from the data presented in FIG. 1, different lots of commercially available human serum show substantial variation (15% to 50%) in total T cell yields. Substantial variation as also found with respect to transduction efficiency (data not shown). The above are some reasons why serum replacements are often used.

[0006] Electroporation is a method by which material can be introduced into cells. A solution containing cells and the material to be introduced are exposed to a brief high intensity electric field. The electric field "porates" the cells, producing transient pores in their outer membranes, allowing diffusion of the material in the solution into the cells.

[0007] One issue with the electroporation of cells is that this process often decreases cells viability. Further, a balance is often sought between the inverse correlation electroporation efficiency and cell viability. Potassium, as an example, in physiological levels equal to intracellular amounts tends to increase viability in electroporated cells (*e.g.,* van den Hoff *et al., Nucleic Acids Res., 20*:2902 (1992)). The presence of calcium ions have been reported to increase viability of cells following electroporation. It is postulated that the reason for the increase in viability is reported to be a contribution by calcium in the resealing process after electroporation. Table 1 of van den Hoff *et al*. (*Nucleic Acids Res., 20*:2902 (1992)) essentially shows that the higher the electrical charge applied to the cells the lower the cell viability, with the highest cell viability measured being around 69%.

[0008] Osmolarity of the electroporation medium affects cell viability and the efficiency of movement of large molecules through cell membranes. For example, van den Hoff *et al*. (*Nucleic Acids Res., 18*:6464 (1990) recommends against the use of hypotonic electroporation media.

[0009] There is a need for electroporation methods that lead to both high levels of introduction of molecules into cells while maintaining high levels of cell viability.

BRIEF SUMMARY

[0010] Provided are compositions and methods for culturing and/or expanding cells (*e.g.,* human cells) with high cell viability. Further provided herein are, *inter alia*, compositions, methods systems, kits, and methods for the introduction of macromolecules into cells where the cells maintain high viability. Thus, in general, provided herein are compositions and methods that relate to cellular processes for the maintenance of high cell viability and the production of cellular compositions where the cells in the compositions maintain a high level of viability.

[0010a] In one aspect, the present disclosure provides a method for preparing a serum free cell culture medium, the method comprising adding a lipoprotein particle composition or a lipoprotein composition to a basal culture medium, wherein the lipoprotein particle composition or a lipoprotein composition is added in an amount to function as a serum replacement.

[0010b] In another aspect, the present disclosure provides a serum free cell culture medium comprising one or more lipoprotein compound, wherein the serum free cell culture medium supports the expansion of mammalian cells and wherein the expansion of the mammalian cells is increased by 10% in the serum free cell culture medium comprising the one or more lipoprotein compound as compared to the same cell expanded in culture medium without the one or more lipoprotein compound but containing serum.

[0010c] In yet another aspect, the present disclosure provides a method for expanding a mammalian cell, the method comprising incubating the mammalian cell in a serum free cell culture medium comprising one or more lipoprotein compound under conditions that allow for expansion of the mammalian cell. In yet another aspect, the present disclosure provides a method for electroporation of a mammalian cell population, the method comprising: (a) contacting the mammalian cell population with one or more lipoprotein compound for at least 12 hours in a serum free culture medium under conditions that allow for expansion of the mammalian cells, and (b) applying one or more electric pulse to the mammalian cell population to thereby electroporate cell membranes of members of the mammalian cell population, wherein the electroporation efficiency is at least 60% and wherein the viability of the cells in the mammalian cell population decreases by less than 10%.

[0010d] In yet another aspect, the present disclosure provides a method for the maintenance of an activated T cell population, the method comprising: (a) generating the activated population of T cells, (b) expanding the activated population of T cells generated in step (a) in the presence of a lipoprotein supplement, (c) exposing the expanded activated population of T cells produced in step (b) to an electric field of sufficient strength to result in a decrease in the rate of cell expansion over the following seven day by at least 30%, and (d) maintaining the activated population of T cells of step (c) under the same conditions as in step (b) for seven days, wherein the viability of the activated population of T cells during steps (a) - (d) remains above 70%.

[0010e] In yet another aspect, the present disclosure provides a method for storing mammalian cells, the method comprising the following steps in order: (a) expanding the mammalian cells in a culture medium comprising one or more lipoprotein compound, (b) exposing the mammalian cells to an electric field, and (c) expanding the mammalian cells in a culture medium comprising one or more lipoprotein compound, wherein the mammalian cells in step (c) expand at a rate that is at least 50% lower than in step (a), and wherein the viability of the mammalian cells remains above 70% during steps (a) – (c).

[0011] In some ~~aspect~~examples, provided herein are methods for preparing serum free cell culture media, as well as composition used in such methods and the resulting culture media prepared by such

methods. Such methods include those in which one or more lipoprotein particle composition and/or one or more lipoprotein is added to a basal culture medium. In many instances, lipoprotein particle compositions and/or lipoprotein are added in amounts to function as a serum replacement.

[0012] Lipoprotein particles used in methods and present in compositions set out here may comprise one or more lipoprotein particle selected from the group consisting of (a) high density lipoprotein particles, (b) low density lipoprotein particles, and (c) very low density lipoprotein particles, as well as other types of lipoprotein particles.

[0013] Lipoprotein particles used in methods and present in compositions set out here may be obtained from a natural source (*e.g.*, the blood or a mammal, such as a human) or may be synthetically produced (*e.g.,* synthetic lipoprotein particles, such as synthetic high density lipoprotein particles). In some instances, synthetic lipoprotein particles may comprise Apolipoprotein AI, Apolipoprotein AII, Apolipoprotein IV, Apolipoprotein-CI, Apolipoprotein III, Apolipoprotein D, Apolipoprotein E and/or a portion of one or more of such apolipoproteins.

[0014] Further, apolipoproteins present in compositions and used in methods set out herein may be obtained from a natural source (*e.g.*, the blood or a mammal, such as a human) and/or recombinantly produced. Additionally, recombinantly production apolipoproteins and/or portions thereof may performed using a non-mammalian cell (*e.g.*, a bacterial cell, a plant cell, and insect cell, etc.).

[0015] In some ~~aspect~~examples, provided herein are serum free cell culture media. Such culture media may comprise one or more lipoprotein. Further, such culture media may support the expansion of mammalian cells, wherein the expansion of the mammalian cells is increased by at least 10% (*e.g.,* from about 10% to about 75%, from about 10% to about 70%, from about 10% to about 55%, from about 10% to about 45%, from about 10% to about 35%, from about 10% to about 25%, from about 20% to about 70%, from about 20% to about 55%, etc.) in the serum free cell culture medium comprising the one or more lipoprotein as compared to the same cell expanded in culture medium without the one or more lipoprotein but containing serum. Serum free cell culture medium set out herein may contain one of the one or more lipoprotein compound (*e.g.,* Apolipoprotein AI, Apolipoprotein AII, Apolipoprotein IV, Apolipoprotein-CI, Apolipoprotein III, Apolipoprotein D, and Apolipoprotein E, etc.) and/or one or more sub-portion of a lipoprotein. Further, lipoprotein and/or lipoprotein sub-portions may be components of a lipoprotein particle (*e.g.*, a high density lipoprotein particle, a low density lipoprotein particles, a very low density lipoprotein particles, etc.).

WHAT IS CLAIMED IS:

1. A method for preparing a serum free cell culture medium, the method comprising adding a lipoprotein particle composition or a lipoprotein composition to a basal culture medium, wherein the lipoprotein particle composition or a lipoprotein composition is added in an amount to function as a serum replacement.

2. The method of claim 1, wherein the lipoprotein particle composition comprises one or more lipoprotein particle selected from the group consisting of:

(a) high density lipoprotein particles,

(b) low density lipoprotein particles, and

(c) very low density lipoprotein particles.

~~3. The method of claims 1 or 2, wherein the lipoprotein particle composition comprises lipoprotein particles obtained from human blood.~~

~~4.~~3. The method of any one of claims 1 through 2, wherein the lipoprotein particle composition comprises a synthetic lipoprotein particle.

~~5. The method of any one of claims 1, 2, or 4, wherein the lipoprotein particle composition comprises a synthetic high density lipoprotein particle.~~

~~6.~~4. The method of claim ~~4~~3, wherein the synthetic lipoprotein particle comprises apolipoprotein AI or apolipoprotein AII.

~~7. The method of claim 5, wherein the synthetic lipoprotein particle comprises a portion of apolipoprotein AI or apolipoprotein AII.~~

~~8. The method of any one of claims 6 through 7, wherein the apolipoprotein AI or apolipoprotein AII is recombinantly produced.~~

~~9. The method of claim 8, wherein the recombinantly production the apolipoprotein AI or apolipoprotein AII is performed using a non-mammalian cell.~~

~~10. The method of claim 9, wherein the non-mammalian cell is an insect cell.~~

~~11.~~5. A serum free cell culture medium comprising one or more lipoprotein compound, wherein the serum free cell culture medium supports the expansion of mammalian cells and wherein the expansion of the mammalian cells is increased by 10% in the serum free cell culture medium comprising the one or more lipoprotein compound as compared to the same cell expanded in culture medium without the one or more lipoprotein compound but containing serum.

~~12.~~6. The serum free cell culture medium of claim ~~11~~5, wherein at least one of the one or more lipoprotein compound is Apolipoprotein AI.

~~13.~~7. The serum free cell culture medium of claim ~~11~~5, wherein at least one of the one or more lipoprotein compound is Apolipoprotein AII.

~~14.~~8. The serum free cell culture medium of any one of claims ~~11~~ 5 through ~~13~~7, wherein at least one of the one or more lipoprotein compound is a component of a lipoprotein particle.

~~15.~~9. The serum free cell culture medium of claim ~~14~~8, wherein the lipoprotein particle is a high density lipoprotein.

~~16.~~10. The serum free cell culture medium of any one of claims ~~14~~ 8 through ~~15~~9, wherein the lipoprotein particle is obtained from a mammal.

~~17.~~11. The serum free cell culture medium of any one of claims ~~14~~ 8 through ~~15~~9, wherein the lipoprotein particle is non-naturally occurring.

~~18.~~12. The serum free cell culture medium of claim ~~17~~11, wherein the non-naturally occurring lipoprotein particle contains either a non-naturally occurring protein, a naturally occurring apolipoprotein, or a portion of a naturally occurring apolipoprotein.

~~19. The serum free cell culture medium of any one of claims 11 through 18, wherein the increase in cell viability is in the range of from 10% to about 75%.~~

~~20. The serum free cell culture medium of claim 11, wherein the mammalian cells are hybridoma cells.~~

~~21. The serum free cell culture medium of claim 20, wherein the mammalian cells are human cells.~~

~~22. The serum free cell culture medium of claim 11, wherein the mammalian cells are immune cells.~~

~~23. The serum free cell culture medium of claim 22, wherein the immune cells are T cells.~~

~~24. The serum free cell culture medium of claim 23, wherein the T cells are one or more T cells selected from the group consisting of:~~

~~(a) regulatory T cells,~~

~~(b) CD4+ T cells,~~

~~(c) CD8+ T cells,~~

~~(d) T_H1 cells,~~

~~(e) T_H2 cells,~~

~~(f) T_H3 cells,~~

~~(g) T_H17 cells,~~

~~(h) T_H9 cells, and~~

~~(i) T_{FH} cells.~~

~~25. The serum free cell culture medium of any one of claims 23 through 24, wherein the T cells are isolated from blood through ligation to a CD3 surface marker.~~

~~26.~~13. A method for expanding a mammalian cell, the method comprising incubating the mammalian cell in a serum free cell culture medium comprising one or more lipoprotein compound under conditions that allow for expansion of the mammalian cell.

~~27.~~14. The method of claim ~~26~~13, wherein the lipoprotein compound comprises one or more lipoprotein particle.

~~28. The method of claim 27, wherein the lipoprotein particle compound comprises one or more lipoprotein particle selected from the group consisting of:~~

~~(a) high density lipoprotein particles,~~

~~(b) low density lipoprotein particles, and~~

~~(c) very low density lipoprotein particles.~~

~~29.~~15. A method for electroporation of a mammalian cell population, the method comprising:

(a) contacting the mammalian cell population with one or more lipoprotein compound for at least 12 hours in a serum free culture medium under conditions that allow for expansion of the mammalian cells, and

(b) applying one or more electric pulse to the mammalian cell population to thereby electroporate cell membranes of members of the mammalian cell population,

wherein the electroporation efficiency is at least 60% and wherein the viability of the cells in the mammalian cell population decreases by less than 10%.

~~30. The method of claim 29, wherein the electroporation efficiency is measured by expression of a detectable marker in members of the mammalian cell population.~~

~~31. The method of claim 30, wherein the detectable marker is a fluorescent protein.~~

~~32.~~16. A method for the maintenance of an activated T cell population, the method comprising:

(a) generating the activated population of T cells,

(b) expanding the activated population of T cells generated in step (a) in the presence of a lipoprotein supplement,

(c) exposing the expanded activated population of T cells produced in step (b) to an electric field of sufficient strength to result in a decrease in the rate of cell expansion over the following seven day by at least 30%, and

(d) maintaining the activated population of T cells of step (c) under the same conditions as in step (b) for seven days,

wherein the viability of the activated population of T cells during steps (a) - (d) remains above 70%.

~~33.~~17. The method of claim ~~32~~16, wherein one or more nucleic acid molecule is introduced in step (c) into individual T cells of the activated population of T cells.

~~34. The method of claim 33, wherein at least one of the one or more nucleic acid molecule encodes a chimeric antigen receptor.~~

~~35. The method of claim 34, wherein the chimeric antigen receptor is stably expressed in individual T cells of the activated population of T cells.~~

~~36. The method of claim 32, wherein the activated population of T cells is expanded for three days in step (b).~~

~~37. The method of claim 32, further comprising:~~

~~(e) washing of the activated population of T cells after step (d), and~~

~~(f) expanding the washed, activated population of T cells generated in step (e) in the absence of a lipoprotein supplement.~~

~~38. The method of claim 37, wherein, over a five day time period, the viability of the washed, activated population of T cells remains above 70% and the washed, activated population of T cells expand at least three fold.~~

~~39.~~18. The method of claim ~~32~~16, wherein the activated population of T cells are shipped to a different location during step (d).

~~40. The method of claim 32, wherein the different location is greater than 100 miles away.~~

~~41.~~19. A method for storing mammalian cells, the method comprising the following steps in order:

(a) expanding the mammalian cells in a culture medium comprising one or more lipoprotein compound,

(b) exposing the mammalian cells to an electric field, and

(c) expanding the mammalian cells in a culture medium comprising one or more lipoprotein compound,

wherein the mammalian cells in step (c) expand at a rate that is at least 50% lower than in step (a), and

wherein the viability of the mammalian cells remains above 70% during steps (a) – (c).

~~42.~~20. The method of claim ~~41~~19, wherein the mammalian cells are T cells.

~~43. The method of claim 41, wherein the mammalian cells are expanded for seven days in step (c).~~

~~44. The method of claim 43, wherein the cell viability of the mammalian cells in step (c) remains above 70% during the seven days of expansion.~~

~~45. The method of claim 41, wherein the mammalian cells in step (c) are washed and transferred to a culture medium comprising at least a 50% lower concentration of the one or more lipoprotein compound.~~

~~46. The method of claim 41, wherein a nucleic acid molecule is introduced into the mammalian cells in step (b).~~

~~47. The method of claim 46, wherein the nucleic acid molecule encodes a chimeric antigen receptor.~~

~~48. The method of claim 47, wherein the mammalian cells are T cells.~~