CLAIMS

What is claimed is:

1. A method for processing cell-derived vesicles (CDVs), comprising:

    a. concentrating CDVs in a biological fluid;
    b. determining a concentration of the CDVs;
    c. contacting the CDVs with a fluorescent staining dye or an antibody for an CDV surface marker;
    d. incubating the contacted CDVs to generate a labeled CDV population;
    e. passing the contacted CDVs through a centrifugal filter comprising a 200-500 kD molecular weight cut off, polyethersulfone filter media, to separate the labeled CDV population from excess fluorescent staining dye or excess antibody; and
    f. recovering the labeled CDV population,

    wherein the CDVs are optionally obtained by a method comprising preparing a suspension of nucleated mammalian cells and conducting a serial extrusion of the nucleated cells by sequentially passing them through filters with diminishing micro-size pores to produce a biological fluid comprising CDVs retaining the same membrane topology as that of the nucleated mammalian cells.

2. The method of claim 1, wherein the serial extrusion comprises serially passing the nucleated mammalian cells through membrane filters with a pore size of about 10 μm, about 3 μm, and about 0.4 μm.

3. The method of claim of claim 1, wherein the concentrating comprises passing the biological fluid through a tangential flow filter.

4. The method of claim 3, wherein the tangential flow filter has a molecular weight cut-off of about 300 kD to about 750 kD.

5. The method of claim 1, wherein the CDVs are contacted with the fluorescent staining dye 6-Carboxyfluorescein succinimidyl ester (CFSE).

6. The method of claim 1, wherein the CDVs are contacted with an anti-CD9 antibody, an anti-CD63 antibody, an anti-CD81 antibody, and/or an anti-IgG1 antibody.

7. The method of any of claims 1-6, wherein the CDVs are produced from human embryonic kidney (HEK) cells, human Caucasian colon adenocarcinoma HT29 cells, mesenchymal stem cells (MSCs), embryonic stem cells and cells derived from embryonic stem cells, induced pluripotent stem cells, endothelial progenitor cells, immature and mature dendritic cells (DC), monocytes, macrophages, T and B lymphocytes, fibroblasts, epithelial cells, endothelial cells, including human umbilical vein endothelial cells (HUVEC), myocytes, cardiomyocytes, neuronal cells, glial cells, kidney cells, pancreatic cells, stromal cells, keratinocytes, or melanocytes

8. The method of any one of claims 1-7, wherein the concentration of CDVs in the biological fluid is determined using a flow cytometer for nanoparticle analysis.

9. The method of any one of claims 1-8, wherein the concentration of CDVs is determined to be at least $1x10^{10}$ CDV/ml, prior to the contacting in (c).

10. The method of any one of claims 1-9, wherein the contacted CDVs are passed through the centrifugal filter for at least 10 minutes at a centrifugal force of at least 10,000 x g.

11. A method for analyzing CDVs, comprising:
    a. concentrating CDVs in a sample with a tangential flow filter;
    b. determining a concentration of the CDVs;
    c. contacting the CDVs with a fluorescent staining dye or an antibody for a CDV surface marker;
    d. incubating the contacted CDVs to generate a labeled CDV population;
    e. passing the contacted CDVs through a centrifugal filter comprising a 300 kD molecular weight cut off, polyethersulfone filter media, to separate the labeled CDV population from excess fluorescent staining dye or excess antibody;
    f. recovering the labeled CDV population; and
    g. analyzing the recovered, labeled CDV population using a flow cytometer for nanoparticle analysis,

wherein the CDVs are optionally obtained by a method comprising preparing a suspension of nucleated mammalian cells, and conducting a serial extrusion of the nucleated cells by sequentially passing them through filters with diminishing micro-size pores to produce a sample comprising CDVs retaining the same membrane topology as that of the nucleated mammalian cells.

12. The method of claim 11, wherein the serial extrusion comprises serially passing the nucleated mammalian cells through membrane filters with a pore size of about 10 μm, about 3 μm, and about 0.4 μm.

13. The method of claim 11, wherein the CDVs are contacted with the fluorescent dye 6-Carboxyfluorescein succinimidyl ester (CFSE).

14. The method of claim 11, wherein the CDVs are contacted with an anti-CD9 antibody, an anti-CD63 antibody, an anti-CD81 antibody, and/or an anti-IgG1 antibody.

15. The method of any one of claims 11-14, wherein the CDVs are produced from human embryonic kidney (HEK) cells, human Caucasian colon adenocarcinoma HT29 cells, or mesenchymal stem cells (MSCs).

16. The method of any one of claims 11-15, wherein the concentration of CDVs in the sample is determined using a flow cytometer for nanoparticle analysis.

17. The method of any one of claims 11-16, wherein the concentration of CDVs is determined to be at least $1x10^{10}$ CDV/ml, prior to the contacting in (c).

18. The method of any one of claims 11-17, wherein the CDVs are concentrated using a 750 kD molecular weight cut-off tangential flow filter in (a).

19. The method of any one of claims 11-18, wherein the CDVs are passed through the centrifugal filter for at least 10 minutes at a centrifugal force of at least 10,000 x g.

20. The method of any one of claims 11-19, wherein the recovered, labeled CDV population is analyzed to determine one or more of labeling efficiency, CDV number, CDV concentration, CDV protein expression, and CDV size.