Improved exosome isolation by sucrose gradient fractionation of ultracentrifuged crude exosome pellets

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Method Article

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Abstract

This protocol describes a method for purifying exosomes using sucrose gradient centrifugation of crude exosome pellets prepared by differential ultracentrifugation. Exosomes isolated by differential ultracentrifugation alone contain both exosomes and aggregated protein and nucleic acid contaminants. Sucrose gradient centrifugation effectively separates exosomes and other extracellular vesicles from these contaminants.

Introduction

Cells not only actively secrete membrane vesicles but also passively release protein, RNA, or membrane complexes/aggregates, especially when cells undergo necrotic or apoptotic cell death \(1). In cell culture supernatant, complexes/aggregates released from dying cells are present together with exosomes secreted from other healthy cells. These aggregates remain in cell culture supernatants even after centrifugation at 10,000 g according to standard extracellular vesicle preparation protocols, and aggregates and exosomes co-sediment during 100,000 g centrifugation. Hence, aggregates contaminate these crude exosome pellets. The contamination of protein/RNA/membrane aggregates is especially problematic when cell death is induced during _in vitro_ cell culture. For example, apoptosis and activation are both induced when macrophages are stimulated by LPS or when T cells are stimulated by α-CD3 and α-CD28 antibodies _in vitro_. Therefore, an optimized strategy for separation of aggregates from exosomes is critical for studying exosomes secreted from immune cells under stimulated conditions. Exosomes have been found to float at densities ranging from 1.15 to 1.19 g/ml on sucrose gradients. By comparison, vesicles purified from the endoplasmic reticulum float at 1.18 to 1.25 g/ml, vesicles from the Golgi at 1.05 to 1.12 g/ml \(2). The density of protein aggregates is 1.22 g/ml \(3). The differing density of the potential contaminants of exosomes make sucrose gradient centrifugation an effective strategy for further purification of exosomes from sedimented crude exosome pellets.

Reagents

PBS PBS/sucrose stock solution \(see recipe)

Equipment

Ultracentrifuge with SW32 Ti rotor Ultracentrifuge with SW41 Ti rotor 38.5-ml ultra-clear tubes \(Beckman coulter, cat no: 344058) 13.2-ml ultra-clear tubes \(Beckman coulter, cat no: 344059)

Procedure

Preparation of crude exosome pellet by differential ultracentrifugation 1. Collect ~200 ml of culture medium into 50-ml conical tubes and centrifuge at 4°C \(1,000 g for 10 min) to remove intact cells. 2. Pipet off the supernatant, transfer it to fresh 50-ml conical tubes, and centrifuge at 4°C \(2,000 g for 20

min) to remove cell debris. 3. Pipet off the supernatant, transfer it to fresh 50-ml conical tubes, and centrifuge at 4°C \(10,000 g for 30 min) to remove microvesicles. 4. Pipet off the supernatant, transfer it to Beckman tubes \(38.5-ml ultra-clear tubes), and ultracentrifuge at 4°C \(100,000 g for 2 hour) using a SW32 Ti or equivalent rotor to remove the crude exosomes. 5. Pour out the supernatant and resuspend the crude exosome pellet \(100,000 g pellet) with 100µl PBS. **Purification of exosomes from crude exosome pellets using sucrose gradient centrifugation** 1. Make 10-90% sucrose stocks using PBS See figure in Figures section. 2. Resuspend the crude exosome preparations \(in 100µl PBS) with 1 ml 90% sucrose stock solution \(final sucrose concentration = 82%). Transfer into 13.2-ml ultra-clear Beckman unltracentrifuge tubes. 3. Overlay the gradient on top of the crude exosome preparation starting with 1 ml 70% sucrose solution. Technical tip: To apply sucrose solutions, place a 200 µl pipet tip on the end of a 1000 µl pipet tip containing 1 ml sucrose solution. Hold both tips steady using gloved fingers. Place the 200 µl tip in contact with the inside wall of the ultracentrifuge tube and allow gravity to feed the solutions into the tube slowly. 4. Repeat step 3 with the remaining sucrose stock solutions in order from the highest to lowest sucrose concentration. 5. Ultracentrifuge at 4°C \(100,000g for 16 hours) in a SW41 Ti rotor or equivalent. 6. Collect 2 ml fractions starting from the top to bottom. Technical tip: To collect fractions correctly without mixing the gradient, touch the side of the tube at the very top of the gradient with the end of 1000 µl pipet tip, carefully take out 1 ml and then transfer it into the Beckman tube \(13.2-ml ultra-clear tube). Repeat this step again so that 2 ml fractions are collected. 7. Repeat step 6 until six fractions of 2 mL each have been collected. 8. Add 9mL PBS to each of the 2 ml fractions. Centrifuge at 4°C \(100,000g for 1 hour) in a SW41 Ti rotor or equivalent. 9. Pour out the supernatant and resuspend the pellet in 50 µl PBS. The third fraction from the top, which contains the interface of the 34% and 40% sucrose solutions, should be enriched for exosomes. The sixth fraction, which contains the interface of the 70% and 82% sucrose solutions, will contain protein/RNA/membrane aggregates.

References

1. Cortez, M.A., et al., MicroRNAs in body fluids – the mix of hormones and biomarkers. _Nat Rev Clin Oncol_ \(2011) **8**: 467-477. 2. Thery, C., et al., Isolation and characterization of exosomes from cell culture supernatants and biological fluids. _Curr Protoc Cell Biol_ \(2006) **3.22**: 1-29. 3. Quillin, M.L. and B.W. Matthews, Accurate calculation of the density of proteins. _Acta Crystallogr D Biol Crystallogr_ \(2000) **56**:791-794.

Figures

stock solution (%)	10	16	22	28	34	40	46	52	58	64	70	90
Sucrose (g)	1.0	1.6	2.2	2.8	3.4	4.0	4.6	5.2	5.8	6.5	7.0	9.0
Add PBS to 10ml												

Figure 1

Table 1 Recipe for PBS/sucrose stock solution