SUMMARY

[0005] In one aspect, the present invention refers to a method of isolating exosomes comprising: conducting at least one purification step in the presence of at least one organic zwitterionic agent having a molecular weight of less than about 350 Daltons, wherein a buffering pK of a negatively charged portion of the organic zwitterionic agent is at least one full pH unit below an operating pH at which the at least one purification step is conducted, and a buffering pK of the positively charged portion of the organic zwitterionic agent is at least one full pH unit above the operating pH, wherein the organic zwitterionic agent is an amino acid or peptide comprising from 2 to 5 amino acids, the amino acid or peptide amino acids being selected from the group consisting of glycine, alanine, *N*,*N*,*N*-trimethylglycine (betaine), and taurine, and wherein the at least one purification step comprises a filtering step, a chromatography step or a field flow fractionation step. Also described herein are exampleswhich relate to methods of isolating exosomes comprising conducting at least one purification step in the presence of an organic zwitterion having a molecular weight of less than about 350 Daltons, wherein a buffering pK of a negatively charged portion of the organic zwitterion is at least one full pH unit below an operating pH at which the at least one purification step is conducted, and a buffering pK of the positively charged portion of the organic zwitterion is at least one full pH unit above the operating pH.

DETAILED DESCRIPTION

[0006] It has been discovered that the combination of exosomes with small organic zwitterions can enhance exosome recovery during purification. Without being bound by theory, enhanced recovery appears to be mediated by a decrease in non-specific interactions between exosomes and surfaces with which they come into contact. This is seemingly counterintuitive because glycine, an example of one appropriate species of zwitterion, is known to promote non-specific interactions between proteins and hydrophobic surfaces, and the lipid component of the outer exosome membrane is understood to be strongly hydrophobic. The apparent ability of qualified zwitterions to reduce non-specific interactions may reflect an increase in exosome solubility mediated by the ability of zwitterions to elevate the polarity of aqueous solutions. Surfaces with which exosomes may interact non-specifically during purification may include filtration media and chromatography media. Whether decreasing non-specific interactions and increasing solubility are distinct phenomena or different aspects of the same phenomenon remains to be elucidated, but in either case combining exosomes with zwitterions, in accordance with embodiments disclosed herein, may lead to improved exosome recovery during purification.

[0007] Small organic zwitterions suitable for practicing the methods disclosed herein particularly include the amino acid glycine, with a molecular weight of about 75 Daltons (Da), or alanine, with a molecular weight of about 89 Da. Glycine consists of a central carbon linked to a primary amino group, a carboxyl group, and two hydrogen atoms, where the charges reside on opposite sides of the molecule. Due to the placement and distance between the amino and carboxyl groups, the zwitterionic form has an elevated dipole moment, which has the effect of increasing the bulk dielectric constant of aqueous solutions in which it resides. Glycine

WHAT IS CLAIMED IS:

1. A method of isolating exosomes comprising: conducting at least one purification step in the presence of at least one organic zwitterionic agent having a molecular weight of less than about 350 Daltons, wherein a buffering pK of a negatively charged portion of the organic zwitterionic agent is at least one full pH unit below an operating pH at which the at least one purification step is conducted, and a buffering pK of the positively charged portion of the organic zwitterionic agent is at least one full pH unit above the operating pH, wherein the organic zwitterionic agent is an amino acid or peptide comprising from 2 to 5 amino acids, the amino acid or peptide amino acids being selected from the group consisting of glycine, alanine, *N*,*N*,*N*-trimethylglycine (betaine), and taurine, and wherein the at least one purification step comprises a filtering step, a chromatography step or a field flow fractionation step.

2. The method of claim 1, wherein the molar dielectric increment of the organic zwitterionic agent under operating conditions of the at least one purification step is greater than about 17.

3. The method of claim 1, wherein the organic zwitterionic agent is *N*,*N*,*N*-trimethylglycine (betaine) or taurine.

4. The method of claim 1, wherein the organic zwitterionic agent is glycine or alanine.

5. The method of claim 1, wherein a concentration of the organic zwitterionic agent is selected from the group consisting of (a) from about 20 mM to about 50 mM, (b) from about 50 mM to about 100 mM, (c) from about 100 mM to about 300 mM, (d) from about 300 mM to about 3 M, and (e) saturated.

6. The method of claim 1, wherein a concentration of the organic zwitterionic agent is selected from the group consisting of (a) from about 5mM to about 20 mM, (b) from about 20 mM to about 50 mM, (c) from about 50 mM to about 100 mM, (d) from about 100 mM to about 300 mM, (e) from about 300 mM to about 3 M, and (f) saturated.

7. The method of claim 1, wherein before conducting the at least one purification step, the exosomes are unpurified, partially purified, or highly purified.

8. The method of claim 1, wherein the osmolarity during the at least one purification step is in a range from about 250 milliOsmoles/kg to about 300 milliOsmoles/kg.

9. The method of claim 1, wherein the osmolarity during the at least one purification step is less than about 250 milliOsmoles/kg.

10. The method of claim 1, wherein the osmolarity during the at least one purification step is greater than about 300 milliOsmoles/kg.