63° SIMPOSIO AFI RIMINI 5·6·7 GIUGNO2024

Associazione Farmaceutici Industria Società Scientifica

TITOLO (maiuscolo)

LYOPHILIZATION AS A TOOL FOR ENHANCING THE STABILITY OF PATIENT-DERIVED EXTRACELLULAR VESICLES

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Riassunto

Carattere: ARIAL
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Extracellular vesicles (EVs) are a mixed population of lipid nanoparticles containing proteins, nucleic acids, and metabolites produced by all cells of the organism and play a key role as natural signaling agents in intercellular communication, thus contributing to several physiological and pathological processes. Recently, it has been postulated that the cellular origin unequivocally determines the homing characteristics of EVs. In another words, EVs derived from tumor cells would exhibit a selective tropism for neoplastic tissue. This feature in combination with the ability to cargo therapeutic and/or diagnostic agents provides new opportunities for personalized medicine, limiting the side effects when drugs are systemically administered to patients. However, to allow their clinical use, many hurdles still need to be overcome. Among them, storage stability is a big challenge since EVs are relatively unstable in liquid state. To standardize the storage and handling procedure, the International Society of EVs supports the storage at -80 °C in phosphate-buffered saline, even if freeze/thaw cycles can affect the biophysical stability depending on the vesicles source.
Considering the experience of pharmaceutical industries, lyophilization is the most common procedure to achieve easy-handling solid formulation. Nevertheless, numerous stresses caused during freezing and drying can result in EV damage unless appropriate stabilizers are added.
Based on these considerations, this work aims to develop a lyophilized formulation for EVs derived from a tumor cell line (MCF-7) with long-term stability. The cryoprotectant/lyoprotectant effect of trehalose, sucrose, or combinations thereof was tested by evaluating changes in particle size and concentration of EVs measured by DLS and NTA. The final composition of medium in which EVs were reconstituted was tuned up in order to satisfy three main requirements: (i) osmolality ranging 285±15 mOsm/Kg, (ii) compatibility and (iii) preservation of the bioactivity. The freeze-drying process was established on the bases of DSC data. Among the tested excipients, only trehalose allowed to retain the size and the particle concentration with respect to the fresh EV, either as cryoprotectant and lyoprotectant of EVs (**Table 1**).

Table 1 – NTA data on fresh, freeze-thawed and lyophilized EVs.

	D_{10} (nm)	D_{50} (nm)	D_{90} (nm)	Part/mL (e+11)
EVs in PBS	123.9±2.9	168.9±5.3	299±20.5	1.43±0.06
thawed EVs in PBS	138.6±2.4	188.1±7.8	320.5±5.9	1.34±0.06
thawed EVs in PBS and trehalose	119.5±1.0	162.4±4.2	260.8±2.4	1.55±0.06
Reconstituted EVs after freeze-drying	123.1±1.0	169.5±0.5	263.5±9.9	1.31±0.13

Upon reconstitution of the freeze-dried products, EV colloidal stability was effectively preserved only by addition of trehalose (**Table 1**). It is worth emphasizing that despite a downregulation in certain protein components, most notably for α-tubulin, lyophilized EVs retained the tumor-targeting property when tested in the murine models of subcutaneous tumors. Interestingly, particle size and concentration of EVs as well as their homing ability was retained over 4 months at 2-8 °C. In conclusion, lyophilization can be proposed to preserve EVs, thus expanding their potential scope of clinical applications.

Aknowledgments: European Union — NextGenerationEU (PNRR M4C2-Investimento 1.4-CN00000041-23 PNRR_CN3RNA_SPOKE8 and PNRR_CN3RNA_SPOKE9)

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