Exosome isolation

Isolation and purification of exosomes from blood plasma by ultracentrifugation using a two-step approach

Introduction

Three types of extracellular vesicles (EVs) are classified according to their morphology and origin: exosomes, microvesicles (MVEs), and apoptotic bodies. Exosomes (30–150 nm in diameter) are generated by inward budding of endosomal membranes, while MVEs (100–1,000 nm in diameter) are formed by outward budding of the plasma membrane (Figure 1) [1-3]. Exosomes can be found in nearly all bodily fluids, including blood, semen, urine, breast milk, saliva, amniotic fluid, sputum, and cerebrospinal fluid.

The characteristics and cargo of EVs are influenced by the type and state of the cells involved in their generation. Their wide variety of cargo includes microRNA, mRNA, lncRNA, DNA, lipids, peptides, and an array of proteins like oncoproteins, tumor suppressors, transcriptional regulators, and splicing factors [1-6].

Since exosomes can cross the blood–brain barrier, they have been studied primarily for their potential as biomarkers and therapeutic delivery systems. Fluid biopsy is a minimally invasive procedure that is required to analyze biomarkers in blood or urine [1]. Therefore, it is common to isolate exosomes from human blood plasma.

It may be difficult to isolate exosomes from plasma because of their similar size (60–200 nm), and lipoprotein precipitation could occur. To ensure that pure exosomes are extracted from blood plasma, a two-step approach is recommended. The primary purpose of this white paper is to provide a workflow for exosome isolation from human blood plasma. Exosomes are first isolated from blood plasma by differential ultracentrifugation. A continuous density gradient is then used to purify the pellets [7-12]. The complete workflow for exosome isolation from plasma and density gradient purification is shown in Figure 2 [11].

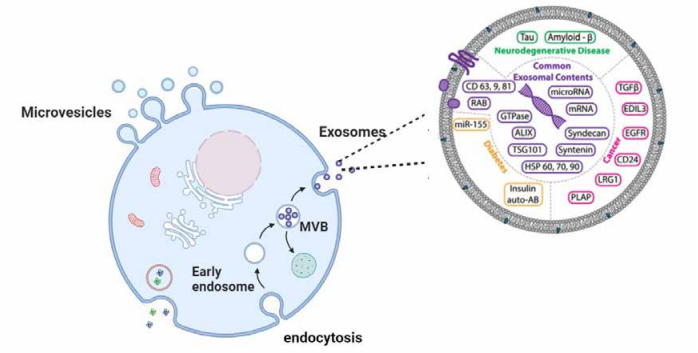

Figure 1. The exosome biogenesis pathway [1]. Exosome biogenesis occurs within multivesicular bodies (MVBs) of the endosomal system. Figure used under terms of the Creative Commons Attribution 4.0 International Public License.

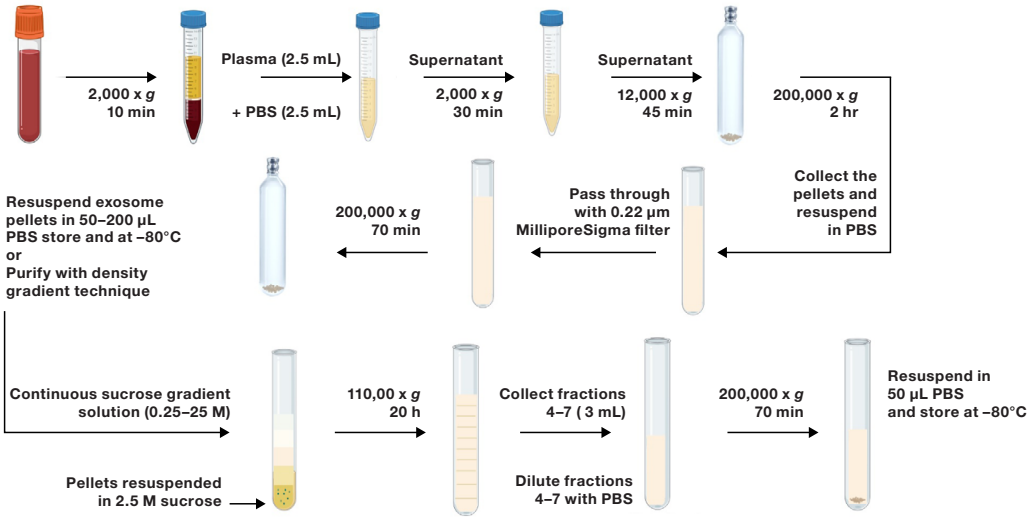

Figure 2. Workflow for exosome isolation and purification [11]. Courtesy of UQ Centre for Clinical Research, Centre for Clinical Diagnostics and Department of Obstetrics and Gynecology, Faculty of Medicine, Universidad de Los Andes, Santiago, Chile.

Methods

Step 1. Isolation of exosomes from blood plasma

To isolate exosomes from blood plasma, blood samples must be treated with EDTA before they can be processed [7-11].

1. Collect blood samples in EDTA-treated tubes (5 mL BD Vacutainer™ Plus plastic plasma tubes).
2. Centrifuge EDTA-treated blood samples at 2,000 x *g* for 10 min at 4°C to separate blood plasma [11].
3. Dilute 2.5 mL of plasma with an equal volume of Gibco™ PBS (pH 7.4) and mix well [11].
4. Centrifuge the mixture of plasma and PBS at 2,000 x *g* for 30 min at 4°C [11].
5. Centrifuge the supernatant at 12,000 x *g* for 45 min at 4°C [11].
6. Pipet off 5 mL of the supernatant and transfer to a Thermo Scientific™ PA Ultracrimp Tube [11].
7. Centrifuge the supernatant at 200,000 x *g* for 2 hr at 4°C in a Thermo Scientific™ Sorvall™ WX+ Ultracentrifuge with a Thermo Scientific™ T-8100 Fixed Angle Rotor [11].
8. Aspirate the supernatant and resuspend the pellets in 5 mL sterile PBS [11].
9. Filter the mixture of supernatant and PBS with a 0.22 µm MilliporeSigma™ Steritop™ syringe filter [11].
10. Centrifuge the filtrate at 200,000 x *g* for 70 min at 4°C in a Sorvall WX+ Ultracentrifuge with a T-8100 Fixed Angle Rotor [11].
11. Wash the pellets and resuspend them in 50–200 µL of PBS. The pellets can be stored at –80°C until the second step of density gradient ultracentrifugation [7-11].

Step 2. Purification of isolated exosomes by continuous sucrose gradient

Isolated exosome pellets can be further purified using buoyant (isopycnic) density gradient ultracentrifugation.

1. Add isolated exosome pellets from step 1 to a 2.5 M sucrose mixture (4 mL) and transfer to a 36 mL thin-walled ultracentrifuge tube [11].
2. Prepare 26 mL of continuous sucrose gradient solution (0.25–25 M) and slowly layer resuspended pellets in PBS on top of the 2.5 M sucrose-containing exosome pellets [11].
3. Centrifuge at 110,000 x *g* for 20 hr at 4°C in a Thermo Scientific™ SureSpin™ 632 Swing-out Ultracentrifuge Rotor [11].
4. After completing centrifugation, collect 4 fractions from the tube automatically using a pulse-free Gilson™ MINIPULS™ 3 Peristaltic Pump at a flow rate of 3 mL/min and the Gilson™ FC 203B fraction collector [11].
5. Measure the density of each fraction (3 mL) by refraction index with a Bellingham and Stanley™ OPTi™ Digital Refractometer [11]. Plot the density of each fraction in g/mL as shown in Figure 3.
6. For further analysis, fractions enriched with isolated exosomes can be combined, diluted with 60 mL PBS, and centrifuged at 200,000 x *g* for 70 min at 4°C with the T-8100 Fixed Angle Rotor.
7. Collect the pellets, resuspend them in 50 µL PBS, and store at –80°C.

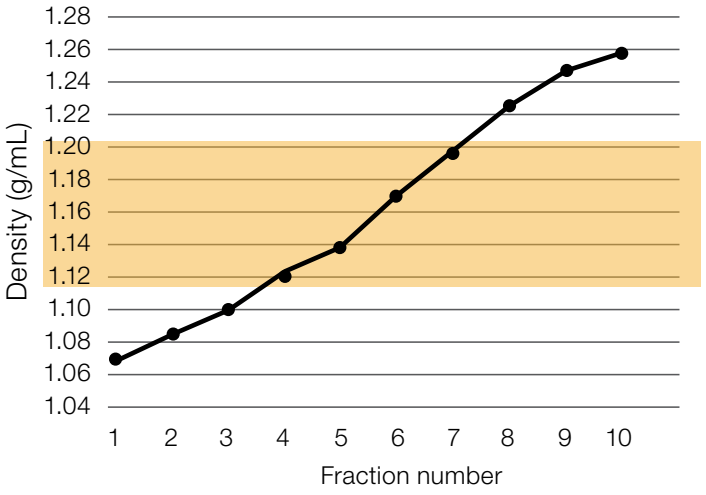

Figure 3. Density profile of fractions in the sucrose gradient. The expected density range for fractions containing isolated exosomes (1.12–1.20 g/mL) is highlighted in orange.

Results

In a nanoparticle tracking analysis (NTA), it was demonstrated that 147 ± 71 nm (mean ± standard deviation) was the average diameter of the exosomes isolated by differential centrifugation (Figure 4, blue curve). This suggested the presence of microsomal particles. Exosomes purified by continuous density gradients were distributed with an average size of 98 ± 39 nm (Figure 4, black curve), confirming the efficiency of the procedure [11].

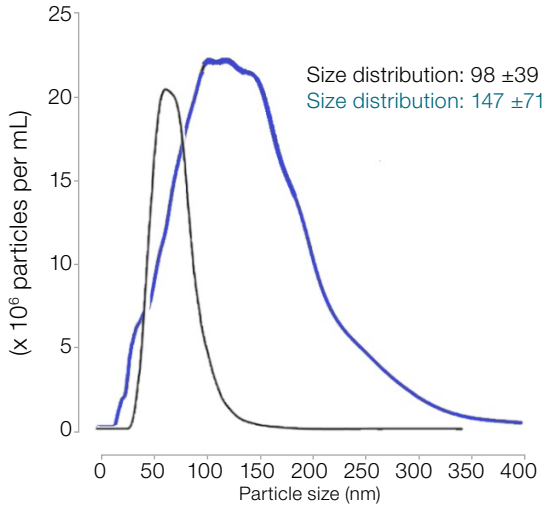

Figure 4. Size distributions of particles isolated by differential centrifugation and fractionation. The size distribution of nanoparticles isolated by differential centrifugation is indicated by the blue curve. The size distribution of exosomes in fractions 4–7 is indicated by the black curve.

Summary

Using different protocols, exosomes can be isolated and enriched from human blood plasma. Exosome vesicles can be difficult to isolate from plasma due to the presence of lipoproteins, such as chylomicrons and very low–density lipoproteins (VLDLs), and microvesicles of similar size. A two-step approach is recommended for isolating and purifying exosomes from blood plasma. A continuous density gradient is used to further purify exosome pellets after they are separated from blood plasma by differential ultracentrifugation. Table 1 summarizes several protocols for isolation and purification of exosome particles from blood plasma.

Table 1. Protocols for exosome isolation and purification reported in the literature.

Sample origin	RCF	Technique	Duration of ultracentrifugation	Rotor	Instrument	Ref.
Blood plasma from patients with prostate cancer and healthy donors	110,000 x *g*	Differential ultracentrifugation	90 min	Thermo Scientific™ Fiberlite™ F50L-24 x 1.5 Fixed-Angle Rotor	Sorvall WX+ Ultracentrifuge	7
Blood plasma from patients with prostate cancer, patients with benign prostatic hyperplasia, and healthy donors	110,000 x *g*	Differential ultracentrifugation	120 min	NA	Sorvall WX+ Ultracentrifuge	8, 10
Maternal blood plasma	200,000 x *g*	Isolation by differential ultracentrifugation	120 min	T-8100 Fixed Angle Rotor	Sorvall WX+ Ultracentrifuge	11
	110,000 x *g*	Purification by density gradient	20 hr	Thermo Scientific™ SureSpin™ 630/360 Swinging Bucket Rotor		
Blood plasma from diabetic mice	100,000 x *g*	Isolation by differential ultracentrifugation	120 min	Thermo Scientific™ S55-S Swinging Bucket Rotor	Sorvall WX+ Ultracentrifuge	12
	100,000 x *g*	Purification by density gradient	18 hr	S55-S Swinging Bucket Rotor	Thermo Scientific™ Sorvall™ M120 SE Micro-Ultracentrifuge	12

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