MICROENCAPSULATION TECHNOLOGY BY NATURE: CELL DERIVED EXTRACELLULAR VESICLES WITH THERAPEUTIC POTENTIAL

Running Title: Extracellular vesicles with therapeutic potential

Á. Kittel[1*], A. Falus[2], E. Buzás[2]

1 Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

2 Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary

*Corresponding author: Agnes Kittel

Department of Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony u. 43. 1083 Budapest, Hungary

E-mail: kittel.agnes@koki.mta.hu

Phone: +36 1 210 9400

Fax: +36 1 210 9423

Cell derived extracellular vesicles are submicron structures surrounded by phospholipid bilayer, and released by both prokaryotic and eukaryotic cells. The sizes of these vesicles roughly fall into the size ranges of microbes, and they represent efficient delivery platforms targeting complex molecular information to professional antigen presenting cells. Critical roles of these naturally formulated units of information have been described in many physiological and pathological processes. Extracellular vesicles are not only potential biomarkers and possible pathogenic factors in numerous diseases, but they are also considered as emerging therapeutic targets and therapeutic vehicles. Strikingly, current drug delivery systems, designed to convey therapeutic proteins and peptides (such as liposomes), show many similarities to extracellular vesicles. Here we review some aspects of therapeutic implementation of natural, cell-derived extracellular vesicles in human diseases. Exploration of molecular and functional details of extracellular vesicle release and action may provide important lessons for the design of future drug delivery systems.

Keywords: microparticles; exosomes; vaccination

Abbreviations: EVs (extracellular vesicles), MVs (microvesicles),

Introduction

Over the past few decades experimental data started to accumulate in support of the existence of extracellular vesicles (EVs) in the size range earlier thought to be occupied only by microorganisms. It is only recently that the universality of EV secretion has been recognized by the exploration of vesiculation also in prokaryotes [1-3]. The microorganism-sized EVs are readily taken up by cells of the immune system. Of note, many of these vesicles are released during apoptosis, and the immune system's "high throughput" homeostatic clearance machinery for the uptake of vesicles of apoptotic origin is highly efficient. This process is mediated by phagocyte receptors (e.g. phosphatidyl serine receptor, TIM-4 or TAM receptors [4, 5] ensuring the rapid internalization of EVs by cells. Importantly, endocytosis is not the only uptake mechanism of EVs. Direct fusion of their membrane with the plasma membrane of specifically recognized cells has been suggested as another uptake mechanism (for recent review see [6]. In this case the content of EVs is released directly into the cytoplasm of the targeted cell [7]. The efficient cellular uptake renders EVs attractive candidate vehicles to deliver selected molecules to cells.

There is a striking analogy between currently used pharmaceutical drug delivery systems such as liposomes or microparticles designed to deliver proteins or peptides (Figures 1 and 2). In general, encapsulation of molecules for targeted delivery provides protection against enzymatic degradation, aggregation, or precipitation. Also, encapsulation ensures high local concentration of substances at a distant, targeted site. It is tempting to speculate that the phospholipid bilayer "capsule" of cell-derived EVs serves similar biological purposes. In line with this hypothesis, recently, EVs have been suggested to function as multipurpose carriers i) to deliver complex information to other cells ii) for safe removal of potentially harmful molecules and iii) aiding and extending functions of the donor cells [8].

In contrast to liposomes, EVs are derived from cells of the body, thus, they are composed of self molecules tolerated by the immune system. They can be used for prolonged time safely. On the other hand, liposomes may be manufactured in large scale synthetically at relatively low expenses.

In the current article, we aim at briefly summarizing some basic concepts and therapeutic implications of EVs to attract attention to a rapidly evolving field that emerges in parallel with development of nanomedicines as drug delivery systems. We propose that novel therapeutic strategies may benefit from lessons of the evolutionary conserved, natural vesicular structures.

Overview of EVs

Classification and nomenclature of cell derived EVs

It has been suggested that major subpopulations of EVs include exosomes, microvesicles and apoptotic bodies [9].

Multivesicular bodies (MVBs) of the endocytotic compartment fuse with the plasma membrane, and release vesicles (50-100 nm in diameter) designated as exosomes [10, 11]. Another pathway of vesicle release involves budding of the plasma membrane with ultimate release of membrane surrounded vesicles referred to as microvesicles (MVs) often referred to as microparticles or ectosomes [12]. While the size range of exosomes roughly overlaps with that of viruses, vesicles, generated by budding, have larger diameter (100-1000 nm) corresponding approximately to the size range of bacteria or insoluble immune complexes [9, 13]. While exosomes are generated both constitutively and upon activation, the release of microvesicles is induced during apoptosis and activation [7, 9]. Both major types of EVs are encapsulated by a phospholipid bilayer membrane rich in cholesterol.

However, recent evidences support that there are numerous further subtypes of EVs. As an example, Théry et al. provided evidence for the existence of diverse populations secreted by different intracellular mechanisms [14].

As yet there is no international consensus regarding the terminology of EVs [15].

Detection methods of EVs

Detection of EVs imposes significant challenge on cell biologists, since conventional/ routine cell biology methodologies can not be applied for the investigation of these subcellular structures. Fluorescence microscopy and flow cytometry cannot be used for the analysis of exosomes, unless the vesicles are bound onto the surface of beads [16]. The analysis of not only exosomes but also of larger sized MVs, have limitations with conventional techniques. Flow cytometry fails to detect structures with less than 2-300 nm in diameter. Methodologies, used to characterize pharmaceutical liposome or microparticle/nanoparticle preparations, are more appropriate for studying cell derived EVs.

Transmission electron microscopy (in particular immune electron microscopy) has proven very useful for the detection and analysis of cell-derived vesicles irrespective of their size [17, 18]. Electron microscopy of exosomes shows a so called “cup shape” after isolation by sucrose gradient/cushion ultracentrifugation (that was suggested to be an artifact of preparation), while microvesicles are characterized by spherical shape with cryo- transmission electron microscopy [19]. Cryo-electron tomography microsopy was shown to be useful to avoid such types of artifacts [20, 21]. Also, scanning electron microscopy [22] single particle electrom microscopy [23] and atomic force microscopy was used successfully to visualize individual EVs [13, 22]. Further non-conventional techniques of analysis (suitable for the analysis of EVs and liposomes) include dynamic light scattering analysis (DLS), nanoparticle

tracking analysis (NTA) [24, 25] and Fluorescence Nanoparticle Tracking Analysis (F-NTA) [26], Raman spectroscopy based techniques, Stimulated Emission Depletion (STED) microscopy, Impedance-based flow cytometry and resistive pulse sensing [27]. Presumably, the increasing demands of this research field will boost the development of further specific methodologies and user-friendly laboratory instruments fitted to the size range of EVs.

Biological functions

EVs are important rescently recognized players of intercellular communication [12, 27, 28]. They are known to disseminate, support, and protect basic biological functions of the releasing cells. Exosomes have been shown to mediate horizontal transfer of mRNA, miRNA [29] and different types of cell surface receptors such as an oncogenic receptor [30] or purinergic P2X7 receptor [31]. One of the most important functions described in association with exosomes, is antigen presentation, a function earlier attributed to antigen presenting cells only [32]. Exosomes display both MHC-I and MHC-II molecules on their surface assembled with antigenic peptides. This feature has significant impact on the ability of EVs (such as exosomes) to induce immune responses upon injection as vaccines [33, 34]. Consequently, immunoregulation (including either stimulation or inhibition) is a principal function of exosomes, depending on the cellular source and target of the vesicles [12]. This feature raises the intriguing possibility of therapeutic immune modulation by exosomes.

Although immune regulatory functions of the larger sized MVs have also been reported (e.g. in the fetomaternal communication [35]), their best characterized function is the one they play in blood coagulation: they have significant procoagulant activity [36-39]. Similarly to exosomes, MVs represent a form of secretion of IL1 beta [31, 40] and have been suggested to contribute to the pathogenesis of rheumatoid arthritis [41, 42]. By their protease [43], and

possibly also by their glycosidase expression [44], MVs may contribute to the proinvasive character of tumors.

Therapeutic targeting of EVs

A few years after that the original concept of liposomes was raised by Bangham et al. in 1965 [45], these artificial lipid vesicles were suggested to be used as drug carriers [46], and a novel drug delivery system, liposomal encapsulation of drugs, has been introduced [47]. Currently, in the „nano era", liposomes are frequently referred to as nanoparticles, and their use represents an organic part of nanomedicine. However, besides all benefits of engineered liposomes (in the case of which biocompatibility and biodegradability is evident), the use of EVs may be more favorable. These nature-encapsulated subcellular structures have been suggested for therapeutical delivery of molecules [48] and it may represent novel tools in future personalized medicine and in efficient and site-specific delivery of therapeutic drugs or nucleic acids.

Moreover, secreted EVs are not only nature-tailored carrier vehicles with potential therapeutic exploitation, but they also represent promising drug targets. As mentioned above, a wide variety of human diseases are characterized by elevated numbers and altered composition of circulating EVs. While in some cases their increased number may reflect general cellular activation or enhanced apoptosis, EVs may also substantially contribute as effectors to disease development. They were shown to contribute to tumor growth, migration and invasion, angiogenesis and tumor escape from immune responses (reviewed recently [49]. Therefore prevention of EV release or therapeutic removal of released vesicles from the circulation might also represent a therapeutic approach.

Tumor-derived exosomes are known biologic messengers in cancers, are mediators of tolerance induction and are shown to spread tumor growth signals that counteract the activity of therapeutic agents [50]. Therefore, therapeutic targeting of tumor cell derived exosomes represent an important therapeutic approach. The extracorporeal haemofiltration of circulating factors as a therapeutic strategy is already approved to be used in cancer patients [50].

EVs of pathogens in health and disease

Functional virus release has been reported to involve several elements from the EV biogenesis patways [51]. EVs have been shown to play either enhancing or blocking roles in infections and and represent removal systems for endogenous retroviruses or retrotransposons [51]. Recently, fraction of Adeno associated virus (AAV) vectors have been shown to be associated with EVs(vector-exosomes) and have been suggested for improved promising strategy to improved gene delivery [52].

EVs have been demonstrated to be secreted by Gram-negative [53, 54] and Gram-positive bacteria [55, 56], as well as eukaryotic parasites of the kinetoplast lineage and opportunistic fungi of both the ascomycetes and basidiomycetes lineages [57].

Outer membrane vesicles (OMVs) of many pathogenic bacteria contribute to the virulence of the releasing bacterial cells. Importantly, OMVs have been recently suggested to serve as a basis of non-replicating vaccines summarized by Ünal et al. [58].

A disease in which EV vaccination was proposed is sepsis, associated with increased proinflammatory cytokine levels and the accumulation of apoptotic cells. In the toxoplasmic model of sepsis, Toxoplasma gondii-pulsed Dex could stimulate a specific and protective T-cell response in CBA/J mice [59]. Although the mechanism remained unclear, and presumably activators of DCs, B-, T or NK cells may have contributed to the efficacy of

exosomes against Toxoplasma gondii infection in this congenital model, Dex appears to be a potentially useful tool for vaccination in sepsis.

In other models, exosomes derived from immature dendritic cells rescued septic animals because of the presence of milk fat globule epidermal growth factor (EGF)-factor VIII (MFG-E8) on their surface. MFG-E8 is required to opsonise cells for phagocytosis, which has to be promoted in septic animals to prevent the release of the potentially harmful substances from dying cells. An increased phagocytosis eventually reduces mortality, and attenuates the release of proinflammatory cytokines in the septic rats [60].

Conclusions

The ubiquitous feature of vesiculation by both eukaryotes and prokaryotes, has been established only recently. Both Gram negative and positive bacteria as well as fungi were shown to release these structure, and more recently, also the significance of plant derived apoplastic exosome-like vesicles have been suggested [61].

It is currently a unique situation that cell-derived EVs can be considered both as novel drug targets and natural drug delivery systems.

Unfolding diseases in which EVs play effector roles, may lead to development of EV targeting therapeutic strategies (such as prevention of vesicle relase or to removal of secreted ones). On the other hand, manufacturing EVs for therapeutic applications is feasible *in vitro* inducing vesicle secretion by various stimuli. *In vitro* manipulation (e.g. transfection) of the releasing cells provides unique opportunity to produce tailored EVs with customized effector or targeting molecules. Vesicles, harvested from tissue culture supernatants, may be injected to modulate immune functions or to vaccinate against epitopes presented on vesicular surfaces in the context of MHC molecules. EVs are of proper size for uptake by cells, non-toxic, biodegradable, carry surface molecules that direct them to targeted cells, and carry complex

information. To date, especially exosomes have been shown to have a great potential. However, larger sized MVs, currently considered as biomarkers in body fluids such as blood plasma, urine or saliva, are far less characterized, and may hold yet unexplored therapeutic or vaccination potential. Vaccination by EVs or removal of circulating exosomes by haemofiltration are the types of exploitation of EVs have been already introduced to clinical practice.

A recent study has directly compared liposomes and exosomes as drug delivery systems for encapsulation curcumin in them. In a proof-of-principle study of Sun et al. it has been demonstrated that encapsulation of the antiinflammatory agent curcumin in exosomes was significantly superior to liposomal delivery as shown by the enhanced stability and higher concentration in the blood as well as higher therapeutic efficacy in (LPS)-induced septic shock mouse model [62].

Even though EVs may offer novel opportunities for prevention or therapeutic intervention in disease states in which patients do not respond to conventional therapies, one has to be aware of the risks also. Given that viruses and exosomes share size distribution and other biophysical parameteres, concerns center on potential contamination of exosome preparations with viruses. Development of safe technologies of large scale production of virus-free exosomal preparations is an absolute prerequisite of their therapeutic exploitation.

What appears to be clear is that is that researchers developing artificial drug delivery systems and those exploring EVs need to have an intense communication, and they should both follow the progress in the other field. The two scientific communities must recognize the possible mutual benefits of such an interaction. EV scientists have already taken advantage of methodologies originally used for the characterization of microbes, liposomes or other nanoparticles (such as DLS, AFM or NTA). Proof for the benefit for drug developers is best

examplified by the recent development of artificial exosomes. These are liposomes coated with MHC/peptide complexes and Fab regions against T cell receptors to mediate cell surface adhesion [63, 64]. Presumably many further experiments will be inspired by lessons of natural EVs that successfully overcame evolutional challenges. Studies focusing on nanomedicinal drug delivery systems and nature-tailored vesicules may cross-fertilize one another, and may lead to novel therapeutical solutions.

Acknowledgements

This work was supported by grants NK 84043 and FP7-PEOPLE-2011-ITN-PITN-GA-2011-289033 "DYNANO". The authors are grateful to Bence György and Tamás G Szabó for careful reading the manuscript.

References

1. Mayrand DGrenier D: Biological activities of outer membrane vesicles. Can J Microbiol, 6, 607-13 (1989)
2. Jones S: Stressed? Time to vesiculate. . Nature Reviews Microbiology, 86-87 (2007)
3. Mashburn-Warren LM, Whiteley M: Special delivery: vesicle trafficking in prokaryotes. Mol Microbiol, 4, 839-46 (2006)
4. Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T et al.: Identification of Tim4 as a phosphatidylserine receptor. Nature, 7168, 435-9 (2007)
5. Lemke G, Rothlin CV: Immunobiology of the TAM receptors. Nat Rev Immunol, 5, 327-36 (2008)
6. D'Asti E, Garnier D, Lee TH, Montermini L, Meehan B et al.: Oncogenic extracellular vesicles in brain tumor progression. Front Physiol, 294 (2012)
7. Cocucci E, Racchetti G, Meldolesi J: Shedding microvesicles: artefacts no more. Trends Cell Biol, 2, 43-51 (2009)
8. Nieuwland R, Sturk A: Why do cells release vesicles? Thromb Res, S49-51 (2010)
9. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P et al.: Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci, 16, 2667-88 (2011)
10. Chaput N, Thery C: Exosomes: immune properties and potential clinical implementations. Semin Immunopathol, 5, 419-40 (2011)
11. Ostrowski M, Carmo NB, Krumeich S, Fanget I, Raposo G et al.: Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol, 1, 19-30; sup pp 1-13 (2010)
12. Théry C, Ostrowski M, Segura E: Membrane vesicles as conveyors of immune responses Nat Rev Immunol, 581-93 (2009)
13. Gyorgy B, Modos K, Pallinger E, Paloczi K, Pasztoi M et al.: Detection and isolation of cell-derived microparticles are compromised by protein complexes resulting from shared biophysical parameters. Blood, 4, e39-48 (2011)
14. Bobrie A, Colombo M, Krumeich S, Raposo G, Théry C: Diverse subpopulations of vesicles secreted by different intracellular mechanisms are present in exosome preparations obtained by differential ultracentrifugation. Journal of Extracellular Vesicles, 1, (2012)
15. Gould SJ, Raposo G: As we wait: coping with an imperfect nomenclature for extracellular vesicles. Journal of Extracellular Vesicles, 20389 (2013)
16. Lasser C, O'Neil SE, Ekerljung L, Ekstrom K, Sjostrand M et al.: RNA-containing exosomes in human nasal secretions. Am J Rhinol Allergy, 2, 89-93 (2011)
17. Hooper C, Sainz-Fuertes R, Lynham S, Hye A, Killick R et al.: Wnt3a induces exosome secretion from primary cultured rat microglia. BMC Neurosci, 144 (2012)
18. van der Pol E, Boing AN, Harrison P, Sturk ANieuwland R: Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev, 3, 676-705 (2012)
19. Cloutier N, Tan S, Boudreau LH, Cramb C, Subbaiah R et al.: The exposure of autoantigens by microparticles underlies the formation of potent inflammatory components: the microparticle-associated immune complexes. EMBO Mol Med, 2, 235-49 (2013)
20. Coleman BM, Hanssen E, Lawson VAHill AF: Prion-infected cells regulate the release of exosomes with distinct ultrastructural features. FASEB J, 10, 4160-73 (2012)
21. Tatischeff I, Larquet E, Falcón-Pérez JM, Turpin P-Y, Kruglik SG: Fast characterisation of cell-derived extracellular vesicles by nanoparticles tracking analysis, cryo-electron microscopy, and Raman tweezers microspectroscopy. Journal of Extracellular Vesicles, 1, (2012)
22. Sharma S, Rasool HI, Palanisamy V, Mathisen C, Schmidt M et al.: Structural-mechanical characterization of nanoparticle exosomes in human saliva, using correlative AFM, FESEM, and force spectroscopy. ACS Nano, 4, 1921-6 (2010)

23. Liu X, Wang HW: Single particle electron microscopy reconstruction of the exosome complex using the random conical tilt method. J Vis Exp, 49, (2011)
24. Gyorgy B, Szabo TG, Turiak L, Wright M, Herczeg P et al.: Improved flow cytometric assessment reveals distinct microvesicle (cell-derived microparticle) signatures in joint diseases. PLoS One, 11, e49726 (2012)
25. Momen-Heravi F, Balaj L, Alian S, Tigges J, Toxavidis V et al.: Alternative methods for characterization of extracellular vesicles. Front Physiol, 354 (2012)
26. Gardiner C, Ferreira YJ, Dragovic RA, Redman CWG, Sargent IL: Extracellular vesicle sizing and enumeration by nanoparticle tracking analysis. 19671 (2013)
27. de Vrij J, Maas SL, van Nispen M, Sena-Esteves M, Limpens RW et al.: Quantification of nanosized extracellular membrane vesicles with scanning ion occlusion sensing. Nanomedicine (Lond), (2013)
28. Raposo G, Stoorvogel W: Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol, 4, 373-83 (2013)
29. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ et al.: Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol, 6, 654-9 (2007)
30. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L et al.: Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol, 5, 619-24 (2008)
31. Dubyak GR: P2X7 receptor regulation of non-classical secretion from immune effector cells. Cell Microbiol, 11, 1697-706 (2012)
32. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV et al.: B lymphocytes secrete antigen-presenting vesicles. J Exp Med, 3, 1161-72 (1996)
33. Xie Y, Wang L, Freywald A, Qureshi M, Chen Y et al.: A novel T cell-based vaccine capable of stimulating long-term functional CTL memory against B16 melanoma via CD40L signaling. Cell Mol Immunol, 1, 72-7 (2013)
34. Ruiss R, Ohno S, Steer B, Zeidler RAdler H: Murine gammaherpesvirus 68 glycoprotein 150 does not contribute to latency amplification in vivo. Virol J, 107 (2012)
35. Pap E, Pallinger E, Falus A, Kiss AA, Kittel A et al.: T lymphocytes are targets for platelet- and trophoblast-derived microvesicles during pregnancy. Placenta, 9, 826-32 (2008)
36. Leroyer AS, Tedgui ABoulanger CM: Role of microparticles in atherothrombosis. J Intern Med, 5, 528-37 (2008)
37. Jayachandran M, Litwiller RD, Lahr BD, Bailey KR, Owen WG et al.: Alterations in platelet function and cell-derived microvesicles in recently menopausal women: relationship to metabolic syndrome and atherogenic risk. J Cardiovasc Transl Res, 6, 811-22 (2011)
38. Aharon A, Tamari TBrenner B: Monocyte-derived microparticles and exosomes induce procoagulant and apoptotic effects on endothelial cells. Thromb Haemost, 5, 878-85 (2008)
39. Briede JJ, Heemskerk JW, Hemker HCLindhout T: Heterogeneity in microparticle formation and exposure of anionic phospholipids at the plasma membrane of single adherent platelets. Biochim Biophys Acta, 1, 163-72 (1999)
40. MacKenzie A, Wilson HL, Kiss-Toth E, Dower SK, North RA et al.: Rapid secretion of interleukin-1beta by microvesicle shedding. Immunity, 5, 825-35 (2001)
41. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS et al.: Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. Science, 5965, 580-3 (2010)
42. Distler JH, Jungel A, Huber LC, Seemayer CA, Reich CF, 3rd et al.: The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. Proc Natl Acad Sci U S A, 8, 2892-7 (2005)
43. Giusti I, D'Ascenzo S, Millimaggi D, Taraboletti G, Carta G et al.: Cathepsin B mediates the pH-dependent proinvasive activity of tumor-shed microvesicles. Neoplasia, 5, 481-8 (2008)

44. Pasztoi M, Nagy G, Geher P, Lakatos T, Toth K et al.: Gene expression and activity of cartilage degrading glycosidases in human rheumatoid arthritis and osteoarthritis synovial fibroblasts. Arthritis Res Ther, 3, R68 (2009)
45. Bangham AD, Standish MMMiller N: Cation permeability of phospholipid model membranes: effect of narcotics. Nature, 5017, 1295-7 (1965)
46. Gregoriadis G, Ryman BE: Fate of protein-containing liposomes injected into rats. An approach to the treatment of storage diseases. Eur J Biochem, 3, 485-91 (1972)
47. Ostro MJ, Cullis PR: Use of liposomes as injectable-drug delivery systems. Am J Hosp Pharm, 8, 1576-87 (1989)
48. O'Loughlin AJ, Woffindale CAWood MJ: Exosomes and the emerging field of exosome-based gene therapy. Curr Gene Ther, 4, 262-74 (2012)
49. Pap E, Pallinger EFalus A: The role of membrane vesicles in tumorigenesis. Crit Rev Oncol Hematol, 3, 213-23 (2011)
50. Marleau AM, Chen CS, Joyce JATullis RH: Exosome removal as a therapeutic adjuvant in cancer. J Transl Med, 134 (2012)
51. Wurdinger T, Gatson NN, Balaj L, Kaur B, Breakefield XO et al.: Extracellular vesicles and their convergence with viral pathways. Adv Virol, 767694 (2012)
52. Maguire CA, Balaj L, Sivaraman S, Crommentuijn MH, Ericsson M et al.: Microvesicle-associated AAV vector as a novel gene delivery system. Mol Ther, 5, 960-71 (2012)
53. Berleman J, Auer M: The role of bacterial outer membrane vesicles for intra- and interspecies delivery. Environ Microbiol, 2, 347-54 (2013)
54. Tashiro Y, Uchiyama HNomura N: Multifunctional membrane vesicles in Pseudomonas aeruginosa. Environ Microbiol, 6, 1349-62 (2012)
55. Keyel PA, Heid ME, Watkins SCSalter RD: Visualization of bacterial toxin induced responses using live cell fluorescence microscopy. J Vis Exp, 68, e4227 (2012)
56. Chernov VM, Chernova OA, Mouzykantov AA, Efimova IR, Shaymardanova GF et al.: Extracellular vesicles derived from Acholeplasma laidlawii PG8. ScientificWorldJournal, 1120-30 (2011)
57. Silverman JM, Reiner NE: Exosomes and other microvesicles in infection biology: organelles with unanticipated phenotypes. Cell Microbiol, 1, 1-9 (2011)
58. Unal CM, Schaar VRiesbeck K: Bacterial outer membrane vesicles in disease and preventive medicine. Semin Immunopathol, 5, 395-408 (2011)
59. Beauvillain C, Juste MO, Dion S, Pierre JDimier-Poisson I: Exosomes are an effective vaccine against congenital toxoplasmosis in mice. Vaccine, 11, 1750-7 (2009)
60. Miksa M, Wu R, Dong W, Komura H, Amin D et al.: Immature dendritic cell-derived exosomes rescue septic animals via milk fat globule epidermal growth factor-factor VIII [corrected]. J Immunol, 9, 5983-90 (2009)
61. Regente M, Pinedo M, Elizalde Mde la Canal L: Apoplastic exosome-like vesicles: a new way of protein secretion in plants? Plant Signal Behav, 5, 544-6 (2012)
62. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S et al.: A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther, 9, 1606-14 (2010)
63. De La Pena H, Madrigal JA, Rusakiewicz S, Bencsik M, Cave GW et al.: Artificial exosomes as tools for basic and clinical immunology. J Immunol Methods, 2, 121-32 (2009)
64. Jubeli E, Moine L, Vergnaud-Gauduchon JBarratt G: E-selectin as a target for drug delivery and molecular imaging. J Control Release, 2, 194-206 (2012)

Figures and Figure Legends

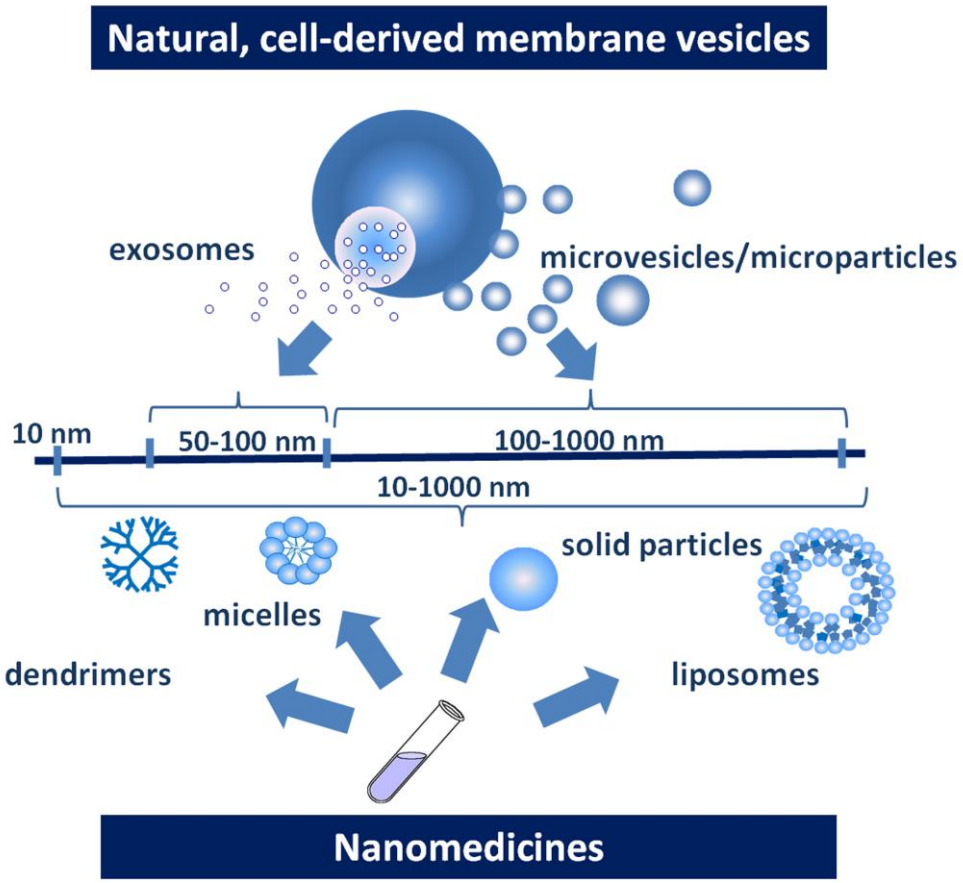

Figure 1. Comparison of size ranges of different natural membrane vesicles and nanomedicines

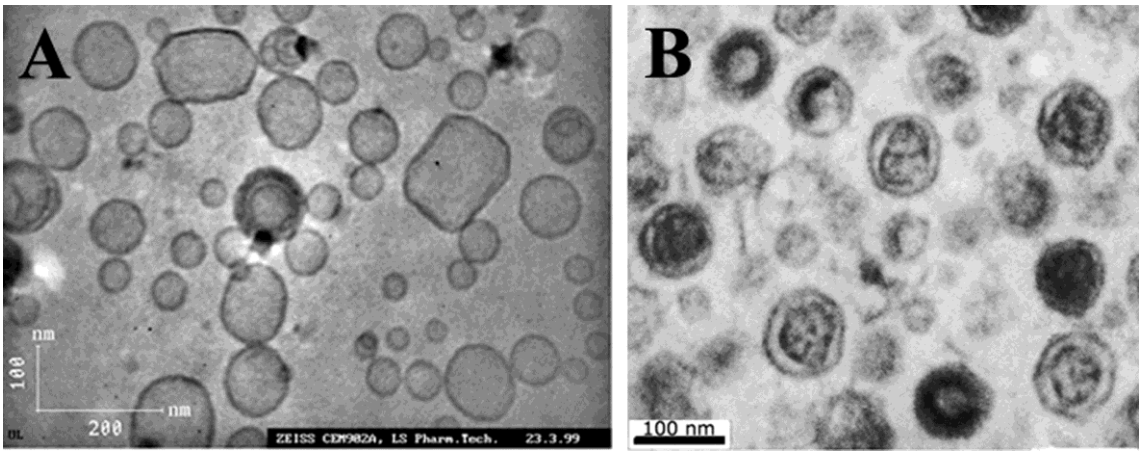

Figure.2. Cryo-electron microscopic image of liposomes (A) (*http://www.mardre.com/homepage/mic/tem/samples/colloid/pc_samples/dmpc_liposome_cryo3.jpg*) and 5/4 T hybridoma cell exosomes (B) show high similarity in both size distribution and their shape