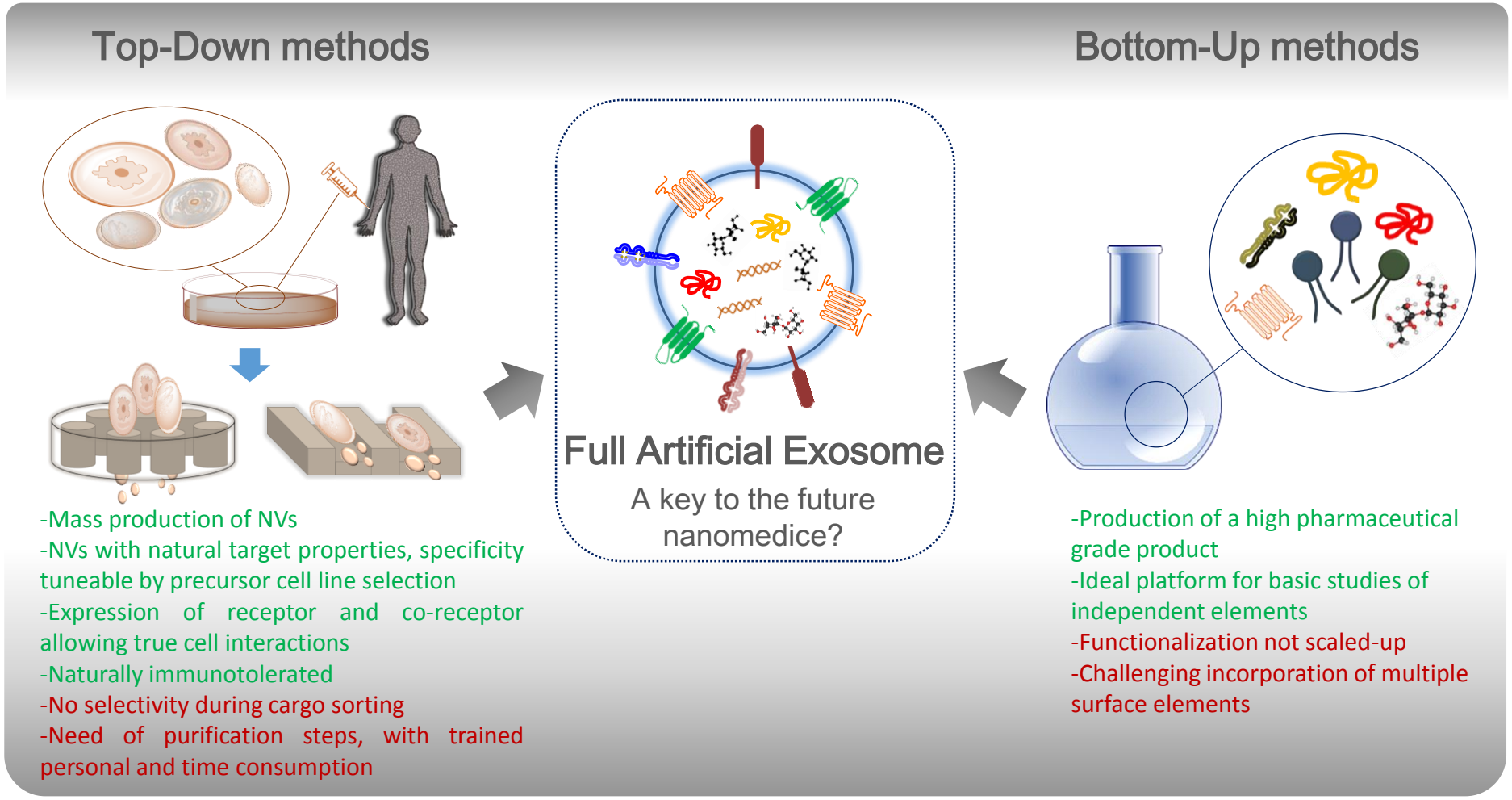
Works based on Top-Down approach				
Mechanism of membrane fragmentation	**Precursor Cell lines**	**Type and examples of material incorporated**	**Application**	**Reference**
Manual extrusion over polycarbonate membrane filters with a device for liposome preparation	Human Monocytes (U937) and Murine mouse Macrophages (Raw 264.7)	Exogenous, chemotherapeutic drugs (Doxorubicin, 5-FU, gemcitabine and carboplatin)	Targeted delivery of chemotherapeutics to an *in vitro* model (TNFα- treated HUVEC) and *in vivo* induced malignant tumours (CT26 mouse colon adenocarcinoma cells)	[4]
	Murine mouse Macrophages (Raw 264.7)	Exogenous, Radiolabelling agent ^{99m}Tc-HMPAO	*In vivo* bio-distribution of exosomes and artificial counterparts	[5]
Centrifugal-induced extrusion over membrane filters in a device designed to be used in lab centrifuges	Murine mousse embryonic stem cell line-D3	Endogenous, Precursor cells characteristic RNA (mOct 3/4 and mNanog)	Gene delivery to NIH-3T3 fibroblast cells	[6]
	Murine mousse embryonic stem cell line-D3	No intention to encapsulate any specific compounds	Enhance *in vitro* cell proliferation for regenerative medicine (Mice skin fibroblasts)	[7]
Pressurization over hydrophilic microchannels array on a microfluidic device	Murine mousse embryonic stem cell line-D3	Endogenous, Precursor cells characteristic RNA (mOct 3/4 and mNanog)	Gene delivery to NIH-3T3 fibroblast cells	[8]
Living cells slicing with silicon nitride blades in a microfluidic device	Murine mousse embryonic stem cell line-D3	Exogenous, Polystyrene beads as representative exogenous material	Material delivery to Mousse embryonic fibroblasts	[9]
Works based on Bottom-Up approach				
Type of formulation	**NVs preparation strategy**	**Proteins for NVs functionalization**	**Application**	**Reference**
Classical liposome, PC:Chol	Thin Film Hydration Method (TFHM) and maleimide based bio-conjugation strategy	MHC Class I peptide complexes and FAB regions against T-Cell receptors for adhesion, early and late activation, and survival.	*Ex vivo* and *In vivo* T-Cell expansion for immunotherapies	[10]
Mimicking exosomes lipid composition, PC:Chol:SM	TFHM and Ni^{2+}/His-Tag protein coordination as bio-conjugation strategy	APO2L/TRIAL-His_{10} recombinant proteins	Down-regulation of T-Cell activation in an autoimmune disease animal model (antigen-induced arthritis) Immunotherapy for apoptosis induction in hematologic tumours	[11-12]
Innovative liposomes, PC:CpEL:DOPE	Micro-emulsion and micelle assembling method for vesicle formation	Monoclonal antibody against DEC205 Dendritic Cell antigen	Probe of concept of artificial antigen presentation to Dendritic Cells	[13]

^{99m}Tc-HMPAO: ^{99m}Tc-Hexamethylpropyleneamineoxime; **Chol**: Cholesterol; **CpEL**: Chemopor EL; **DOPE**: Dioleoyl-Phosphoethanolamine; **HUVEC**: Human Umbilical Vein Endothelial Cells; **MHC**: Major Histocompatibility Complex; **NVs**: Artificial exosomes, here referred as the general term Nanovesicles; **PC**: Phosphatidylcholine; **SM**: Sphingomyelin; **TFHM**: Thin Film Hydration Method;

Figure captions:

Fig.1. Advantages (green) and disadvantages (red) of the two approaches for bio-nanotechnological development of full artificial exosomes as theranostic agents.

Figure


Top-Down methods
Bottom-Up methods
Full Artificial Exosome
A key to the future nanomedice?
-Mass production of NVs
-NVs with natural target properties, specificity tuneable by precursor cell line selection
-Expression of receptor and co-receptor allowing true cell interactions
-Naturally immunotolerated
-No selectivity during cargo sorting
-Need of purification steps, with trained personal and time consumption
-Production of a high pharmaceutical grade product
-Ideal platform for basic studies of independent elements
-Functionalization not scaled-up
-Challenging incorporation of multiple surface elements