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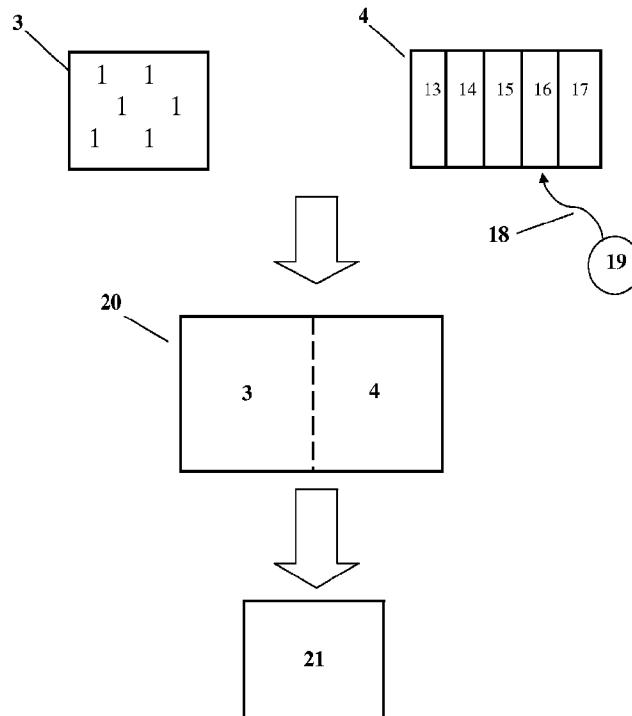
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(54) Title: SYSTEM AND SUBSTANCES FOR CRYOPRESERVATION OF VIABLE CELLS



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An improved cryopreservation process and substances can involve a cellular collection (1) in a cryopreservation fluid (4) that has been conditioned or treated (7) to enhance the cryopreservation process by adding (18) energy (19) such as in the surface energy of a substance in the cryopreservation fluid (4) prior to reducing energy for that same cryopreservation media for freezing. This can offer enhanced- post-cryogenic viability of the cryopreserved structures or a more optimum cooling curve (22) for a specific cell type.

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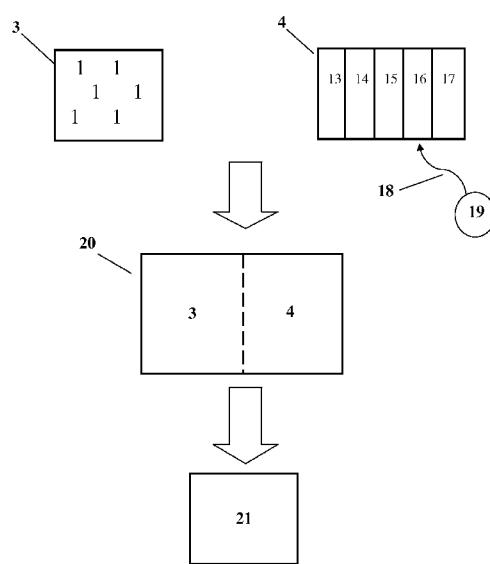
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(54) Title: SYSTEM AND SUBSTANCES FOR CRYOPRESERVATION OF Viable CELLS



(57) Abstract: An improved cryopreservation process and substances can involve a cellular collection (1) in a cryopreservation fluid (4) that has been conditioned or treated (7) to enhance the cryopreservation process by adding (18) energy (19) such as in the surface energy of a substance in the cryopreservation fluid (4) prior to reducing energy for that same cryopreservation media for freezing. This can offer enhanced- post-cryogenic viability of the cryopreserved structures or a more optimum cooling curve (22) for a specific cell type.

Fig. 1

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SYSTEM AND SUBSTANCES FOR CRYOPRESERVATION OF VIABLE CELLS

I. TECHNICAL FIELD

5 The present invention relates to the field of cryopreservation of biological substances such as cells, tissue, and the like. In particular it relates to processes, substances, and apparatus that can enhance and achieve cryopreservation and the freezing of biological substances, tissues, and cells in a manner that achieves enhanced results for the substances themselves, such as enhancing the viability of the frozen biological substances or perhaps enhancing the cryopreservation process
10 itself to allow inclusion of additional substances, speed the process, or otherwise afford technical or economic advantages.

II. BACKGROUND

15 The need and desire to maintain the functionality of cells and/or cellular collections has been known for a long period of time. Preservation has been approached by a variety of methods including desiccation, and freezing. Freezing or cryopreservation is now a common method to preserve cells. Cellular collections are cooled then frozen to a final temperature perhaps such as -80°C or -196°C in liquid nitrogen. Of course freezing by definition is an exothermic reaction (reducing heat energy) and is explained by a phase change from a liquid to a solid by removing
20 energy. Cryopreservation can include the use of very low temperatures to preserve structurally intact living cells. However while they may be structurally intact, the same cells are often not viable upon thawing. In fact, cryopreservation is wrought with challenges in that thawed cells are often damaged in some sense. For example frozen neurons have a post-cryogenic viability rate of about 30%. Live births from frozen oocytes can average about 20%. Nearly 50% of
25 cryopreserved sperm cells are often not viable after cryopreservation. Similarly in cells such as umbilical cord blood, depending on the technique used, only 40% or less of the cells may be viable.

Often four primary factors can affect cryopreservation success. These may include
30 cryopreservation media, temperature, freezing profiles and viability assessment. Moreover, post cryogenic viability can even be affected by handling after thawing. Common methods to allay some of the cryopreservation damage include addition of cryoprotectants such as glycerol which

may increase the viscosity of the solution and vitrification. Large molecules are known to have a positive effect of increasing viscosity and therefore serve to protect cells. A variety of cryoprotective agents are known to those skilled in the art as explained below. Further, rapid cooling or rapid removal of thermal energy, can promote vitrification. Generally it is understood 5 that vitrification can positively affect the viability of the cells. Although desired rates of cooling can vary by cell or tissue type, removing energy from the system at a rate of about -1 to -3°C /min can be considered a general rule of thumb. Controlling the rate of freezing by removing the energy out of the system at a predictable, repeatable rate appears to reduce some of the damage caused by freezing and thawing. Some substances in the cryopreservation fluid may affect the 10 rate of freezing and of thawing. Of course, viability is affected by both by the removal of thermal energy then later by the endothermic reaction, or addition of energy to return the cell to its normal metabolic status. Adding energy to the system, for example exposing a cryovial to room temperature air, can cause a 25 degree temperature increase (-75 to -50°C) in one minute. Such an increase in energy can be beneficial during thawing of cells as a rapid 60-90 second 15 temperature increase to around 37°C may decrease damage related to thawing. Cryopreservation fluid can also be customized to the type of cellular collection involved.

A component of significant influence on cryopreservation success can be the measure of viability. Of course, viability depends on the type of cellular collection and the parameter(s) to 20 be measured. Cryopreservation induced damage that may affect viability might include induction of apoptosis or apoptotic cascade, necrosis, damage to external membrane proteins, DNA damage, oxidative damage, membrane damage, disruption in the functional areas of the cell or tissue as well as impaired growth capacity. In addition cells may experience biochemical toxicity. The effects of toxicity can include cytoskeletal reorganization, suppression of normal 25 metabolism and membrane composition shifts. Upon thawing, if the cell has survived the effects of the toxicity, often the biochemical markers are modified such that the cell is no longer functional. Measuring these functionalities can provide a measure of the viability of the cell after cryopreservation.

30 The apparent limitations of cryopreservation and less than optimum anticipated results are even to some degree accepted by current technology. Several patents speak to methods of limiting

cryopreservation damage by rendering the cells immotile (US7892725 and 7838210), protecting cells by using a cold shock method (US7820425), microcapsulation of embryos or cells in an alginate or similar material prior to vitrification and inclusion of an ice nucleator (US2010/0311036).

5

It has been postulated that antioxidants and protective components may be beneficial to cells as they go through cryopreservation, long term storage, thawing and secondary use. Interestingly it may be more economical to store cells at -80°C than in liquid nitrogen but metabolic activity is not completely inhibited at -80°C and therefore post cryogenic viability can be affected not only 10 by inherent cryopreservation issues but also metabolic byproducts. These same metabolites can be problematic when cells are thawed then held in their cryopreservation fluid. Such activities can increase the potential value of compounds such as vitamin E, linoleic acid, triterpenes, phenylpropanoid, alkaloids and other hydrophobic or water insoluble structures. However, the use of hydrophobic moieties such as tocopherol and the like has been less satisfactory, and not 15 generally addressed except in a limited sense. Examples of such include, but are not limited to, addition of tocopherol (vitamin E) to sperm cells wherein data has shown it to be beneficial in one instance, and detrimental in the next. Moreover the addition of other hydrophobic moieties such as lipids to cell culture media may be toxic to cultured cells perhaps because of problems with toxicity of free lipids in solution. This toxicity may ultimately affect the viability of the 20 cells.

Cryopreservation is an effective method to store cells, tissues, organs, and genetic materials for the long term. However, concerns about post-cryogenic viability assemblage of biological structures can lessen the applicability and can leave many industries wanting for solutions to aid 25 in the viability of cryopreserved items. The present invention discloses a system which overcomes many challenges associated with cryopreservation, with viability, and with adding certain substances to cryopreservation fluids in a practical fashion. It provides a method to achieve cryopreservation and to add hydrophobic moieties to cryopreservation fluid in a manner that does not reduce, and may improve the post-cryogenic viability of a plurality of biological 30 samples.

III. DISCLOSURE OF INVENTION

Accordingly, the present invention includes a variety of aspects or embodiments which may be selected in different combinations to suit the needs of the user. First, it may be understood that attempts at adding lipids or any hydrophobic moieties, has been fraught with hurdles, including

5 rendering the cells non-viable, and/or failing to confer protective benefits. The ability to remove variability associated with the addition of hydrophobic moieties to cryopreservation media while enhancing post-cryogenic viability is one general goal of this invention. Aspects of the invention may enable a full spectrum of protective benefits to be enhanced or perhaps even conferred upon cryopreserved cellular compositions. It can function with lipid-based antioxidants, membrane
10 stabilizers, and other lipid soluble compounds in such a manner as to benefit not only the cells but also the thermodynamic or other processes of cryopreservation.

The invention can accomplish various goals that can be implemented either alone or in combinations to achieve a variety of objectives. In one general goal, it can function for a large
15 variety of hydrophobic moieties and/or for a large variety of cells, cellular collections, mixtures of cell types, tissues, tissue samples, organs and other samples to be cryopreserved that may benefit such as from the use of hydrophobic substances in the cryopreservation solution.

In another general objective, the present technology can include the counter-intuitive process of
20 adding energy to a cryopreservation solution prior to removing energy to accomplish freezing. Adding energy before removing it has now shown to be beneficial. The invention can even now allow the addition of certain lipids or certain amounts of lipids to cell storage media. Further this objective may help to prevent some damage to cells imparted by thermal energy removal during the standard cryopreservation process.

25

Generally embodiments of the process can permit the use of new and novel oils, new hydrophobic moieties, new lipids, or multiple lipids, that can beneficial substances in a cryopreservation fluid. These may include generally recognized as safe (GRAS) ingredients that can be utilized with food substances, and those substances approved for use with cryopreserved
30 tissues and cells to be transplanted into humans. The hydrophobic substances to be added might

also include those that could provide beneficial effects to the subject in which the cryopreserved cellular compositions are transferred or utilized.

5 The present invention may also enable addition of lipids not previously suitable for use with cellular collections. The present invention may also provide a method to surmount problems with cooling curve and other aspects of traditional cryopreservation processes.

10 Another broad objective is to enable such additions of surface energy that may in turn permit the freezing process to occur with less damage to viability than occurs during traditional cryopreservation. Moreover such an addition of energy may enable thawing to occur with less damage than during traditional process thawing. The addition of surface energy to the thermal system may be achieved by the multiplication in the number of discrete lipid droplets in the solution without changing the total concentration (v/v or w/v) of the lipid relative to the remaining cryopreservation fluid components.

15

One more broad objective of the present invention could include addition of energy such that unnaturally small hydrophobic moiety congregations can be realized. Such a composition may be considered as a non-natural fluidic cryopreservation composite. It can be understood that lipids and other hydrophobic moieties do not exist in a small independent droplets state unless 20 confined by a membrane or other such barrier and therefore this state can be considered unnatural for lipids and other hydrophobic moieties. In the present invention, these unnaturally small congregations of lipids may also exist in a skewed size distribution that favors smaller size droplets like a positively skewed distribution, and which may not represent a normal Gaussian distribution of droplet size. The distribution of the unnaturally small droplet size may be such 25 that the distribution is strongly unimodal; that is, strongly favoring a mode of one droplet size. This type of distribution of lipid droplet size may also create a situation where repeatability of cryopreservation is improved thereby providing a more reliable, non-variable cryopreservation process. Modification of the droplet variation, and associated limitation of droplet size may occur after or as a result of the addition of surface energy to the cryopreservation media (perhaps 30 in situ) thereby maintaining the prescribed concentration of hydrophobic moiety while also enabling the addition without negative effect on the thermal profile necessitated by the particular

cellular collection. Removal of, or limitation of, droplets of a specific size profile outside of the cryopreservation fluid may make it difficult to maintain or measure the appropriate lipid composition and/or concentration therefore in situ sizing may provide improvements over current technologies.

5

Another general objective of the present invention may also provide a more homogeneous crystallization process than expected with the addition of substances outside the standard cryopreservation media substance list.

10 In embodiments, the goals of the invention may be achieved by structurally or physically altering the hydrophobic substances in the cryopreservation fluid in such a manner as to add surface energy to cryopreservation media before addition of cellular collections and before thermal energy removal. The drop transformation of lipids may enable addition of surface energy to the cryopreservation media in such a manner as to provide, enact and/or enable beneficial thermal
15 energy removal during freezing. Surprisingly, the addition of energy and improved thermal energy removal may improve post-cryogenic viability of cells. For substances that form drops (through surface tension or such) there is a physical relationship between the amount of surface energy of the substance and droplet size. By increasing the number of droplets in the cryopreservation media prior to adding a cellular collection, the surface energy, perhaps measured as joules per kilogram can be increased. Of course, depending on the hydrophobic
20 moiety to be added and the surface tension of such larger congregations, the surface energy added to the system may vary. Such variation could be between lipid compositions not within the fluidic cryopreservation composites made with the same lipid and lipid concentration assuming all other variables remain static. Surprisingly, an increase in surface energy may,
25 among other aspects, even enable a more uniform decrease in thermal energy perhaps thereby providing an effective cryopreservation process which may also be more non-variable due to such controlled energy addition. Further, included in a broad objective it is understood that hydrophobic moieties including lipids, by their nature are immiscible in water, or water-based solutions such as cryopreservation media. The tendency of lipids in a solution is to coalesce into
30 large droplets as such coalescence may increase the kinetic stability. Rending large droplets into smaller droplets is an unnatural state for the hydrophobic moieties and usually does not occur

naturally. Small lipid droplets may generally be unstable and may coalesce perhaps quickly. In the present invention however creating unnaturally small droplets may increase the overall, general stability of the cellular system. In addition, perhaps creating substantially uniform maximum droplet size of a hydrophobic substance will provide enhanced stability both prior to, 5 during and post-cryopreservation. In this invention the increased stability imparted by unnaturally small droplets may also affect or perhaps even enhance the shelf life stability of cryopreservation fluid.

These hydrophobic substances, rendered as described, may also then be more bioavailable for 10 cellular collection utilization. This may provide additional benefits to the post-cryogenic viability of said collection.

Another broad objective of the invention may enable the use of most water-insoluble organic molecules which may fall into such broad general classes as fats, phospholipids, waxes, sterols 15 and steroids which may be of plant or animal origin. The addition of such a broad class of substances may enable significant improvements in cryopreservation. It should be understood that the present invention may include modifications which make allowances for the different physical properties of the different classes of lipids and lipid-products.

20 According to an aspect of the invention is process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cells;
- establishing a lipid containing cryopreservation fluid to be incorporated with said biological cells in cryopreservation;
- adding at least 270 J/kg surface energy to the lipids in said lipid containing cryopreservation fluid to create a lipid surface energy increased cryopreservation fluid by the multiplication in the 25 number of discrete lipid droplets in the solution without changing the total concentration of lipids;
- mixing said lipid surface energy increased cryopreservation fluid and said biological cells to 30 form an energy increased fluidic cryopreservation composite;
- removing thermal energy from said fluidic cryopreservation composite; and

- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water..

5 According to another aspect of the invention is a process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cell structures;
- establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;

10 - adding energy to said cryopreservation fluid to create an energy increased cryopreservation fluid;

- mixing said energy increased cryopreservation fluid and said biological cell structures to form an energy increased fluidic cryopreservation composite;

- removing thermal energy from said fluidic cryopreservation composite; and

15 - freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water.

According to another aspect of the invention there is provided a process to enhance the cryopreservation of biological cells comprising the steps of:

20 - assembling a cellular collection containing a plurality of biological cell structures;

- establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;

- *in situ* sizing droplets of at least one substance within said cryopreservation fluid;

- mixing said cryopreservation fluid and said biological cell structures to form a fluidic cryopreservation composite;

- removing thermal energy from said fluidic cryopreservation composite; and

25 - freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water.

I. BRIEF DESCRIPTION OF DRAWINGS

Figure 1 represents a schematic of equipment and substances as they may be used in
5 embodiments of the present invention.

Figure 2 represents one process flow as may exist in accomplishing processes according to
embodiments of the present invention.

10 Figure 3 represents a schematic depiction of before and after example of a conceptual substance
that has been rendered according to embodiments of the present invention.

Figure 4 represents a general cooling curve such as might be used in the cryopreservation
process according to an embodiment of the present invention.

Figure 5 represents size distribution depiction showing how distributions can be altered in an embodiment of the present invention.

5 **V. MODE(S) FOR CARRYING OUT THE INVENTION**

The basic concepts of the present invention may be embodied in a variety of ways. It involves treatment techniques and methods, compositions and combinations of naturally derived and synthetic hydrophobic moieties, as well as equipment to accomplish the appropriate treatment. In this application, the treatment techniques and equipment are disclosed as part of the results 10 shown to be achieved by the various devices described and as steps which are inherent to utilization. In addition, while some devices are disclosed, it would be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facts should be understood to be encompassed by this disclosure.

15 Cryopreservation can involve assembling (2) a subject cellular collection (1) to create an assemblage (3) of cells, tissue, seeds, or the like. In addition, a cryopreservation fluid (4) can be collected (5). Substances of this cryopreservation fluid (4) can be combined (6) to create the typical composite cryopreservation fluid (4). According to embodiments of the present invention, the cryopreservation fluid (4) may be conditioned or treated (7) to enhance the 20 process. The subject cellular collection (1) can be mixed (8) with some type of cryopreservation fluid (4) to form a cryopreservation composite (20) and then have thermal energy removed (9) from it to accomplish freezing (26) to form a frozen cryopreservate (21). To use the cells or the like, thermal energy can be added (10) to effect thawing. The thawed composite can have items separated (11) for ultimate use (12) hopefully presenting viable, functional cells.

25

As mentioned earlier, the cryopreservation fluid (4) can have various elements (13)-(17). One element can be a cryoprotectant or cryoprotective agent (13). A variety of cryoprotective agents are known to those skilled in the art. These can include the following: acetamide, agaroses, alginates, alanine albumin, ammonium acetate, butanediol, chondroitin sulfate, 30 chloroform, choline, cyclohexanediols, dextrnas, diethylene glycol, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide*, erythritol, lethonol, ethylene glycol, monomethyl

ether, formamide, glucose, glycerol, glycerophosphate, glycylmonoacetate, glycine, hydroxyethyl starch, inositol, lactose, magnesium chloride, magnesium sulfate, maltose, mannitol, mannose, methanol, methoxypropanediol, methyl acetamide, methyl formamide, methyl ureas, methyl glucose, methyl glycerol, phenol, pluronicpolyos, olyethylene glycol, 5 polyvinylpyrrolidone, proline, propanediol, propylene glycol, pyridine N-oxide, ribose, serine, sodium bromide, sodium chloride, sodium iodide, sodium nitrate, sodium nitrite, sodium sulfate, sorbitol, sucrose, trehalose, triethylene glycol, trimethylamine acetate, urea, valine and xylose. Of course, each agent or the overall cryopreservation fluid (4) can be customized for the application or cellular collection (1) or individual cells involved.

10

The overall cryopreservation fluid (4) may also commonly contain a foundation medium (14) perhaps such as water, an energy source (15), and the cryoprotectant (13). Other ingredients (16) and (17) can be included as well and as one skilled in the art would well understand these can vary by cell type or process. For example, media may be 90% serum +10% cryoprotectant 15 (glycerol or DMSO, dimethylsulfoxide). In general, cryoprotectant concentrations may range from 2 to 10% or 20%. Serum may include HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), sodium bicarbonate, fetal bovine serum, bovine serum albumin, fetal serum albumin and the like. Another general media for suspension cell types can include 45% fresh medium used to grow the cells plus protein, 45% used (depleted) medium plus 10% 20 cryopreservative. Cryopreservative concentrations may vary but, as mentioned, often range from 2-10% such as for DMSO or the like, and 2-20% for glycerol and the like, again dependent on cell type as some cryopreservatives are cytotoxic for some cells. For example, sperm freezing medium may contain serum albumin, glycerol and sucrose and may contain egg yolk or milk (20%) plus 6% to 10% glycerol (final concentration). The solution may include HEPES, sodium 25 bicarbonate, fetal bovine serum, bovine serum albumin, fetal serum albumin and the like. Another commercial freezing medium may contain high-glucose Dulbecco's modified eagle medium (DMEM) or Earle's balanced salt solution, Hank's balanced salt solution, nutrient mixture F-12 (Ham's), Leibovitz's L-15 plus 10% serum (such as fetal bovine serum), 10% DMSO. Such solutions may also include D-glucose, L-glutamine, sodium pyruvate, phosphate 30 buffered saline, calcium and magnesium salts. For embryos equilibration and vitrification media may include: 7.5% DMSO, 7.5% ethylene glycol, 20% DSS, 0.5M sucrose in HEPES buffered

medium. For sperm cells equilibration and freezing media may include: Sodium citrate, 20% egg yolk, or 4% milk fat, fructose and/or 7% glycerol.

The principal components of lipid modified cryopreservation can be cryopreservation media
5 customized for a specific cell, tissue, organ and/or cellular composition, the biological cellular composition, a hydrophobic extract that is not generally miscible with the water-based cryopreservation media, a method to condition or treat (7) the cryopreservation fluid (4), such method may include adding (18) energy (19) such as through surface energy, to the cryopreservation fluid (4), a method to decrease or reduce thermal energy (9) from the
10 cryopreservation media, and a method to assess post-cryogenic viability of the cryopreserved structures. Lipids not previously considered adequately suitable for use can now be used. From one perspective, the reasons they may have been unsuitable include the inadvertent and/or unintended modification of the optimum cooling curve (22) for a specific cell type. In an embodiment of this invention the addition of energy (19) may work with a variety of commercial
15 cooling devices and commercial freezing devices and techniques without requiring undue modification.

In another embodiment of the invention the addition of hydrophobic substances can be achieved via the addition of energy (19) to a cryopreservation fluid (4) or media. This energy input may
20 be achieved using one or a combination of commercially available pieces of equipment designed to rend large items into smaller items. These pieces of equipment are well known to those skilled in the art.

In one embodiment of the invention the surface area of hydrophobic moieties is increased by
25 rending (25) large drops (23) to create sufficient surface energy within the lipid containing cryopreservation fluid so as to provide enhanced post-cryogenic viability of the cryopreserved cellular collection. Rending (25) the lipids or hydrophobic moieties may include increasing the number of initial large drops (23) to droplets (24) increased in number by a multiple of perhaps 50 times or many more times. Rending (25) should be understood to be dependent on the
30 particular hydrophobic solution and specific surface tension for said solution. Rending (25) may be achieved by a variety of methods including physically dispersing large droplets, shearing,

cavitation, sonication, pressing, chemical means, relatively energetic mechanical mixing, using a detergent, salts, common emulsifying or similar methods and many other commercially available methods. The present invention is independent of the method utilized to rend and may instead focus on the apparent surface energy imparted by said rending. In one embodiment the 5 number of droplets may be specific to a particular lipid and lipid surface tension and the same number of droplets may represent different additions of energy to the substance in the cryopreservation fluid.

In still another embodiment, the effect and a measurement of the viability can be specific for a 10 specific cell type and in general can be understood to include at least one of a variety of attributes including reducing the amount of apoptosis, reducing the quantity of necrotic cells, decreasing damage to external membrane proteins, decreasing DNA damage, decreasing oxidative damage, decreasing membrane damage, decreasing the amount of disruption to functional areas of the cellular collection, limiting biochemical toxicity, suppressing cytoskeletal 15 reorganization and membrane composition rearrangement. Viability improvement can be compared to traditional post-cryogenic cells of a similar character and thermal treatment. Methods of assessing viability may be known to vary also so viability assessment could optimally utilize the same methods, techniques and protocols to assess viability.

20 In another embodiment of the invention the addition of lipids may not cause a negative change in the kinetics of the thermal energy decrease that is necessary for said cellular collection to experience cryopreservation down to the target temperature of either about -80°C or about -196°C. Likewise in another embodiment of the invention lipids may have a droplet size that enables appropriate rate of thermal energy input of said cellular collections to be thawed. Such 25 enablements should be understood to not diminish or positively affect the post-cryogenic viability of the cellular collection.

In still another embodiment the addition of the lipid may comprise an antioxidant that protects 30 said cells from oxidative damage during processing pre- and post- cryopreservation. Moreover, the lipids or hydrophobic moieties might comprise or may be selected such that they serve as antioxidants or a free-radical scavenger for the specific stresses experienced by a specific cellular

composition type. Lipids might also comprise or contain membrane stabilizers such as cholesterol, lipid cryoprotectants, and other types of lipids as may be beneficial to the particular cellular composition or the particular cryopreservation media.

5 In still another embodiment the lipids may be derived from any number of sources including animal fats, lipids from reproductive structures such as egg or seeds, lipids from animal byproducts such as milk and other bodily fluids, plants, fruit, roots, leaves, seeds, whole plants, fruits, fruit pulp, cotyledons of plant embryo, endosperm, seeds, leaves, nuts, milk, roots, bark, algal and fungal derived lipids, and other plant parts, milk, algae, fungus, yeast cells, synthetic 10 lipids, mixed oils and the like, and may include multiple lipid sources within one cryopreservation fluids to achieve the goals of protecting the cryopreserved cell. Lipids may also be synthetically created or be bio-identical in order to emulate naturally produced lipids or the functionality of lipids such as synthetic vitamin E versus naturally extracted vitamin E. Lipids may be any substance which can be extracted using such compounds as ether, benzene, or other 15 nonpolar solvents. Lipids for inclusion in the present invention may also be derived using physical means such as centrifugation, pressing and other methods common to those practicing the art.

In one more embodiment the extracts may include lipids from the following gross classes of 20 lipids: triacylglycerols, polyphenolics, tocopherol, ascorbic acid, poly-unsaturated fatty acids, saturated fatty acids, anthraquinones, phospholipids, sterols, sterol esters, carotenoids, liquid waxes and glycerolipids perhaps more specifically including the following: monagalactosyldiacylglycerol, digalatosyldiacylglycerol, sulfoquinovosyldiacyllglycerol, triacylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, 25 phosphatidylglycerol, glycosyldiacylglycerols and others. Fatty acids may include 16:0, 18:0, 18:1, 18:2, 18:3, plus C₂₀-C₂₂ but may also include any of the other known or unknown saturated and/or unsaturated fatty acids.

In one more embodiment additional substances such as weighting agents and emulsifiers may be 30 added to the blend of lipids to further enable a uniform population of lipid droplets and to further enable emulsion stability. Similarly the lipid droplet size may be such that it is unnaturally

small. It may also be selected so it is not unstable and thus not requiring additional substances. In a similar embodiment, the emulsion may be stabilized by solid particles such as colloidal silica, latex particles or proteins such as casein (milk fat proteins) such that a pickering emulsion is formed and droplet stability is further enhanced.

5

In another embodiment the emulsion may be classified as an emulsifier stabilized and/or nanoemulsion indicating the small droplet size. The size distribution (26) of the numbers of droplets (24) can vary in different ways. The size value selected, desired, or resulting can present a maximum so that no substantial numbers of drops or droplets exist with significantly

10 larger sizes, so that particular maximum percentages of droplets with larger sizes (by number or even by volume) is established. It can also represent an average, mean (27), median (28), or just a non-normal distribution as well. In one embodiment, the unnaturally small or other droplets of the lipid or other substance may also exist in a skewed size distribution (30) such as may favor smaller size droplets (24), perhaps as in a positively skewed distribution (30), as opposed to a 15 normal Gaussian distribution of droplet size. The distribution may also be strongly unimodal, strongly favoring a mode (29) of one droplet size, perhaps a reduced mode droplet size. These, of course, can have specified values as explained below.

20 In one embodiment the invention may provide a method for input or use of at least one lipid into a cryopreservation fluid. Such embodiments may include methods to size, size exclude, and size limit droplets of said lipid in the cryopreservation media. These may be utilized to achieve an unnaturally small droplet size and/or distribution that provides the appropriate surface energy to the cryopreservation fluid.

25 In some embodiments, the method may include techniques for utilizing multiple hydrophobic substances from disparate sources including plants, animals and synthetic sources and which may easily be exemplified by egg yolk, milk lipids and soybean oil. The method may also include use of substances from multiple sources such as egg yolk and plant derived olive oil. In the next embodiment lipids may serve as an antioxidant or radical-scavenger for said cells. In 30 another embodiment said lipids may be selected such that they may serve as a membrane stabilizer for the specific cell type being frozen. In another embodiment the combination of

lipids may be selected such that they serve as both antioxidant or radical-scavenger and/or membrane protectant for the specific cell type being frozen.

In an embodiment of the invention a variety of lipids could be added to a variety of cellular compositions including, but not limited to, skin, oocytes, sperm, stem cells, embryonic stem cells, neural stem cells, epithelial stem cells, cardiac stem cells, muscle stem cells, connective stem cells, epithelial cells, cardiac cells, muscle cells, connective cells, nerve cells, umbilical cord blood, blood, histological samples, plant seeds, plant shoots, ovarian tissue, testicular tissue, embryos, tumorous tissue, yeast cells, bacterial cells, algal cells, fungal cells, mesenchymal cells, keratinocytes, melanocytes, hepatocytes, liver tissue, and the like.

An example of the benefits of embodiments of the present invention can be shown by a comparison of processes for an application to sperm cells as follows. Initially as a control, one can create a solution of Tris extender for 1000 mls (pH 6.8, osmotic pressure 322-325 mmol/kg) perhaps with the following components: 10.53 g Tris, 4.38 g fructose, 5.53 g citric acid, and 870 ml H₂O. To 500 mls Tris, one can add 22% egg yolk (all white removed; Part A extender) and mix by hand or using a stir bar. One can dilute concentrated ejaculate using Part A extender so sperm cells reach a final concentration of 15×10^6 cells/ml. This mixture can be cooled to about 4°C then one may add a part B extender (Part A plus 14% glycerol) to a final concentration of 7% glycerol to cooled sperm cells. This can be allowed to equilibrate and then packaged as the cooled suspension is put into straws. These straws may be cooled over liquid nitrogen vapor at a rate of about 10-20°C/min then the straws may be plunged into the liquid nitrogen. This may be stored at the -196°C temperature of liquid nitrogen until needed. To utilize these straws, they may be removed from the liquid nitrogen and submerged in a 37°C water bath for about 60 seconds.

As an improvement that modifies the cryopreservation fluid (4), lipids can be added to the part A extender of the above example prior to addition of the cells. This addition may be of the 22% egg yolk with added 5% mineral oil. Energy can be added (18) wherein these lipids may then be sonicated at 30% for 2 times before using the solution to dilute the sperm cells. Other options

for energy addition (18) can include mechanical mixing, physical dispersal and the like. The energy addition can rend (25) the lipid as explained.

As can be seen from the following data (Table 1, experiment 1), clearly the addition of lipids without modifying (7) to the cryopreserved cells negatively affected the viability of the cells. In this example total lipid content is 20% egg yolk plus the varying percentage of hydrophilic (lipid) extracts results in a total lipid concentration of up to 29% total lipid (v/v). This additional lipid causes a statistically significant 70% decrease in viability. With modifying (7) the addition of ≥ 270 j/kg or ≥ 330 j/kg (ex. 2 and 3) as energy additions (18) to the lipid containing cryopreservation fluid (4), the viability is equal to, or superior to 20% total lipid example. As can be seen by example 2, the type of lipid also has an effect on energy required to achieve superior viability. For example, oil A may benefit from higher energy addition. Note, the post-cryogenic viability difference from traditional post-cryogenic cells (0% extra lipid) is >29 percentage points in example 1, while in example 2, where energy is added, the difference is <5 percentage points, a significant improvement in post-cryogenic cell viability. Comparing the viability of cells with 20% total lipids in example 3, one sees an improvement in viability by adding >270 j/kg energy and increasing the total percentage of lipid is beneficial. Note, this table only reflects one measure of viability for these cells but does not reflect benefits such as a 30% increase in DNA quality when using 9% oil B as compared to traditional post-cryogenic cells of a similar character and thermal treatment.

Table 1:

| Oil | A | B | C | A | B | C | A | B | C |
|---|-----|---|---|----|----|---|----|----|---|
| Additional hydrophobic extracts (percent v/v) | 0 | | | 3 | | | 9 | | |
| Total lipid concentration (percent v/v) | 20* | | | 23 | | | 29 | | |
| EXAMPLE 1 Post cryogenic viability | 41 | | | 30 | 30 | | 12 | 18 | |

| (Motility) | | | | | | | |
|---|----|-----------------|----|----|----|----|----|
| EXAMPLE 2 Post-cryogenic viability (motility) with addition of ≥ 270 j/kg energy** | 26 | | 28 | 32 | 21 | 20 | 26 |
| Post-cryogenic viability (motility) with addition of ≥ 330 j/kg energy** | 26 | | 27 | 29 | 27 | 19 | 27 |
| EXAMPLE 3 Post-cryogenic viability (motility) with addition of ≥ 270 j/kg energy | 57 | 59 [#] | | 57 | 60 | 57 | 38 |
| | | | | | | 57 | 55 |

*contains only egg yolk lipids, no additional lipids.

** No energy addition to the sample with 20% total lipid concentration.

Energy addition to sample with 20% total lipid concentration

5 Semen from 10 production bulls (example 1, 7 production bulls (example 2) or 5 production bulls (example 3) was collected and initially evaluated using industry standard procedure. Ejaculates were extended to 56×10^6 sperm/ml in egg yolk citrate extender (part A) containing 20% egg yolk plus oil extract A, B or C at 0, 2, 6, 10, 14 or 18% and held for a minimum of 2 hours at 4°C. After equilibration at 4°C, an equal amount of part B extender (egg yolk citrate + 14% glycerol) was then added, the sperm packaged in 0.5 cc straws at a final concentration of 28×10^6 sperm/ml resulting in a final concentration of 0, 1,3,5,9 or 9% oil A, B or C . Oil A = Olive oil, Oil B = Sea buckthorn, Oil C = Mineral oil. The sperm was frozen over nitrogen vapor then plunged into liquid nitrogen using industry standard procedure.

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These sperm cells were thawed by immersing in a 37°C water bath for a minimum of 45 seconds.

Three replicates of each treatment/bull were analyzed after thawing and warming for 30 minutes for motility. Motility parameters including total and progressive motility, velocity parameters were assessed by Hamilton-Thorne IVOS system with the following standard settings:

5 Progressive cells: path velocity (VAP) 500 μ m/s, straightness 70%; image capture: frames per sec 60 Hz, 40 frames. Slow cells-static: VAP cutoff 30 μ m/s, ASL cutoff 15 μ m/s. Cell size 5 px, cell intensity 70. 2x-CEL slides (Hamilton-throne) were used for all evaluations.

Motility above is the average of 10 bulls per treatment (ex. 1) or 7 bulls per treatment (ex. 2), 5

10 bulls per treatment (ex. 3) in extenders where the addition of 270 j/kg energy (ex. 1, 2 and 3) or \geq 330 j/kg energy (ex. 2) was added to the extender prior to the addition of sperm cells.

As can be understood from the above examples, the modifying of the cryopreservation fluid (4) and particularly the addition of surface energy to the fluid prior to the removal of thermal energy

15 has a very beneficial effect. While the above examples indicate some values and substances used, naturally, these can be varied to achieve the desired result in any application. For example, the variations can be accomplished to achieve set values or thresholds of cell viability improvement. Of course, embodiments can achieve as much improvement as possible, but levels of improvement in viability can provide or present a substance with at least about 5%, 10%, 20

15%, 20%, or even 25% higher post-cryogenic viability for the biological cell structures or the like as compared to or over traditional post-cryogenic cell structures of a similar character and thermal treatment. Similarly, embodiments can provide or present a substance with not less than about 80%, 70%, 60%, 50%, 40%, 30%, and 20% of the pre-cryogenic viability, however measured, for the biological cell structures or the like.

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Another aspect that can be varied in embodiments of the invention can be the amount of energy or even surface energy added. This can be varied so that the values achieved can result in adding at least about, or result in a cryopreservation fluid (4) containing a substance having its surface

30 energy increased by at least about 100 j/kg, 270 j/kg, 320 j/kg, 650 j/kg, 1050 j/kg, 3600 j/kg, or even 5200 j/kg. This can even be mainly the lipid in the cryopreservation fluid (4). Similarly, the surface energy of a substance in the cryopreservation fluid (4) can be increased to at least

about 200 j/kg, 420 j/kg, 760 j/kg, 1200 j/kg, 3800 j/kg, or even 5400 j/kg of surface energy. From another perspective, the surface energy of a substance in the cryopreservation fluid (4) can be increased by at least about 30%, 50%, 100%, 3 times, 5 times, 10 times, 30 times, or even 50 times that of the preexisting state.

5

Yet another aspect that can be varied in embodiments of the invention can be the transformation in the number of drops or droplets present. This can be varied so that the values achieved can result in causing droplets that number, or presenting a substance having droplets numbering at least about 30 times, 50 times, 100 times, 500 times, 1000 times, 5000 times, 10000 times, 50000 times, and even 100000 times the number of initial drops of the substance in the cryopreservation fluid (4) or of an typical fluid of such character.

Still another aspect that can be varied in embodiments of the invention can be the size of the droplets resulting. This can be varied so that the values achieved can present a cryopreservation fluid (4) containing a substance having no substantial number of droplets with a diameter larger than about, or rending larger drops so that there are no substantial amount of droplets larger than about 1000nm, 900nm, 700nm, 500nm, 300nm, 100nm, 70nm, 50nm, or even 30nm in that substance in the cryopreservation fluid (4). Even the distribution of these droplets of the substance in the cryopreservation fluid (4) can be a varied effect. Here, size distributions of the above sizes can be altered so that these sizes or smaller are present for, and droplets as small as exist for at least about 20%, 40%, 60%, 80%, and even 90% of the droplets of the substance in the cryopreservation fluid (4). The activity can cause droplets so that there is no substantial amount of droplets larger than the above sizes perhaps even for only a specified lipid or the like. The distribution can also be just presenting a substantially uniform sizing with or without the above sizes indicated. The distribution can be skewed toward smaller sizes as well. Here even the mode value of size distribution for a substance or even a specified lipid can be less than about the above sizes. Even sizes larger than about the above sizes can be eliminated or even rended so that there are no substantial amount of droplets larger than any of the above sizes. This can occur in situ as well so concentration remains largely unvaried.

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Another aspect that can be varied in embodiments of the invention can be the composition of the substances themselves. Even concentrations can be presented that are relatively high concentrations for that substance (as compared to traditional uses and also as compared to unconditioned substances). These concentrations can be at least about 10%, 20%, 40%, 60%, 5 and even 80% higher than traditional lipid concentration cryopreservation fluids as compared to similar fluid of similar character for similar thermal treatment perhaps with similar cells. The substances themselves can now be varied even so that substances can now include lipids, lipid soluble substances, or other substances that may not have previously worked, worked as well, worked at concentrations desired, or been combinable with other substances. Through the 10 present invention processes can now include oils, lipids, hydrophobic substances, or lipids at appropriate concentrations or efficacies, from the following list: sea buckthorn lipids, saturated free fatty acids, unsaturated free fatty acids, lauric acid, myristic acid, palmitic acid, stearic acid, arachadonic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachadonic acid, 15 lecithin, triglycerides, spermaceti, bees wax, carnuba wax, sphingomyelins, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, cholic acid, oleic acid, β -sitosterol, β -amyrin, γ -carotene, α -amyrin, β -carotene, lycopene, lutein, tocopherol, ubiquinol, tocotrienols, eugenol, phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, ceramide phosphorylcholine, ceramine phosphorylglycerol, 20 digalactosyldiacylglycerol, monogalactosymonoacylglycerol, 16:1 fatty acids, 18:1 fatty acids, 18:2 fatty acids, 18:3 fatty acids, natural rubber, and gutta-percha.

Through the present invention processes can now include substances combinable with lipids or other substances such as from the following list: thiols, ascorbic acid, polyphenols, superoxide 25 dismutase, catalase, peroxidase, lipoic acid, uric acid, hydrophobic substances (non-lipid), silicones, fluorocarbons, glutathione, melatonin, peroxiredoxins, resveratrol, phytic acid, flavonoids, vitamin A, vitamin E, vitamin D2, vitamin K1, lipid soluble substances, and lipid soluble vitamins.

30 The above, and other substances can also be used alone or in combination, at different levels, from different forms, plant sources, plant components and enantiomers such as: plants, plant

components, lipids, *Hippophea*, sea buckthorn lipids, seed oil, pulp (fruit)oil, saturated fatty acids, unsaturated fatty acids, lauric acid, natural rubber, leaf alcohol extract, myristic acid, gutta-percha, palmitic acid, *Euterpe oleracea*, açai, fruit, pulp, skin, stearic acid, vitamin A, (perhaps in suspension as well), glycine, soybean, milk, arachadonic acid, vitamin E, lycium, 5 goji/wolfberry, berry, palmitoleic acid, vitamin D2, , blueberry, oleic acid, vitamin K1, carya, pecan, nut pulp, linoleic acid, raspberries, fruit pulp, linolenic acid, *Rosaceae*, strawberry, thiols, *Rubus*, blackberry, triglycerides, ascorbic acid, *Amelanchier* (saskatoon), *Prunus*, plum, spermaceti, polyprenols, litchi, lychee, seeds, bees wax, superoxide dismutase, *Psidium*, guava, leaves, carnuba wax, catalase, sphingomyelins, peroxidase, *Vitis*, grape, monoterpenes, lipoic 10 acid, *Eugenia*, alcohol extract fruit, sesquiterpenes, uric acid, *Olea*, olive, oil and fruit, diterpenes, hydrophobic substances, *Persea*, avocado, *Gymnema inodorum*, australian cowplant, triterpenes, silicones, *Sechium edule*, chayote, cholic acid, fluorocarbons, *Mentha*, mint, oleic acid, glutathione, *Leucanea leucocephala*, lead tree, shoot tips, β -sitosterol, melatonin, piper, 15 pepper, lecithin, β -amyrin, α -amyrin, peroxiredoxins, eryngium, coriander, carotene, resveratrol, *Oenanthe*, celery, phytic acid, *Zingiber*, ginger, β -carotene, γ -carotene, flavonoids, *Cucrcuma longa*, turmeric, lycopene, lutein, fish, tocopherol, whale, ubiquinol, bee, tocotrienols, fowl 20 eggs, fowl body fat, eugenol, beef body fat, phosphatidic acid, pork body fat, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, ceramide phosphorylcholine, ceramine phosphorylglycerol, digalactosyldiacylglycerol, and monogalactosymonoacylglycerol. While it should be understood that each of these can have varying degrees of effect as a result of the present invention, it is anticipated that advantages for each and for combinations of each exist through the present invention. It is also expected that both particular substances and especially combinations not previously viable are now possible with positive results.

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While the invention has been described in connection with a preferred embodiment, it is not intended to limit the scope of the invention to the particular form set forth, but on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the statements of invention.

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Examples of alternative claim may include:

1. A process to enhance the cryopreservation of biological cells comprising the steps of:
 - assembling a cellular collection containing a plurality of biological cell structures;
 - establishing a lipid containing cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;
 - adding at least about 270 j/kg surface energy to at least one lipid in said lipid containing cryopreservation fluid to create a lipid surface energy increased cryopreservation fluid;
 - mixing said lipid surface energy increased cryopreservation fluid and said biological cell structures to form an energy increased fluidic cryopreservation composite;
 - removing thermal energy from said fluidic cryopreservation composite;
 - freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water; and
 - providing an enhanced post-cryogenic viability for said biological cell structures.
- 15 2. A process to enhance the cryopreservation of biological cells as described in clause 1 or any other clause wherein said step of providing an enhanced post-cryogenic viability for said biological cell structures comprises the step of providing at least about 10% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cells of a similar character and thermal treatment.
- 20 3. A process to enhance the cryopreservation of biological cells as described in clause 2 or any other clause wherein said step of adding at least about 270 j/kg surface energy to at least one lipid in said lipid containing cryopreservation fluid to create a lipid surface energy increased cryopreservation fluid comprises the step of adding at least about 650 j/kg lipid surface energy to at least one lipid in said cryopreservation fluid.
- 25 4. A process to enhance the cryopreservation of biological cells as described in clause 2 or any other clause and further comprising the step of drop transforming at least one lipid in said cryopreservation fluid into droplets numbering at least about 50 times the number of initial drops of said lipid in said cryopreservation fluid.
5. A process to enhance the cryopreservation of biological cells as described in clause 2 or 3 or any other clause and further comprising the step of drop transforming at least one lipid

in said cryopreservation fluid into droplets numbering at least about 50000 times the number of initial drops of said lipid in said cryopreservation fluid.

6. A process to enhance the cryopreservation of biological cells as described in clause 2 or
5 any other clause wherein said step of adding at least about 270 j/kg surface energy to at least one lipid in said lipid containing cryopreservation fluid to create a lipid surface energy increased cryopreservation fluid comprises the step of rending larger drops of said at least one lipid in said cryopreservation fluid to form smaller droplets of said at least one lipid in said cryopreservation fluid.
- 10 7. A process to enhance the cryopreservation of biological cells as described in clause 6 or any other clause wherein said step of rending larger drops of said at least one lipid in said cryopreservation fluid to form smaller droplets of said at least one lipid in said cryopreservation fluid comprises the step of rending larger drops of said at least one lipid in said cryopreservation fluid to contain no substantial number of droplets having a diameter larger than about 1000nm in said at least one lipid in said cryopreservation fluid.
- 15 8. A process to enhance the cryopreservation of biological cells as described in clause 6 or any other clause wherein said step of rending larger drops of said at least one lipid in said cryopreservation fluid to form smaller droplets of said at least one lipid in said cryopreservation fluid comprises the step of rending larger drops of said at least one lipid in said cryopreservation fluid to no substantial number of droplets having a diameter larger than about 200nm in said at least one lipid in said cryopreservation fluid.
- 20 9. A process to enhance the cryopreservation of biological cells as described in clause 2, 4, 7, or 8 or any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:
 - assembling a cellular collection containing a plurality of blood cell structures,
 - assembling a cellular collection containing a plurality of stem cell structures,
 - assembling a cellular collection containing a plurality of skin cells,
 - assembling a cellular collection containing a plurality of embryonic stem cells,
 - assembling a cellular collection containing a plurality of neural stem cells,

- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- assembling a cellular collection containing a plurality of connective stem cells
- 5 -assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells
- assembling a cellular collection containing a plurality of connective cells
- assembling a cellular collection containing a plurality of nerve cells
- 10 - assembling a cellular collection containing a plurality of umbilical cord blood cells,
- assembling a cellular collection containing a plurality of histological sample cells,
- assembling a cellular collection containing a plurality of plant seed cells,
- 15 - assembling a cellular collection containing a plurality of plant shoot cells,
- assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- assembling a cellular collection containing a plurality of testicular tissue cell structures,
- assembling a cellular collection containing a plurality of embryo cells,
- 20 - assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
- assembling a cellular collection containing a plurality of algal cells,
- 25 - assembling a cellular collection containing a plurality of fungal cells,
- assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,
- assembling a cellular collection containing a plurality of melanocyte cells,
- assembling a cellular collection containing a plurality of hepatocyte cells,
- 30 - assembling a cellular collection containing a plurality of oocyte cells,
- assembling a cellular collection containing a plurality of sperm cells, and

10. A cryopreservation composition comprising:
 - an enhanced cryogenic viability assemblage of biological cell structures; and
 - an increased lipid surface energy content cryopreservation fluid mixed with said enhanced cryogenic viability assemblage of biological cell structures, containing a lipid that has had its surface energy increased by at least about 270 j/kg surface energy, and that has at some point has been frozen at temperature below the freezing point of water.
11. A cryopreservation composition as described in clause 10 or any other clause wherein said enhanced post-cryogenic viability assemblage of biological cell structures comprises an at least about 10% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cells of a similar character and thermal treatment.
12. A cryopreservation composition as described in clause 11 or any other clause wherein said increased lipid surface energy content cryopreservation fluid comprises a cryopreservation fluid containing a lipid having its surface energy increased by at least about 650 j/kg surface energy.
13. A cryopreservation composition as described in clause 11 or any other clause wherein said increased lipid surface energy content cryopreservation fluid comprises a cryopreservation fluid containing a lipid having its number of drops in said cryopreservation fluid increased by at least about 50 times.
14. A cryopreservation composition as described in clause 11 or 12 or any other clause wherein said increased lipid surface energy content cryopreservation fluid comprises a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50000 times.
- 25 15. A cryopreservation composition as described in clause 11 or any other clause wherein said increased lipid surface energy content cryopreservation fluid comprises a larger drop rended cryopreservation fluid.
16. A cryopreservation composition as described in clause 15 or any other clause wherein said larger drop rended cryopreservation fluid comprises a cryopreservation fluid containing a substance having no substantial number of droplets having a diameter larger than about 1000nm.

17. A cryopreservation composition as described in clause 15 or any other clause wherein said larger drop rended cryopreservation fluid comprises a cryopreservation fluid containing a substance having no substantial number of droplets having a diameter larger than about 200nm.

5 18. A cryopreservation composition as described in clause 11, 12, 16, or 17, or any other clause wherein said enhanced cryogenic viability assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- 10 - an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- 15 - an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability muscle cells
- 20 - an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability of nerve cells- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- 25 - an assemblage of enhanced cryogenic viability plant shoot cells,
- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- 30 - an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,

- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- 5 - an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures,

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10 19. A process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cell structures;
- establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;
- adding energy to said cryopreservation fluid to create an energy increased cryopreservation fluid;
- mixing said energy increased cryopreservation fluid and said biological cell structures to form an energy increased fluidic cryopreservation composite;
- removing thermal energy from said fluidic cryopreservation composite; and
- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water.

20 20. A process to enhance the cryopreservation of biological cells as described in clause 19 or any other clause and further comprising the step of providing an enhanced post-cryogenic viability for said biological cell structures.

21. A process to enhance the cryopreservation of biological cells as described in clause 19 or any other clause wherein said step of providing an enhanced post-cryogenic viability for said biological cell structures comprises the step of providing relatively high post-cryogenic viability for said biological cell structures.

25 22. A process to enhance the cryopreservation of biological cells as described in clause 20 or any other clause wherein said step of providing relatively high post-cryogenic viability for said biological cell structures comprises a step selected from a group consisting of the steps of:

- providing at least about 5% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

5 - providing at least about 10% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

- providing at least about 15% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

10 - providing at least about 20% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment, and

- providing at least about 25% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment.

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23. A process to enhance the cryopreservation of biological cells as described in clause 21 or any other clause wherein said step of providing relatively high post-cryogenic viability for said biological cell structures comprises a step selected from a group consisting of the steps of:

20 - providing not less than about 80% of the pre-cryogenic viability for said biological cell structures,

- providing not less than about 70% of the pre-cryogenic viability for said biological cell structures,

- providing not less than about 60% of the pre-cryogenic viability for said biological cell structures,

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- providing not less than about 50% of the pre-cryogenic viability for said biological cell structures,

- providing not less than about 40% of the pre-cryogenic viability for said biological cell structures,

30 - providing not less than about 30% of the pre-cryogenic viability for said biological cell structures, and

- providing not less than about 20% of the pre-cryogenic viability for said biological cell structures.

24. A process to enhance the cryopreservation of biological cells as described in clause 21 or any other clause wherein said step of adding energy to said cryopreservation fluid to create an energy increased cryopreservation fluid comprises the step of adding surface energy to a substance in said cryopreservation fluid to create a surface energy increased cryopreservation fluid.

5 25. A process to enhance the cryopreservation of biological cells as described in clause 24 or any other clause wherein said step of adding surface energy to a substance in said cryopreservation fluid to create a surface energy increased cryopreservation fluid comprises the step of adding substantial surface energy to a substance in said cryopreservation fluid to create a substantially surface energy enhanced cryopreservation fluid.

10 26. A process to enhance the cryopreservation of biological cells as described in clause 25 or any other clause wherein said step of adding substantial surface energy to a substance in said cryopreservation fluid to create a substantially surface energy enhanced cryopreservation fluid comprises a step selected from a group consisting of the steps of:

15 - adding at least about 100 j/kg surface energy to a substance in said cryopreservation fluid,
20 - adding at least about 270 j/kg surface energy to a substance in said cryopreservation fluid,
- adding at least about 320 j/kg surface energy to a substance in said cryopreservation fluid,
- adding at least about 650 j/kg surface energy to a substance in said cryopreservation fluid,
25 - adding at least about 1050 j/kg surface energy to a substance in said cryopreservation fluid,
- adding at least about 3600 j/kg surface energy to a substance in said cryopreservation fluid, and
30 - adding at least about 5200 j/kg surface energy to a substance in said cryopreservation fluid.

27. A process to enhance the cryopreservation of biological cells as described in clause 25 or any other clause wherein said step of adding substantial surface energy to a substance in said cryopreservation fluid to create a substantially surface energy enhanced cryopreservation fluid comprises a step selected from a group consisting of the steps of:

5 - increasing a surface energy of a substance in said cryopreservation fluid to at least about 200 j/kg surface energy,

 - increasing a surface energy of a substance in said cryopreservation fluid to at least about 420 j/kg surface energy,

 - increasing a surface energy of a substance in said cryopreservation fluid to at least about 760 j/kg surface energy,

10 - increasing a surface energy of a substance in said cryopreservation fluid to at least about 1200 j/kg surface energy,

 - increasing a surface energy of a substance in said cryopreservation fluid to at least about 3800 j/kg surface energy, and

15 - increasing a surface energy of a substance in said cryopreservation fluid to at least about 5400 j/kg surface energy.

28. A process to enhance the cryopreservation of biological cells as described in clause 25 or any other clause wherein said step of adding substantial surface energy to a substance in said cryopreservation fluid to create a substantially surface energy enhanced cryopreservation fluid comprises a step selected from a group consisting of the steps of:

20 - increasing the surface energy of a substance in said cryopreservation fluid by at least about 30%,

 - increasing the surface energy of a substance in said cryopreservation fluid by at least about 50%,

25 - increasing the surface energy of a substance in said cryopreservation fluid by at least about 100%,

 - increasing the surface energy of a substance in said cryopreservation fluid by at least about 3 times,

 - increasing the surface energy of a substance in said cryopreservation fluid by at least about 5 times,

30

- increasing the surface energy of a substance in said cryopreservation fluid by at least about 10 times,

- increasing the surface energy of a substance in said cryopreservation fluid by at least about 30 times, and

5 - increasing the surface energy of a substance in said cryopreservation fluid by at least about 50 times.

29. A process to enhance the cryopreservation of biological cells as described in clause 19,25,27, 28 or any other clause wherein said step of adding energy to said cryopreservation fluid to create an energy increased cryopreservation fluid comprises the 10 step of structurally altering a substance in said cryopreservation fluid.

30. A process to enhance the cryopreservation of biological cells as described in clause 29 or any other clause wherein said step of structurally altering a substance in said cryopreservation fluid comprises the step of structurally storing energy in a substance in said cryopreservation fluid.

15 31. A process to enhance the cryopreservation of biological cells as described in clause 29 or any other clause wherein said step of structurally altering a substance in said cryopreservation fluid comprises the step of drop transforming a substance in said cryopreservation fluid.

32. A process to enhance the cryopreservation of biological cells as described in clause 31 or 20 any other clause wherein said step of drop transforming a substance in said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- transforming a substance in said cryopreservation fluid into droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,

25 - transforming a substance in said cryopreservation fluid into droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,

- transforming a substance in said cryopreservation fluid into droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,

- transforming a substance in said cryopreservation fluid into droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

5 - transforming a substance in said cryopreservation fluid into droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,

- transforming a substance in said cryopreservation fluid into droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,

10 - transforming a substance in said cryopreservation fluid into droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,

- transforming a substance in said cryopreservation fluid into droplets numbering at least about 50000 times the number of initial drops of said substance in said

15 cryopreservation fluid, and

- transforming a substance in said cryopreservation fluid into droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

33. A process to enhance the cryopreservation of biological cells as described in clause 24 or
20 any other clause wherein said step of adding energy to said cryopreservation fluid to
create an energy increased cryopreservation fluid comprises the step of rending larger
drops of a substance in said cryopreservation fluid to form smaller droplets of said
substance in said cryopreservation fluid.

34. A process to enhance the cryopreservation of biological cells as described in clause 33 or
25 any other clause wherein said step of rending larger drops of a substance in said
cryopreservation fluid to form smaller droplets of said substance in said cryopreservation
fluid comprises a step selected from a group consisting of the steps of:

30 - rending larger drops of a substance in said cryopreservation fluid so there are no
substantial amount of droplets larger than about 1000nm in said substance in said
cryopreservation fluid,

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 900nm in said substance in said cryopreservation fluid,

5 - rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 700nm in said substance in said cryopreservation fluid,

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 500nm in said substance in said cryopreservation fluid,

10 - rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 300nm in said substance in said cryopreservation fluid,

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 100nm in said substance in said

15 cryopreservation fluid,

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 70nm in said substance in said cryopreservation fluid,

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 50nm in said substance in said cryopreservation fluid, and

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 30nm in said substance in said cryopreservation fluid.

20 25 35. A process to enhance the cryopreservation of biological cells as described in clause 34 or any other clause wherein said step of rendering larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- rendering said substance in said cryopreservation fluid so that at least about 20% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,

- rendering said substance in said cryopreservation fluid so that at least about 40% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,

5 - rendering said substance in said cryopreservation fluid so that at least about 60% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,

- rendering said substance in said cryopreservation fluid so that at least about 80% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size, and

10 - rendering said substance in said cryopreservation fluid so that at least about 90% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size.

36. A process to enhance the cryopreservation of biological cells as described in clause 19, 24, 26, 27, 28, 34 or 35 or any other clause wherein said step of establishing a 15 cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation comprises a step selected from a group consisting of the steps of:

- establishing a sea buckthorn lipid containing cryoprotective fluid to be incorporated with said biological cell structures,

- establishing an unsaturated fatty acid containing cryoprotective fluid to be incorporated 20 with said biological cell structures

- establishing a saturated fatty acid containing cryoprotective fluid to be incorporated with said biological cell structures

- establishing a palmitic acid containing cryoprotective fluid to be incorporated with said biological cell structures,

- establishing a palmitoleic acid containing cryoprotective fluid to be incorporated with 25 said biological cell structures,

- establishing a oleic acid containing cryoprotective fluid to be incorporated with said biological cell structures,

- establishing a linoleic acid containing cryoprotective fluid to be incorporated with 30 said biological cell structures,

- establishing a linolenic acid containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a lecithin containing cryoprotective fluid to be incorporated with said biological cell structures,
- 5 - establishing a β -sitosterol containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a β -amyrin containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a γ -carotene containing cryoprotective fluid to be incorporated with said 10 biological cell structures,
- establishing a α -amyrin containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a β -carotene containing cryoprotective fluid to be incorporated with said biological cell structures,
- 15 - establishing a lycopene containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a lutein containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a tocopherol containing cryoprotective fluid to be incorporated with said 20 biological cell structures,
- establishing a phosphatidylethanolamine containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a digalactosyldiacylglycerol lipid containing cryoprotective fluid to be incorporated with said biological cell structures,
- 25 - establishing a monogalactosymonoacylglycerol containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a 16:1 fatty acid containing cryoprotective fluid to be incorporated with said biological cell structures,
- establishing a 18:1 fatty acid containing cryoprotective fluid to be incorporated with 30 said biological cell structures,

- establishing a 18:2 fatty acid containing cryoprotective fluid to be incorporated with said biological cell structures, and
- establishing a 18:3 fatty acid containing cryoprotective fluid to be incorporated with said biological cell structures.

5 37. A process to enhance the cryopreservation of biological cells as described in clause 36 or any other clause wherein said step of establishing a lipid containing cryoprotective fluid to be incorporated with said biological cell structures comprises the step of establishing a high lipid concentration cryopreservation fluid to be incorporated with said biological cell structures.

10 38. A process to enhance the cryopreservation of biological cells as described in clause 37 or any other clause wherein said step of establishing a high lipid concentration cryopreservation fluid to be incorporated with said biological cell structures comprises a step selected from a group consisting of the steps of:

15 - establishing an at least about 10% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures,

- establishing an at least about 20% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures,

20 - establishing an at least about 40% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures,

- establishing an at least about 60% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures, and

25 - establishing an at least about 80% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures.

39. A process to enhance the cryopreservation of biological cells as described in clause 37 or any other clause wherein said step of establishing a high lipid concentration cryopreservation fluid to be incorporated with said biological cell structures comprises

the step of establishing a component selected from a group consisting of: a relatively high concentration sea buckthorn lipid, a relatively high concentration unsaturated fatty acid, a relatively high concentration saturated fatty acid, a relatively high concentration palmitic acid, a relatively high concentration palmitoleic acid, a relatively high concentration oleic acid, a relatively high concentration linoleic acid, a relatively high concentration linolenic acid, a relatively high concentration lecithin, a relatively high concentration β -sitosterol, a relatively high concentration β -amyrin, a relatively high concentration γ -carotene, a relatively high concentration α -amyrin, a relatively high concentration β -carotene, a relatively high concentration lycopene, a relatively high concentration lutein, a relatively high concentration tocopherol, a relatively high concentration digalactosyldiacylglycerol, a relatively high concentration monogalactosymonoacylglycerol, a relatively high concentration 16:1 fatty acids, a relatively high concentration 18:1 fatty acids, a relatively high concentration 18:2 fatty acids, and a relatively high concentration 18:3 fatty acid.

40. A process to enhance the cryopreservation of biological cells as described in clause 19 or

15 any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,
- assembling a cellular collection containing a plurality of stem cell structures,
- assembling a cellular collection containing a plurality of skin cells,
- assembling a cellular collection containing a plurality of embryonic stem cells,
- assembling a cellular collection containing a plurality of neural stem cells,
- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells
- assembling a cellular collection containing a plurality of connective cells

-assembling a cellular collection containing a plurality of nerve cells - assembling a cellular collection containing a plurality of umbilical cord blood cells,

- assembling a cellular collection containing a plurality of histological sample cells,

- assembling a cellular collection containing a plurality of plant seed cells,

5 - assembling a cellular collection containing a plurality of plant shoot cells,

- assembling a cellular collection containing a plurality of ovarian tissue cell structures,

- assembling a cellular collection containing a plurality of testicular tissue cell structures,

- assembling a cellular collection containing a plurality of embryo cells,

10 - assembling a cellular collection containing a plurality of tumorous tissue cell structures,

- assembling a cellular collection containing a plurality of yeast cells,

- assembling a cellular collection containing a plurality of bacterial cells,

- assembling a cellular collection containing a plurality of algal cells,

15 - assembling a cellular collection containing a plurality of fungal cells,

- assembling a cellular collection containing a plurality of mesenchymal cells,

- assembling a cellular collection containing a plurality of keratinocyte cells,

- assembling a cellular collection containing a plurality of melanocyte cells,

- assembling a cellular collection containing a plurality of hepatocyte cells,

20 - assembling a cellular collection containing a plurality of oocyte cells, and

- assembling a cellular collection containing a plurality of sperm cells.

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41. A process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cell structures;

25 - establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;

- establishing non-naturally occurring lipid droplets within said cryopreservation fluid to create a non-natural lipid containing cryopreservation fluid;

- mixing said non-natural lipid containing cryopreservation fluid and said biological cell structures to form a non-natural fluidic cryopreservation composite;

- removing thermal energy from said non-natural fluidic cryopreservation composite;
and

- freezing said non-natural fluidic cryopreservation composite by reducing the temperature of said non-natural fluidic cryopreservation composite below the freezing point of water.

5 42. A process to enhance the cryopreservation of biological cells as described in clause 41 or any other clause wherein said step of said establishing non-natural lipid containing cryopreservation fluid and said biological cell structures to form a non-natural fluidic cryopreservation composite comprises the step of creating unstably sized lipid droplets
10 within said cryopreservation fluid.

15 43. A cryopreservation composition as described in clause 41 or any other clause wherein said step of establishing non-natural lipid containing cryopreservation fluid and said biological cell structures to form a non-natural fluidic cryopreservation composite comprises the step of modifying the size of said lipid droplets contained within said cryopreservation fluid.

44. A cryopreservation composition as described in clause 43 or any other clause wherein said step of creating modified sized lipid droplets within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

20 - creating a substance in said cryopreservation fluid with droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,

25 - creating a substance in said cryopreservation fluid with droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,

5 - creating a substance in said cryopreservation fluid with droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,

10 - creating a substance in said cryopreservation fluid with droplets numbering at least about 50000 times the number of initial drops of said substance in said cryopreservation fluid, and

- creating a substance in said cryopreservation fluid with droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

15 45. A process to enhance the cryopreservation of biological cells as described in clause 41 or any other clause wherein said step of establishing non-naturally occurring lipid droplets within said cryopreservation fluid to create a non-natural lipid containing cryopreservation fluid comprises the step of creating a substantial number of relatively small lipid droplets in a substance contained within said cryopreservation fluid.

20 46. A process to enhance the cryopreservation of biological cells as described in clause 45 or any other clause wherein said step creating of a substantial number of relatively small lipid droplets in a substance contained within said cryopreservation fluid comprises the step of rending larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid.

25 47. A process to enhance the cryopreservation of biological cells as described in clause 44, 45, or 46 or any other clause wherein said step of creating a substantial number of relatively small lipid droplets in a substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 1000nm,

5 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 900nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 700nm,

10 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 500nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 300nm,

15 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 100nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 70nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 50nm, and

20 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 30nm.

25 48. A process to enhance the cryopreservation of biological cells as described in clause 45 or 46 or any other clause wherein said step of creating a substantial number of relatively small lipid droplets in a substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- creating said droplets in said cryopreservation fluid so that at least about 20% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,
- creating said droplets in said cryopreservation fluid so that at least about 40% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,
- 5 - creating said droplets in said cryopreservation fluid so that at least about 60% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,
- creating said droplets in said cryopreservation fluid so that at least about 80% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size, and
- creating said droplets in said cryopreservation fluid so that at least about 90% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size.

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49. A process to enhance the cryopreservation of biological cells as described in clause 45 or 46 or any other clause wherein said step of creating a substantial number of relatively small lipid droplets in a substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- creating a substance in said cryopreservation fluid with droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,
- creating a substance in said cryopreservation fluid with droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,
- 20 - creating a substance in said cryopreservation fluid with droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,
- creating a substance in said cryopreservation fluid with droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

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- creating a substance in said cryopreservation fluid with droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,
- creating a substance in said cryopreservation fluid with droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,
- creating a substance in said cryopreservation fluid with droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,
- creating a substance in said cryopreservation fluid with droplets numbering at least about 50000 times the number of initial drops of said substance in said cryopreservation fluid, and
- creating a substance in said cryopreservation fluid with droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

50. A process to enhance the cryopreservation of biological cells as described in clause 41, 44, or 46 or any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,
- assembling a cellular collection containing a plurality of stem cell structures,
- assembling a cellular collection containing a plurality of skin cells,
- assembling a cellular collection containing a plurality of embryonic stem cells,
- assembling a cellular collection containing a plurality of neural stem cells,
- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells

-assembling a cellular collection containing a plurality of connective cells
-assembling a cellular collection containing a plurality of nerve cells
- assembling a cellular collection containing a plurality of umbilical cord blood cells,
- assembling a cellular collection containing a plurality of histological sample cells,
5 - assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- assembling a cellular collection containing a plurality of testicular tissue cell structures,
10 - assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
15 - assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,
- assembling a cellular collection containing a plurality of melanocyte cells,
20 - assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- assembling a cellular collection containing a plurality of sperm cells.
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51. A process to enhance the cryopreservation of biological cells as described in clause 41 or
25 any other clause wherein said step of establishing non-naturally occurring lipid droplets
within said cryopreservation fluid to create a non-natural lipid cryopreservation fluid
comprises the step of creating a substantially uniform maximum droplet size of a
substance contained within said cryopreservation fluid.

52. A process to enhance the cryopreservation of biological cells as described in clause 51 or
30 any other clause wherein said step of creating a substantially uniform maximum droplet

size of a substance contained within said cryopreservation fluid comprises the step selected from a group consisting of:

- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 1000nm,
- 5 - causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 900nm,
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 700nm,
- 10 - causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 500nm,
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 300nm,
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 100nm,
- 15 - causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 70nm,
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 50nm, and
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 30nm.

53. A process to enhance the cryopreservation of biological cells as described in clause 51 or any other clause wherein said step of creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- 25 - eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 1000nm,
- eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 900nm,
- eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 700nm,

- eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 500nm,

- eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 300nm,

5 - eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 100nm,

- e eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 70nm,

10 - eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 50nm, and

- eliminating droplets of a substance contained within said cryopreservation fluid so there are no substantial amount of droplets larger than at least about 30nm.

54. A process to enhance the cryopreservation of biological cells as described in clause 41 or any other clause wherein said step of establishing non-naturally occurring lipid droplets

15 within said cryopreservation fluid to create a non-natural lipid cryopreservation fluid comprises the step of causing a substance contained within said cryopreservation fluid to have a droplet size with a skewed size distribution that favors smaller size droplets.

55. A process to enhance the cryopreservation of biological cells as described in clause 54 or any other clause wherein the step of causing a substance contained within said

20 cryopreservation fluid to have a droplet size with a skewed size distribution that favors smaller size droplets a step selected from a group consisting of the steps of:

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 1000nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 900nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 700nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 500nm,

30 - modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 300nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 100nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 70nm,

5 - modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 50nm, and

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 30nm.

56. A process to enhance the cryopreservation of biological cells comprising the steps of:

10 - assembling a cellular collection containing a plurality of biological cell structures;

- establishing a high lipid concentration cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;

- altering at least one characteristic of a lipid substance in said high lipid concentration cryopreservation fluid in a manner that enhances cell viability;

15 - mixing said altered high lipid concentration cryopreservation fluid and said biological cell structures to form a vitality enhanced fluidic cryopreservation composite;

- removing thermal energy from said fluidic cryopreservation composite; and

- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water.

20 57. A process to enhance the cryopreservation of biological cells as described in clause 56 or any other clause wherein said step of establishing a high lipid concentration cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation comprises a step selected from a group consisting of the steps of:

25 - establishing an at least about 10% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures,

- establishing an at least about 20% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures,

- establishing an at least about 40% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures,

5 - establishing an at least about 60% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures, and

- establishing an at least about 80% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment to be incorporated with said biological cell structures.

10 58. A process to enhance the cryopreservation of biological cells as described in clause 56 or any other clause wherein said step of establishing a high lipid concentration cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation comprises the step of establishing a component selected from a group consisting of: a relatively high concentration sea buckthorn lipid, a relatively high

15 concentration saturated fatty acid, a relatively high concentration unsaturated fatty acid, a relatively high concentration palmitic acid, a relatively high concentration palmitoleic acid, a relatively high concentration oleic acid, a relatively high concentration linoleic acid, a relatively high concentration linolenic acid, a relatively high concentration

16 lecithin, a relatively high concentration β -sitosterol, a relatively high concentration β -amyrin, a relatively high concentration γ -carotene, a relatively high concentration α -amyrin, a relatively high concentration β -carotene, a relatively high concentration

lycopene, a relatively high concentration lutein, a relatively high concentration

20 tocopherol, a relatively high concentration digalactosyldiacylglycerol, a relatively high concentration monogalactosymonoacylglycerol, a relatively high concentration 16:1 fatty acids, a relatively high concentration 18:1 fatty acids, a relatively high concentration 18:2 fatty acids, and a relatively high concentration 18:3 fatty acid.

25 59. A process to enhance the cryopreservation of biological cells as described in clause 56 or any other clause and further comprising the step of providing an enhanced post-cryogenic viability for said biological cell structures.

30 60. A process to enhance the cryopreservation of biological cells as described in clause 59 or any other clause wherein said step of providing an enhanced post-cryogenic viability for

said biological cell structures comprises the step of providing relatively high post-cryogenic viability for said biological cell structures.

61. A process to enhance the cryopreservation of biological cells as described in clause 60 or any other clause wherein said step of providing relatively high post-cryogenic viability for said biological cell structures comprises a step selected from a group consisting of the steps of:

- providing at least about 5% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

10 - providing at least about 10% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

- providing at least about 15% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

15 - providing at least about 20% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment, and

- providing at least about 25% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment.

20. A process to enhance the cryopreservation of biological cells as described in clause 60 or any other clause wherein said step of providing relatively high post-cryogenic viability for said biological cell structures comprises a step selected from a group consisting of the steps of:

- providing not less than about 80% of the pre-cryogenic viability for said biological cell structures,

- providing not less than about 70% of the pre-cryogenic viability for said biological cell structures,

25 - providing not less than about 60% of the pre-cryogenic viability for said biological cell structures,

- providing not less than about 50% of the pre-cryogenic viability for said biological cell structures,

- providing not less than about 40% of the pre-cryogenic viability for said biological cell structures,

5 - providing not less than about 30% of the pre-cryogenic viability for said biological cell structures, and

- providing not less than about 20% of the pre-cryogenic viability for said biological cell structures.

63. A process to enhance the cryopreservation of biological cells as described in clause 56,

10 57, 60, or 62 or any other clause wherein said step of altering at least one characteristic of a lipid substance in said high lipid concentration cryopreservation fluid in a manner that enhances cell viability comprises the step of rending larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid.

15 64 A process to enhance the cryopreservation of biological cells as described in clause 63 or any other clause wherein said step of rending larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

20 - rending larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 1000nm for said substance in said cryopreservation fluid,

- rending larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 900nm for said substance in said cryopreservation fluid,

25 - rending larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 700nm for said substance in said cryopreservation fluid,

- rending larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 500nm for said substance in said cryopreservation fluid,

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- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 300nm for said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 100nm for said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 70nm for said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 50nm for said substance in said cryopreservation fluid, and
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 30nm for said substance in said cryopreservation fluid.

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65. A process to enhance the cryopreservation of biological cells as described in clause 56 or any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,
- assembling a cellular collection containing a plurality of stem cell structures,
- assembling a cellular collection containing a plurality of skin cells,
- assembling a cellular collection containing a plurality of embryonic stem cells,
- assembling a cellular collection containing a plurality of neural stem cells,
- assembling a cellular collection containing a plurality of umbilical cord blood cells,
- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells

- assembling a cellular collection containing a plurality of muscle cells
- assembling a cellular collection containing a plurality of connective cells
- assembling a cellular collection containing a plurality of nerve cells
- assembling a cellular collection containing a plurality of histological sample cells,
- 5 - assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- assembling a cellular collection containing a plurality of testicular tissue cell structures,
- 10 - assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
- 15 - assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,
- assembling a cellular collection containing a plurality of melanocyte cells,
- 20 - assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- assembling a cellular collection containing a plurality of sperm cells.
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66. A process to enhance the cryopreservation of biological cells as described in clause 64
25 wherein the step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,
- assembling a cellular collection containing a plurality of stem cell structures,
- assembling a cellular collection containing a plurality of skin cells,
- assembling a cellular collection containing a plurality of embryonic stem cells,
- 30 - assembling a cellular collection containing a plurality of neural stem cells,

- assembling a cellular collection containing a plurality of umbilical cord blood cells,
- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- 5 - assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells
- assembling a cellular collection containing a plurality of connective cells
- 10 - assembling a cellular collection containing a plurality of nerve cells
- assembling a cellular collection containing a plurality of histological sample cells,
- assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- 15 - assembling a cellular collection containing a plurality of testicular tissue cell structures,
- assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
- assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- assembling a cellular collection containing a plurality of mesenchymal cells,
- 20 - assembling a cellular collection containing a plurality of keratinocyte cells,
- assembling a cellular collection containing a plurality of melanocyte cells,
- assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- assembling a cellular collection containing a plurality of sperm cells.

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30 67. A process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cell structures;
- establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;
- mixing said cryopreservation fluid and said biological cell structures to form a fluidic cryopreservation composite;
- subjecting said biological cell structures to said cryopreservation fluid in said fluidic cryopreservation composite in a manner that reduces cryogenic damage to said biological cell structures;
- removing thermal energy from said fluidic cryopreservation composite; and
- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water while reducing cryogenic damage to said biological cell structures.

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68. A process to enhance the cryopreservation of biological cells as described in clause 67 or any other clause wherein said step of subjecting said biological cell structures to said cryopreservation fluid in said fluidic cryopreservation composite in a manner that reduces cryogenic damage to said biological cell structures comprises the step of configuring at least one lipid contained within said cryopreservation fluid.
69. A process to enhance the cryopreservation of biological cells as described in clause 67 or any other clause wherein said step of subjecting said biological cell structures to said cryopreservation fluid in said fluidic cryopreservation composite in a manner that reduces cryogenic damage to said biological cell structures comprises the step of altering at least one lipid contained within said cryopreservation fluid.
70. A process to enhance the cryopreservation of biological cells as described in clause 69 or any other clause said step of altering at least one lipid contained within said cryopreservation fluid comprises the step of physically altering at least one lipid contained within said cryopreservation fluid.
71. A process to enhance the cryopreservation of biological cells as described in clause 69 or any other clause said step of altering at least one lipid contained within said cryopreservation fluid comprises the step of in situ physically altering at least one lipid contained within said cryopreservation fluid.

72. A process to enhance the cryopreservation of biological cells as described in clause 71 or any other clause wherein said step of physically altering at least one lipid contained within said cryopreservation fluid comprises the step of rending larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid.

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73. A process to enhance the cryopreservation of biological cells as described in clause 67 or any other clause and further comprising the step of thawing said cryopreservation fluid by increasing its temperature above the freezing point of water

74. A process to enhance the cryopreservation of biological cells as described in clause 67 or 10 any other clause and further comprising the step of providing an enhanced post-cryogenic viability for said biological cell structures.

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75. A process to enhance the cryopreservation of biological cells as described in clause 74 or any other clause wherein said step of providing an enhanced post-cryogenic viability for said biological cell structures comprises the step of providing relatively high post-cryogenic viability for said biological cell structures.

76. A process to enhance the cryopreservation of biological cells as described in clause 75 or any other clause wherein said step of providing relatively high post-cryogenic viability for said biological cell structures comprises a step selected from a group consisting of the steps of:

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- providing at least about 5% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,
- providing at least about 10% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

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- providing at least about 15% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,
- providing at least about 20% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment, and

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- providing at least about 25% higher post-cryogenic viability for said biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment.

77. A process to enhance the cryopreservation of biological cells as described in clause 75 or
5 any other clause wherein said step of providing relatively high post-cryogenic viability
for said biological cell structures comprises a step selected from a group consisting of the
steps of:

- providing not less than about 80% of the pre-cryogenic viability for said biological
cell structures,

10 - providing not less than about 70% of the pre-cryogenic viability for said biological
cell structures,

- providing not less than about 60% of the pre-cryogenic viability for said biological
cell structures,

- providing not less than about 50% of the pre-cryogenic viability for said biological
15 cell structures,

- providing not less than about 40% of the pre-cryogenic viability for said biological
cell structures,

- providing not less than about 30% of the pre-cryogenic viability for said biological
cell structures, and

20 - providing not less than about 20% of the pre-cryogenic viability for said biological
cell structures.

78. A process to enhance the cryopreservation of biological cells as described in clause 67,
75, or 77 or any other clause wherein said step of subjecting said biological cell structures
to said cryopreservation fluid in said fluidic cryopreservation composite in a manner that
25 reduces cryogenic damage to said biological cell structures comprises the step of rending
larger drops of a substance in said cryopreservation fluid to form smaller droplets of said
substance in said cryopreservation fluid.

79. A process to enhance the cryopreservation of biological cells as described in clause 78 or
any other clause wherein said step of rending larger drops of a substance in said
30 cryopreservation fluid to form smaller droplets of said substance in said cryopreservation
fluid comprises a step selected from a group consisting of the steps of:

- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 1000nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 900nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 700nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 500nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 300nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 100nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 70nm in said substance in said cryopreservation fluid,
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 50nm in said substance in said cryopreservation fluid, and
- rendering larger drops of a substance in said cryopreservation fluid so there are no substantial amount of droplets larger than about 30nm in said substance in said cryopreservation fluid.

80. A process to enhance the cryopreservation of biological cells as described in clause 67 or any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,
- assembling a cellular collection containing a plurality of stem cell structures,
- assembling a cellular collection containing a plurality of skin cells,
- assembling a cellular collection containing a plurality of embryonic stem cells,
- 5 - assembling a cellular collection containing a plurality of neural stem cells,
- assembling a cellular collection containing a plurality of umbilical cord blood cells,
- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- 10 - assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells
- assembling a cellular collection containing a plurality of connective cells
- 15 - assembling a cellular collection containing a plurality of nerve cells

- assembling a cellular collection containing a plurality of histological sample cells,
- assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- 20 - assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- assembling a cellular collection containing a plurality of testicular tissue cell structures,
- assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- 25 - assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
- assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- 30 - assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,

- assembling a cellular collection containing a plurality of melanocyte cells,
- assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- assembling a cellular collection containing a plurality of sperm cells.

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81. A process to enhance the cryopreservation of biological cells as described in clause 79 wherein the step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,
- assembling a cellular collection containing a plurality of stem cell structures,
- assembling a cellular collection containing a plurality of skin cells,
- assembling a cellular collection containing a plurality of embryonic stem cells,
- assembling a cellular collection containing a plurality of neural stem cells,
- assembling a cellular collection containing a plurality of umbilical cord blood cells,
- assembling a cellular collection containing a plurality of epithelial stem cells
- assembling a cellular collection containing a plurality of cardiac stem cells
- assembling a cellular collection containing a plurality of muscle stem cells
- assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells
- assembling a cellular collection containing a plurality of connective cells
- assembling a cellular collection containing a plurality of nerve cells - assembling a cellular collection containing a plurality of histological sample cells,
- assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- assembling a cellular collection containing a plurality of testicular tissue cell structures,
- assembling a cellular collection containing a plurality of embryo cells,

- assembling a cellular collection containing a plurality of tumorous tissue cell structures,

- assembling a cellular collection containing a plurality of yeast cells,

- assembling a cellular collection containing a plurality of bacterial cells,

5 - assembling a cellular collection containing a plurality of algal cells,

- assembling a cellular collection containing a plurality of fungal cells,

- assembling a cellular collection containing a plurality of mesenchymal cells,

- assembling a cellular collection containing a plurality of keratinocyte cells,

- assembling a cellular collection containing a plurality of melanocyte cells,

10 - assembling a cellular collection containing a plurality of hepatocyte cells,

- assembling a cellular collection containing a plurality of oocyte cells, and

- assembling a cellular collection containing a plurality of sperm cells.

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82. A process to enhance the cryopreservation of biological cells comprising the steps of:

15 - assembling a cellular collection containing a plurality of biological cell structures;

- establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;

- sizing droplets of at least one substance within said cryopreservation fluid;

20 - mixing said cryopreservation fluid and said biological cell structures to form a fluidic cryopreservation composite;

- removing thermal energy from said fluidic cryopreservation composite;

- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water; and

- providing an enhanced post-cryogenic viability for said biological cell structures.

25 83. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises the step of rending at least some drops of at least one substance in said cryopreservation fluid.

30 84. A process to enhance the cryopreservation of biological cells as described in clause 83 or any other clause wherein said step of sizing droplets of at least one substance within said

cryopreservation fluid comprises the step of rendering at least some lipid drops of at least one substance in said cryopreservation fluid.

85. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

5 - creating a substance in said cryopreservation fluid with droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,

10 - creating a substance in said cryopreservation fluid with droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,

15 - creating a substance in said cryopreservation fluid with droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,

20 - creating a substance in said cryopreservation fluid with droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

25 - creating a substance in said cryopreservation fluid with droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,

 - creating a substance in said cryopreservation fluid with droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,

 - creating a substance in said cryopreservation fluid with droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,

 - creating a substance in said cryopreservation fluid with droplets numbering at least about 50000 times the number of initial drops of said substance in said cryopreservation fluid, and

- creating a substance in said cryopreservation fluid with droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

86. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises the step of creating a substantial number of relatively small droplets in a substance contained within said cryopreservation fluid.

5 87. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises the step of in situ creating a substantial number of relatively small droplets in a substance contained within said cryopreservation fluid.

10 88. A process to enhance the cryopreservation of biological cells as described in clause 86 or any other clause wherein said step creating of a substantial number of relatively small droplets in a substance contained within said cryopreservation fluid comprises the step of rendering larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid.

15 89. A process to enhance the cryopreservation of biological cells as described in clause 85, 86, 87, or 88 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

20 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 1000nm,

25 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 900nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 700nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 500nm,

5 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 300nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 100nm,

10 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 70nm,

- creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 50nm, and

15 - creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 30nm.

90. A process to enhance the cryopreservation of biological cells as described in clause 89 or
20 any other clause said step of sizing droplets of at least one substance within said
cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- creating said substance in said cryopreservation fluid so that at least about 20% of
said droplets of said substance in said cryopreservation fluid are droplets at least about
as small as said desired size,

25 - creating said substance in said cryopreservation fluid so that at least about 40% of
said droplets of said substance in said cryopreservation fluid are droplets at least about
as small as said desired size,

- creating said substance in said cryopreservation fluid so that at least about 60% of
said droplets of said substance in said cryopreservation fluid are droplets at least about
30 as small as said desired size,

- creating said substance in said cryopreservation fluid so that at least about 80% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size, and

5 - creating said substance in said cryopreservation fluid so that at least about 90% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size.

91. A process to enhance the cryopreservation of biological cells as described in clause 86, 87, or 88 or any other clause wherein said step of sizing droplets of at least one substance contained within said cryopreservation fluid comprises a step selected from a group 10 consisting of the steps of:

- creating a substance in said cryopreservation fluid with droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,

15 - creating a substance in said cryopreservation fluid with droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,

20 - creating a substance in said cryopreservation fluid with droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,

25 - creating a substance in said cryopreservation fluid with droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,

- creating a substance in said cryopreservation fluid with droplets numbering at least about 50000 times the number of initial drops of said substance in said cryopreservation fluid, and

5 - creating a substance in said cryopreservation fluid with droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

92. A process to enhance the cryopreservation of biological cells as described in clause 82, 85, 88 or any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting 10 of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,

- assembling a cellular collection containing a plurality of stem cell structures,

- assembling a cellular collection containing a plurality of skin cells,

- assembling a cellular collection containing a plurality of embryonic stem cells,

- assembling a cellular collection containing a plurality of neural stem cells,

15 - assembling a cellular collection containing a plurality of umbilical cord blood cells,

- assembling a cellular collection containing a plurality of epithelial stem cells

- assembling a cellular collection containing a plurality of cardiac stem cells

- assembling a cellular collection containing a plurality of muscle stem cells

20 - assembling a cellular collection containing a plurality of connective stem cells

- assembling a cellular collection containing a plurality of epithelial cells

- assembling a cellular collection containing a plurality of cardiac cells

- assembling a cellular collection containing a plurality of muscle cells

- assembling a cellular collection containing a plurality of connective cells

25 - assembling a cellular collection containing a plurality of nerve cells

- assembling a cellular collection containing a plurality of histological sample cells,

- assembling a cellular collection containing a plurality of plant seed cells,

- assembling a cellular collection containing a plurality of plant shoot cells,

- assembling a cellular collection containing a plurality of ovarian tissue cell structures,

30 - assembling a cellular collection containing a plurality of testicular tissue cell structures,

- assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- assembling a cellular collection containing a plurality of yeast cells,
- 5 - assembling a cellular collection containing a plurality of bacterial cells,
- assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,
- 10 - assembling a cellular collection containing a plurality of melanocyte cells,
- assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- assembling a cellular collection containing a plurality of sperm cells.

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15 93. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises the step of creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid.

94. A process to enhance the cryopreservation of biological cells as described in clause 93 or any other clause wherein said step of creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid comprises the step selected from a group consisting of:

- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 1000nm,
- 20 - causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 900nm,
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 700nm,
- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 500nm,

- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 300nm,

- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 100nm,

5 - causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 70nm,

- causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 50nm, and

10 - causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 30nm.

95. A process to enhance the cryopreservation of biological cells as described in clause 93 or any other clause wherein said step of creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

15 - establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 1000nm,

- establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 900nm,

20 - establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 700nm,

- establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 500nm,

25 - establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 300nm,

- establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 100nm,

- establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 70nm,

30 - establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 50nm, and

- establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 30nm.

96. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said cryopreservation fluid comprises the step of causing a substance contained within said cryopreservation fluid to have a droplet size with a skewed size distribution that favors smaller size droplets.

5 97. A process to enhance the cryopreservation of biological cells as described in clause 96 or any other clause wherein the step of causing a substance contained within said cryopreservation fluid having a droplet size with a skewed size distribution that favors 10 smaller size droplets a step selected from a group consisting of the steps of:

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 1000nm,

- 15 modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 900nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 700nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 500nm,

- 20 modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 300nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 100nm,

- 25 modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 70nm,

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 50nm, and

- modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 30nm.

30 98. A process to enhance the cryopreservation of biological cells as described in clause 82 or any other clause wherein said step of sizing droplets of at least one substance within said

cryopreservation fluid comprises the step of eliminating droplets from said cryopreservation fluid.

99. A process to enhance the cryopreservation of biological cells as described in clause 98 or any other clause wherein said step of eliminating droplets from said cryopreservation fluid comprises the step of size eliminating droplets from said cryopreservation fluid.

5 100. A process to enhance the cryopreservation of biological cells as described in clause 99 or any other clause wherein said step of size eliminating droplets from said cryopreservation fluid comprises the step of size eliminating at least some lipid droplets of said cryopreservation fluid.

10 101. A process to enhance the cryopreservation of biological cells as described in clause 100 or any other clause wherein said step of eliminating at least some lipid droplets of said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 1000nm,
- eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 900nm,
- eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 700nm,
- eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 500nm,
- eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 300nm,
- eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 100nm,

20 - eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 70nm,

25 - eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 50nm, and

30 - eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 30nm.

102. A process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cell structures;
- establishing a cryopreservation fluid to be incorporated with said biological cell structures in cryopreservation;
- in situ sizing droplets of at least one substance within said cryopreservation fluid;
- 5 - mixing said cryopreservation fluid and said biological cell structures to form a fluidic cryopreservation composite;
- removing thermal energy from said fluidic cryopreservation composite; and
- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water.

10 103. A process to enhance the cryopreservation of biological cells as described in clause 102 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises the step of in situ rending at least some drops of at least one substance in said cryopreservation fluid

15 104. A process to enhance the cryopreservation of biological cells as described in clause 103 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises the step of in situ rending at least some lipid drops of at least one substance in said cryopreservation fluid.

105. A process to enhance the cryopreservation of biological cells as described in clause 102 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises a step selected from a group consisting of

20 the steps of:

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,
- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,
- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,

25

30

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

5 - in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,

10 - in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 50000 times the number of initial drops of said substance in said

15 cryopreservation fluid, and

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

106. A process to enhance the cryopreservation of biological cells as described in clause 102

20 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises the step of in situ creating a substantial number of relatively small droplets in a substance contained within said cryopreservation fluid.

107. A process to enhance the cryopreservation of biological cells as described in clause 106

25 or any other clause wherein said step in situ creating of a substantial number of relatively small droplets in a substance contained within said cryopreservation fluid comprises the step of in situ rending larger drops of a substance in said cryopreservation fluid to form smaller droplets of said substance in said cryopreservation fluid.

108. A process to enhance the cryopreservation of biological cells as described in clause 129,

30 106, or 107 or any other clause wherein said step of in situ sizing droplets of at least one

substance within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 1000nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 900nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 700nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 500nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 300nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 100nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 70nm,
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 50nm, and
- in situ creating a substantial number of droplets of a substance contained within said cryopreservation fluid with no substantial amount of droplets larger than at least about 30nm.

109. A process to enhance the cryopreservation of biological cells as described in clause 108 or any other clause said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- in situ creating said substance in said cryopreservation fluid so that at least about 20% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,
- 5 - in situ creating said substance in said cryopreservation fluid so that at least about 40% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,
- in situ creating said substance in said cryopreservation fluid so that at least about 60% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size,
- 10 - in situ creating said substance in said cryopreservation fluid so that at least about 80% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size, and
- in situ creating said substance in said cryopreservation fluid so that at least about 90% of said droplets of said substance in said cryopreservation fluid are droplets at least about as small as said desired size.

110. A process to enhance the cryopreservation of biological cells as described in clause 106 or 107 or any other clause wherein said step of in situ sizing droplets of at least one substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 30 times the number of initial drops of said substance in said cryopreservation fluid,
- 20 - in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 50 times the number of initial drops of said substance in said cryopreservation fluid,
- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 100 times the number of initial drops of said substance in said cryopreservation fluid,
- 25
- 30

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 500 times the number of initial drops of said substance in said cryopreservation fluid,

5 - in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 1000 times the number of initial drops of said substance in said cryopreservation fluid,

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 5000 times the number of initial drops of said substance in said cryopreservation fluid,

10 - in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 10000 times the number of initial drops of said substance in said cryopreservation fluid,

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 50000 times the number of initial drops of said substance in said

15 cryopreservation fluid, and

- in situ creating a substance in said cryopreservation fluid with droplets numbering at least about 100000 times the number of initial drops of said substance in said cryopreservation fluid.

111. A process to enhance the cryopreservation of biological cells as described in clause 102,

20 105, or 107 or any other clause wherein said step of assembling a cellular collection containing a plurality of biological cell structures comprises a step selected from a group consisting of the steps of:

- assembling a cellular collection containing a plurality of blood cell structures,

- assembling a cellular collection containing a plurality of stem cell structures,

25 - assembling a cellular collection containing a plurality of skin cells,

- assembling a cellular collection containing a plurality of embryonic stem cells,

- assembling a cellular collection containing a plurality of neural stem cells,

- assembling a cellular collection containing a plurality of umbilical cord blood cells,

- assembling a cellular collection containing a plurality of epithelial stem cells

30 - assembling a cellular collection containing a plurality of cardiac stem cells

- assembling a cellular collection containing a plurality of muscle stem cells

- assembling a cellular collection containing a plurality of connective stem cells
- assembling a cellular collection containing a plurality of epithelial cells
- assembling a cellular collection containing a plurality of cardiac cells
- assembling a cellular collection containing a plurality of muscle cells
- 5 -assembling a cellular collection containing a plurality of connective cells
- assembling a cellular collection containing a plurality of nerve cells
- assembling a cellular collection containing a plurality of histological sample cells,
- assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- 10 - assembling a cellular collection containing a plurality of ovarian tissue cell structures,
- assembling a cellular collection containing a plurality of testicular tissue cell structures,
- assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cell structures,
- 15 - assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
- assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- 20 - assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,
- assembling a cellular collection containing a plurality of melanocyte cells,
- assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- 25 - assembling a cellular collection containing a plurality of sperm cells.
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112. A process to enhance the cryopreservation of biological cells as described in clause 102 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises the step of creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid.

113. A process to enhance the cryopreservation of biological cells as described in clause 112 or any other clause wherein said step of in situ creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid comprises the step selected from a group consisting of:

5 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 1000nm,

10 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 900nm,

15 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 700nm,

20 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 500nm,

25 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 300nm,

30 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 100nm,

 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 70nm,

 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 50nm, and

 - in situ causing a substance contained within said cryopreservation fluid to have no substantial number of droplets larger than at least about 30nm.

114. A process to enhance the cryopreservation of biological cells as described in clause 112 or any other clause wherein said step of in situ creating a substantially uniform maximum droplet size of a substance contained within said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

25 - in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 1000nm,

30 - in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 900nm,

- in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 700nm,
- in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 500nm,
- 5 - in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 300nm,
- in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 100nm,
- in situ establishing droplets of a substance contained within said cryopreservation
- 10 fluid so there is no substantial amount of droplets larger than at least about 70nm,
- in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 50nm, and
- in situ establishing droplets of a substance contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 30nm.

15 115. A process to enhance the cryopreservation of biological cells as described in clause 102 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises the step of in situ causing a substance contained within said cryopreservation fluid to have a droplet size with a skewed size distribution that favors smaller size droplets.

20 116. A process to enhance the cryopreservation of biological cells as described in clause 115 or any other clause wherein the step of in situ causing a substance contained within said cryopreservation fluid having a droplet size with a skewed size distribution that favors smaller size droplets a step selected from a group consisting of the steps of:

- in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 1000nm,
- 25 - in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 900nm,
- in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 700nm,
- in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 500nm,

- in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 300nm,

- in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 100nm,

5 - in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 70nm,

- in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 50nm, and

10 - in situ modifying a substance contained within said cryopreservation fluid to have a mode drop size of less than at least about 30nm.

117. A process to enhance the cryopreservation of biological cells as described in clause 102 or any other clause wherein said step of in situ sizing droplets of at least one substance within said cryopreservation fluid comprises the step of in situ eliminating droplets from said cryopreservation fluid.

15 118. A process to enhance the cryopreservation of biological cells as described in clause 117 or any other clause wherein said step of in situ eliminating droplets from said cryopreservation fluid comprises the step of in situ size eliminating droplets from said cryopreservation fluid.

20 119. A process to enhance the cryopreservation of biological cells as described in clause 118 or any other clause wherein said step of in situ size eliminating droplets from said cryopreservation fluid comprises the step of in situ size eliminating at least some lipid droplets of said cryopreservation fluid.

25 120. A process to enhance the cryopreservation of biological cells as described in clause 119 or any other clause wherein said step of in situ eliminating at least some lipid droplets of said cryopreservation fluid comprises a step selected from a group consisting of the steps of:

- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 1000nm,

- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 900nm,

- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 700nm,
- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 500nm,
- 5 - in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 300nm,
- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 100nm,
- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 70nm,
- 10 - in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 50nm, and
- in situ eliminating droplets of a lipid contained within said cryopreservation fluid so there is no substantial amount of droplets larger than at least about 30nm.

15 121. A cryopreservation composition comprising:

- an assemblage of biological cell structures; and
- an increased surface energy content cryopreservation fluid mixed with said biological cell structures.

122. A cryopreservation composition as described in clause 121 or any other clause wherein
20 said increased surface energy content cryopreservation fluid comprises an increased surface energy content cryopreservation fluid that has been frozen at temperature below the freezing point of water.

123. A cryopreservation composition as described in clause 122 or any other clause wherein
25 said increased surface energy content cryopreservation fluid comprises an increased surface energy content cryopreservation fluid that has been thawed by increasing its temperature above the freezing point of water.

124. A cryopreservation composition as described in clause 122 or 123 or any other clause wherein said assemblage of biological cell structures comprises an enhanced post-cryogenic viability assemblage of biological cell structures.

125. A cryopreservation composition as described in clause 124 or any other clause wherein
said enhanced post-cryogenic viability assemblage of biological cell structures comprises
a relatively high post-cryogenic viability assemblage of biological cell structures.

126. A cryopreservation composition as described in clause 125 or any other clause wherein
5 said relatively high post-cryogenic viability assemblage of biological cell structures
comprises an assemblage of biological cell structures selected from a group consisting of:
- an at least about 5% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,
10 - an at least about 10% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,
- an at least about 15% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
15 thermal treatment,
- an at least about 20% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment, and
- an at least about 25% higher post-cryogenic viability assemblage of biological cell
20 structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment.

127. A cryopreservation composition as described in clause 125 or any other clause wherein
said relatively high post-cryogenic viability assemblage of biological cell structures
comprises an assemblage of biological cell structures selected from a group consisting of:
25 - an assemblage of biological cell structures having not less than about 80% of the pre-
cryogenic viability for said biological cell structures,
- an assemblage of biological cell structures having not less than about 70% of the pre-
cryogenic viability for said biological cell structures,
- an assemblage of biological cell structures having not less than about 60% of the pre-
30 cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 50% of the pre-cryogenic viability for said biological cell structures,
- an assemblage of biological cell structures having not less than about 40% of the pre-cryogenic viability for said biological cell structures,
- 5 - an assemblage of biological cell structures having not less than about 30% of the pre-cryogenic viability for said biological cell structures, and
- an assemblage of biological cell structures having not less than about 20% of the pre-cryogenic viability for said biological cell structures.

128. A cryopreservation composition as described in clause 121 or any other clause wherein
10 said increased surface energy content cryopreservation fluid comprises a substantially increased surface energy cryopreservation fluid.

129. A cryopreservation composition as described in clause 128 or any other clause wherein
15 said substantially increased surface energy cryopreservation fluid comprises a substantially increased surface energy cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having its surface energy increased by at least about 100 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 270 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 320 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 650 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 1050 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 3600 j/kg surface energy, and
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 5200 j/kg surface energy.

20 25 30 130. A cryopreservation composition as described in clause 128 or any other clause wherein said substantially increased surface energy cryopreservation fluid comprises a

substantially increased surface energy cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 200 j/kg surface energy,
- 5 - a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 420 j/kg surface energy,
- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 760 j/kg surface energy,
- 10 - a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 1200 j/kg surface energy,
- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 3800 j/kg surface energy, and
- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 5400 j/kg surface energy.

15 131. A cryopreservation composition as described in clause 128 or any other clause wherein said substantially increased surface energy cryopreservation fluid comprises a substantially increased surface energy cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having its surface energy increased by 20 at least about 30%,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 50%,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 100%,
- 25 - a cryopreservation fluid containing a substance having its surface energy increased by at least about 3 times,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 5 times,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 10 times,

- a cryopreservation fluid containing a substance having its surface energy increased by at least about 30 times, and
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 50 times.

5 132. A cryopreservation composition as described in clause 121,101.1,130, or 131 or any other clause wherein said increased surface energy content cryopreservation fluid comprises a structurally altered cryopreservation fluid.

10 133. A cryopreservation composition as described in clause 132 or any other clause wherein said structurally altered cryopreservation fluid comprises an increased structurally stored energy cryopreservation fluid.

134. A cryopreservation composition as described in clause 132 or any other clause wherein said structurally altered cryopreservation fluid comprises a drop transformed cryopreservation fluid.

15 135. A cryopreservation composition as described in clause 134 or any other clause wherein said drop transformed cryopreservation fluid comprises a drop transformed cryopreservation fluid selected from a group consisting of:

20 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 30 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50 times,

25 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 500 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 1000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 5000 times,

30 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 10000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50000 times, and
- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100000 times.

5 136. A cryopreservation composition as described in clause 128 or any other clause wherein
said increased surface energy content cryopreservation fluid comprises a larger drop
rended cryopreservation fluid.

137. A cryopreservation composition as described in clause 136 or any other clause wherein
said larger drop rended cryopreservation fluid comprises a larger drop rended
10 cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 1000nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 900nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 700nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 500nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 300nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 100nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 70nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 50nm, and
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 30nm.

15 138. A cryopreservation composition as described in clause 137 or any other clause wherein
said larger drop rended cryopreservation fluid comprises a larger drop rended
20 cryopreservation fluid selected from a group consisting of:

25

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- a cryopreservation fluid containing a substance having at least about 20% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size,

5 - a cryopreservation fluid containing a substance having at least about 40% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size,

- a cryopreservation fluid containing a substance having at least about 60% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size,

10 - a cryopreservation fluid containing a substance having at least about 80% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size, and

- a cryopreservation fluid containing a substance having at least about 90% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size.

15 139. A cryopreservation composition as described in clause 121, 128, 129, 130, , 131, 137, or 138 or any other clause wherein said increased surface energy content cryopreservation fluid comprises a lipid containing cryoprotective fluid selected from a group consisting of:

20 - a sea buckthorn lipid containing cryoprotective fluid incorporated with said biological cell structures,

- an unsaturated fatty acid containing cryoprotective fluid with said biological cell structures

- a saturated fatty acid containing cryoprotective fluid with said biological cell structures - a palmitic acid containing cryoprotective fluid incorporated with said biological cell structures,

- a palmitoleic acid containing cryoprotective fluid incorporated with said biological cell structures,

- an oleic acid containing cryoprotective fluid incorporated with said biological cell structures,

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- a linoleic acid containing cryoprotective fluid incorporated with said biological cell structures,
- a linolenic acid containing cryoprotective fluid incorporated with said biological cell structures,
- 5 - a lecithin containing cryoprotective fluid incorporated with said biological cell structures,
- a β -sitosterol containing cryoprotective fluid incorporated with said biological cell structures,
- a β -amyrin containing cryoprotective fluid incorporated with said biological cell structures,
- 10 - a γ -carotene containing cryoprotective fluid incorporated with said biological cell structures,
- an α -amyrin containing cryoprotective fluid incorporated with said biological cell structures,
- a β -carotene containing cryoprotective fluid incorporated with said biological cell structures,
- 15 - a lycopene containing cryoprotective fluid incorporated with said biological cell structures,
- a lutein containing cryoprotective fluid incorporated with said biological cell structures,
- 20 - a tocopherol containing cryoprotective fluid incorporated with said biological cell structures,
- a phosphatidylethanolamine containing cryoprotective fluid incorporated with said biological cell structures,
- a digalactosyldiacylglycerol lipid containing cryoprotective fluid incorporated with said biological cell structures,
- 25 - a monogalactosymonoacylglycerol containing cryoprotective fluid incorporated with said biological cell structures,
- a 16:1 fatty acid containing cryoprotective fluid incorporated with said biological cell structures,
- 30 -

- a 18:1 fatty acid containing cryoprotective fluid incorporated with said biological cell structures,

- a 18:2 fatty acid containing cryoprotective fluid incorporated with said biological cell structures, and

5 - a 18:3 fatty acid containing cryoprotective fluid incorporated with said biological cell structures.

140. A cryopreservation composition as described in clause 139 or any other clause wherein said lipid containing cryoprotective fluid incorporated with said biological cell structures comprises a high lipid concentration cryopreservation fluid incorporated with said

10 biological cell structures.

141. A cryopreservation composition as described in clause 140 or any other clause wherein said high lipid concentration cryopreservation fluid incorporated with said biological cell structures comprises a high lipid concentration cryopreservation fluid incorporated with said biological cell structures selected from a group consisting of:

15 - an at least about 10% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment,

- an at least about 20% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment,

- an at least about 40% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment,

20 - an at least about 60% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment, and

- an at least about 80% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment.

25 142. A cryopreservation composition as described in clause 140 or any other clause wherein said high lipid concentration cryopreservation fluid incorporated with said biological cell structures comprises a high lipid concentration cryopreservation fluid incorporated with said biological cell structures having a component selected from a group consisting of: a relatively high concentration sea buckthorn lipid, a relatively high saturated fatty acid concentration, a relatively high concentration unsaturated fatty acid concentration, a relatively high concentration palmitic acid, a relatively high concentration palmitoleic

acid, a relatively high concentration oleic acid, a relatively high concentration linoleic acid, a relatively high concentration linolenic acid, a relatively high concentration lecithin, a relatively high concentration β -sitosterol, a relatively high concentration β -amyrin, a relatively high concentration γ -carotene, a relatively high concentration α -amyrin, a relatively high concentration β -carotene, a relatively high concentration lycopene, a relatively high concentration lutein, a relatively high concentration tocopherol, a relatively high concentration digalactosyldiacylglycerol, a relatively high concentration monogalactosymonoacylglycerol, a relatively high concentration 16:1 fatty acids, a relatively high concentration 18:1 fatty acids, a relatively high concentration 18:2 fatty acids, and a relatively high concentration 18:3 fatty acid.

143. A cryopreservation composition as described in clause 121 or any other clause wherein said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,

- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- 5 - an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- 10 - an assemblage of enhanced cryogenic viability keratinocyte cells,
- an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures.

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144. A cryopreservation composition comprising:

- an assemblage of biological cell structures; and
- a collection of non-naturally occurring lipid droplets contained within a cryopreservation fluid and mixed with said biological cell structures.

20 145. A cryopreservation composition as described in clause 144 or any other clause wherein said collection of non-naturally occurring lipid droplets contained within a cryopreservation fluid and mixed with said biological cell structures comprises unstably sized lipid droplets contained within said cryopreservation fluid

146. A cryopreservation composition as described in clause 144 or any other clause wherein 25 said collection of non-naturally occurring lipid droplets contained within a cryopreservation fluid and mixed with said biological cell structures comprises size-modified lipid droplets contained within said cryopreservation fluid.

147. A cryopreservation composition as described in clause 146 wherein said size-modified lipid droplets contained within said cryopreservation fluid comprises a drop transformed 30 cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 30 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50 times,

5 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 500 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 1000 times,

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- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 5000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 10000 times,

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- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50000 times, and

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100000 times.

148. A cryopreservation composition as described in clause 144 or any other clause wherein

20 said collection of non-naturally occurring lipid droplets contained within a

cryopreservation fluid and mixed with said biological cell structures comprises a

cryopreservation fluid containing a lipid having a substantial number of relatively small droplets.

149. A cryopreservation composition as described in clause 148 or any other clause wherein

25 said cryopreservation fluid containing a lipid having a substantial number of relatively small droplets comprises a larger drop rended cryopreservation fluid.

150. A cryopreservation composition as described in clause 147, 148, or 149 or any other

clause wherein said cryopreservation fluid containing a lipid having a substantial number of relatively small droplets comprises a lipid selected from a group consisting of:

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- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 1000nm,

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 900nm,

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 700nm,

5 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 500nm,

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 300nm,

10 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 100nm,

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 70nm,

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 50nm, and

15 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 30nm.

151. A cryopreservation composition as described in clause 150 or any other clause wherein said cryopreservation fluid containing a lipid having a substantial number of relatively small droplets comprises a fluid selected from a group consisting of:

20 - a cryopreservation fluid containing a substance having at least about 20% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size,

- a cryopreservation fluid containing a substance having at least about 40% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size,

25 - a cryopreservation fluid containing a substance having at least about 60% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size,

- a cryopreservation fluid containing a substance having at least about 80% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size, and

30 - a cryopreservation fluid containing a substance having at least about 100% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size, and

- a cryopreservation fluid containing a substance having at least about 90% of said droplets of said substance in said cryopreservation fluid at least about as small as said desired size.

152. A cryopreservation composition as described in clause 148 or 149 or any other clause

5 wherein said cryopreservation fluid containing a lipid having a substantial number of relatively small droplets comprises a fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 30 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50 times,

10 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 500 times,

15 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 1000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 5000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 10000 times,

20 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50000 times, and

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100000 times.

25 153. A cryopreservation composition as described in clause 144, 147, or 149 or any other

clause wherein said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,

- an assemblage of enhanced cryogenic viability stem cell structures,

30 - an assemblage of enhanced cryogenic viability skin cells,

- an assemblage of enhanced cryogenic viability embryonic stem cells,

- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- 5 - an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- 10 - an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- 15 - an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- 20 - an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- 25 - an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures.

30 154. A cryopreservation composition as described in clause 144 wherein said collection of non-naturally occurring lipid droplets contained within a cryopreservation fluid and

mixed with said biological cell structures comprises a substance having a substantially uniform maximum size of droplets while contained within said cryopreservation fluid.

155. A cryopreservation composition as described in clause 154 wherein said substance having a substantially uniform maximum size of droplets while contained within said cryopreservation fluid comprises a substance selected from a group consisting of:

- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 1000nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 900nm,
- 10 - a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 700nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 500nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 300nm,
- 15 - a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 100nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 70nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 50nm, and
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 30nm.

156. A cryopreservation composition as described in clause 154 or any other clause wherein said substance having a substantially uniform maximum size of droplets while contained within said cryopreservation fluid comprises comprises a lipid selected from a group consisting of:

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 1000nm,
- 30 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 900nm,

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 700nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 500nm,
- 5 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 300nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 100nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 70nm,
- 10 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 50nm, and
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 30nm.

15 157. A cryopreservation composition as described in clause 144 wherein said collection of non-naturally occurring lipid droplets contained within a cryopreservation fluid and mixed with said biological cell structures comprises a substance contained within said cryopreservation fluid having a droplet size with a skewed size distribution that favors smaller size droplets.

20 158. A cryopreservation composition as described in clause 157 or any other clause wherein said substance contained within said cryopreservation fluid having a droplet size with a skewed size distribution that favors smaller size droplets comprises a substance selected from a group consisting of:

- a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 1000nm,
- a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 900nm,
- a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 700nm,
- 25 - a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 500nm,

- a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 300nm,

- a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 100nm,

5 - a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 70nm,

- a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 50nm, and

10 - a substance contained within said cryopreservation fluid having a mode drop size of less than at least about 30nm.

159. A cryopreservation composition comprising:

- an assemblage of enhanced post-cryogenic viability biological cell structures; and

- a high lipid concentration cryopreservation fluid mixed with said enhanced post-cryogenic viability biological cell structures.

15 160. A cryopreservation composition as described in clause 159 or any other clause wherein said high lipid concentration cryopreservation fluid incorporated with said biological cell structures comprises a high lipid concentration cryopreservation fluid incorporated with said biological cell structures selected from a group consisting of:

20 - an at least about 10% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment,

- an at least about 20% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment,

- an at least about 40% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment,

25 - an at least about 60% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment, and

- an at least about 80% higher than traditional lipid concentration cryopreservation fluid as compared to similar fluid of similar character for similar thermal treatment.

161. A cryopreservation composition as described in clause 159 or any other clause wherein said high lipid concentration cryopreservation fluid incorporated with said biological cell structures comprises a high lipid concentration cryopreservation fluid incorporated

with said biological cell structures having a component selected from a group consisting of: a relatively high concentration sea buckthorn lipid, a relatively high concentration unsaturated fatty acid, a relatively high concentration saturated fatty acid, a relatively high concentration palmitic acid, a relatively high concentration palmitoleic acid, a relatively high concentration oleic acid, a relatively high concentration linoleic acid, a relatively high concentration linolenic acid, a relatively high concentration lecithin, a relatively high concentration β -sitosterol, a relatively high concentration β -amyrin, a relatively high concentration γ -carotene, a relatively high concentration α -amyrin, a relatively high concentration β -carotene, a relatively high concentration lycopene, a relatively high concentration lutein, a relatively high concentration tocopherol, a relatively high concentration digalactosyldiacylglycerol, a relatively high concentration monogalactosymonoacylglycerol, a relatively high concentration 16:1 fatty acids, a relatively high concentration 18:1 fatty acids, a relatively high concentration 18:2 fatty acids, and a relatively high concentration 18:3 fatty acid.

15 162. A cryopreservation composition as described in clause 159 or any other clause wherein
said enhanced post-cryogenic viability assemblage of biological cell structures comprises
a relatively high post-cryogenic viability assemblage of biological cell structures.

163. A cryopreservation composition as described in clause 162 or any other clause wherein
said relatively high post-cryogenic viability assemblage of biological cell structures
20 comprises an assemblage of biological cell structures selected from a group consisting of:
- an at least about 5% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,
- an at least about 10% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,
- an at least about 15% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,

- an at least about 20% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment, and

5 - an at least about 25% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment.

164. A cryopreservation composition as described in clause 162 or any other clause wherein said relatively high post-cryogenic viability assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

10 - an assemblage of biological cell structures having not less than about 80% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 70% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 60% of the pre-cryogenic viability for said biological cell structures,

15 - an assemblage of biological cell structures having not less than about 50% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 40% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 30% of the pre-cryogenic viability for said biological cell structures, and

20 - an assemblage of biological cell structures having not less than about 20% of the pre-cryogenic viability for said biological cell structures.

165. A cryopreservation composition as described in clause 159, 160, 162, or 164 or any other clause wherein said increased surface energy content cryopreservation fluid comprises a larger drop rended cryopreservation fluid.

25 166. A cryopreservation composition as described in clause 165 or any other clause wherein said larger drop rended cryopreservation fluid comprises a larger drop rended cryopreservation fluid selected from a group consisting of:

30 - a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 1000nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 900nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 700nm,

5 - a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 500nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 300nm,

- a cryopreservation fluid containing a substance having no substantial number of 10 droplets with a diameter larger than about 100nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 70nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 50nm, and

15 - a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 30nm.

167. A cryopreservation composition as described in clause 159 or any other clause wherein said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

20 - an assemblage of enhanced cryogenic viability blood cell structures,

- an assemblage of enhanced cryogenic viability stem cell structures,

- an assemblage of enhanced cryogenic viability skin cells,

- an assemblage of enhanced cryogenic viability embryonic stem cells,

- an assemblage of enhanced cryogenic viability neural stem cells,

25 - an assemblage of enhanced cryogenic viability umbilical cord blood cells,

- an assemblage of enhanced cryogenic viability epithelial stem cells

- an assemblage of enhanced cryogenic viability cardiac stem cells

- an assemblage of enhanced cryogenic viability muscle stem cells

- an assemblage of enhanced cryogenic viability connective stem cells

30 - an assemblage of enhanced cryogenic viability epithelial cells

- an assemblage of enhanced cryogenic viability cardiac cells

- an assemblage of enhanced cryogenic viability of muscle cells
- an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,
- 5 - an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- 10 - an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- 15 - an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- 20 - an assemblage of enhanced cryogenic viability sperm cell structures.

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168. A cryopreservation composition as described in clause 166 or any other clause wherein said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- 25 - an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- 30 - an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells

- an assemblage of enhanced cryogenic viability cardiac stem cells
- an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells

5 - an assemblage of enhanced cryogenic viability cardiac cells

- an assemblage of enhanced cryogenic viability of muscle cells
- an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,

10 - an assemblage of enhanced cryogenic viability plant seed cells,

- an assemblage of enhanced cryogenic viability plant shoot cells,
- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,

15 - an assemblage of enhanced cryogenic viability tumorous tissue cell structures,

- an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,

20 - an assemblage of enhanced cryogenic viability mesenchymal cells,

- an assemblage of enhanced cryogenic viability keratinocyte cells,
- an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and

25 - an assemblage of enhanced cryogenic viability sperm cell structures.

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169. A cryopreservation composition comprising:

- an assemblage of biological cell structures; and
- a reduced damage lipid content cryopreservation fluid mixed with said biological cell structures.

170. A cryopreservation composition as described in clause 169 or any other clause wherein said reduced damage lipid content cryopreservation fluid mixed with said biological cell structures comprises a configured lipid content cryopreservation fluid.
171. A cryopreservation composition as described in clause 169 or any other clause wherein 5 reduced damage lipid content cryopreservation fluid mixed with said biological cell structures comprises an altered lipid content cryopreservation fluid.
172. A cryopreservation composition as described in clause 171 or any other clause wherein said altered lipid content cryopreservation fluid comprises a physically altered lipid content cryopreservation fluid.
- 10 173. A cryopreservation composition as described in clause 172 or any other clause wherein said physically altered lipid content cryopreservation fluid comprises a drop rended cryopreservation fluid.
174. A cryopreservation composition as described in clause 169 or any other clause wherein 15 said reduced damage lipid content cryopreservation fluid comprises a cryopreservation fluid that has been frozen by reducing its temperature below the freezing point of water.
175. A cryopreservation composition as described in clause 174 or any other clause wherein said reduced damage lipid content cryopreservation fluid comprises a cryopreservation fluid that has been thawed by increasing its temperature above the freezing point of water.
- 20 176. A cryopreservation composition as described in clause 169, 404 or any other clause wherein said assemblage of biological cell structures comprises an enhanced post-cryogenic viability assemblage of biological cell structures.
177. A cryopreservation composition as described in clause 176 or any other clause wherein 25 said enhanced post-cryogenic viability assemblage of biological cell structures comprises a relatively high post-cryogenic viability assemblage of biological cell structures.
178. A cryopreservation composition as described in clause 177 or any other clause wherein said relatively high post-cryogenic viability assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:
 - an at least about 5% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

- an at least about 10% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

5 - an at least about 15% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment,

- an at least about 20% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment, and

10 - an at least about 25% higher post-cryogenic viability assemblage of biological cell structures over traditional post-cryogenic cell structures of a similar character and thermal treatment.

179. A cryopreservation composition as described in clause 177 or any other clause wherein said relatively high post-cryogenic viability assemblage of biological cell structures

15 comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of biological cell structures having not less than about 80% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 70% of the pre-cryogenic viability for said biological cell structures,

20 - an assemblage of biological cell structures having not less than about 60% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 50% of the pre-cryogenic viability for said biological cell structures,

25 - an assemblage of biological cell structures having not less than about 40% of the pre-cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 30% of the pre-cryogenic viability for said biological cell structures, and

- an assemblage of biological cell structures having not less than about 20% of the pre-cryogenic viability for said biological cell structures.

180. A cryopreservation composition as described in clause 169, 412, 417 or any other clause wherein said reduced damage lipid content cryopreservation fluid comprises a larger drop rended cryopreservation fluid.

181. A cryopreservation composition as described in clause 180 or any other clause wherein said larger drop rended cryopreservation fluid comprises a larger drop rended cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 1000nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 900nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 700nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 500nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 300nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 100nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 70nm,
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 50nm, and
- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 30nm.

182. A cryopreservation composition as described in clause 169 or any other clause wherein said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,

- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- 5 - an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- 10 - an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- 15 - an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- 20 - an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- 25 - an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures/
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183. A cryopreservation composition as described in clause 181 or any other clause wherein
said assemblage of biological cell structures comprises an assemblage of biological cell
structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,
- 5 - an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- 10 - an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- 15 - an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,
- 20 - an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- 25 - an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- 30 - an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,

- an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures.

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184. A cryopreservation composition comprising:
 - an enhanced post-cryogenic viability assemblage of biological cell structures; and
 - a sized droplet cryopreservation fluid mixed with said biological cell structures.
185. A cryopreservation composition as described in clause 184 or any other clause wherein
10 said sized droplet cryopreservation fluid mixed with said biological cell structures
comprises a drop rended cryopreservation fluid.
186. A cryopreservation composition as described in clause 185 or any other clause wherein
said sized droplet cryopreservation fluid mixed with said biological cell structures
comprises a lipid drop rended cryopreservation fluid.
187. A cryopreservation composition as described in clause 184 or any other clause wherein
15 said fluid comprises a sized droplet cryopreservation fluid that has been frozen by
reducing its temperature below the freezing point of water.
188. A cryopreservation composition as described in clause 187 or any other clause wherein
said sized droplet cryopreservation fluid comprises a sized droplet cryopreservation fluid
20 that has been thawed by increasing its temperature above the freezing point of water.
189. A cryopreservation composition as described in clause 184 or 185 or any other clause
wherein said sized droplet cryopreservation fluid mixed with said biological cell
structures comprises a cryopreservation fluid selected from a group consisting of:
 - a cryopreservation fluid containing a substance having no substantial number of
25 droplets with a diameter larger than about 1000nm,
 - a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 900nm,
 - a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 700nm,
 - a cryopreservation fluid containing a substance having no substantial number of
30 droplets with a diameter larger than about 500nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 300nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 100nm,

5 - a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 70nm,

- a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 50nm, and

10 - a cryopreservation fluid containing a substance having no substantial number of droplets with a diameter larger than about 30nm.

190. A cryopreservation composition as described in clause 185 or any other clause wherein said sized droplet cryopreservation fluid comprises a cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 30 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100 times,

20 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 500 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 1000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 5000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 10000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50000 times, and

30 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100000 times.

191. A cryopreservation composition as described in clause 184 or any other clause wherein said sized droplet cryopreservation fluid mixed with said biological cell structures comprises a cryopreservation fluid containing a lipid having a substantial number of relatively small droplets.
- 5 192. A cryopreservation composition as described in clause USa230 or any other clause wherein said cryopreservation fluid containing a lipid having a substantial number of relatively small droplets comprises a larger drop rended cryopreservation fluid.
- 10 193. A cryopreservation composition as described in clause 190, 191 or 192 or any other clause wherein said cryopreservation fluid containing a lipid having a substantial number of relatively small droplets comprises a cryopreservation fluid selected from a group consisting of:
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 1000nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 900nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 700nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 500nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 300nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 100nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 70nm,
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 50nm, and
 - a lipid containing cryopreservation fluid having no substantial number of droplets larger than at least about 30nm.
- 15
- 20
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194. A cryopreservation composition as described in clause 193 or any other clause wherein
said lipid containing cryopreservation fluid comprises a cryopreservation fluid selected
from a group consisting of:

- a cryopreservation fluid containing a substance having at least about 20% of said
5 droplets of said substance in said cryopreservation fluid at least about as small as said
desired size,
- a cryopreservation fluid containing a substance having at least about 40% of said
droplets of said substance in said cryopreservation fluid at least about as small as said
desired size,
- a cryopreservation fluid containing a substance having at least about 60% of said
10 droplets of said substance in said cryopreservation fluid at least about as small as said
desired size,
- a cryopreservation fluid containing a substance having at least about 80% of said
droplets of said substance in said cryopreservation fluid at least about as small as said
15 desired size, and
- a cryopreservation fluid containing a substance having at least about 90% of said
droplets of said substance in said cryopreservation fluid at least about as small as said
desired size.

195. A cryopreservation composition as described in clause 191 or 192 or any other clause
20 wherein said lipid containing cryopreservation fluid comprises a cryopreservation fluid
selected from a group consisting of:

- a cryopreservation fluid containing a lipid having its number of drops in said
cryopreservation fluid increased by at least about 30 times,
- a cryopreservation fluid containing a lipid having its number of drops in said
25 cryopreservation fluid increased by at least about 50 times,
- a cryopreservation fluid containing a lipid having its number of drops in said
cryopreservation fluid increased by at least about 100 times,
- a cryopreservation fluid containing a lipid having its number of drops in said
cryopreservation fluid increased by at least about 500 times,
- a cryopreservation fluid containing a lipid having its number of drops in said
30 cryopreservation fluid increased by at least about 1000 times,

- a cryopreservation fluid containing a lipid having its number of drops in said cryopreservation fluid increased by at least about 5000 times,
- a cryopreservation fluid containing a lipid having its number of drops in said cryopreservation fluid increased by at least about 10000 times,
- 5 - a cryopreservation fluid containing a lipid having its number of drops in said cryopreservation fluid increased by at least about 50000 times, and
- a cryopreservation fluid containing a lipid having its number of drops in said cryopreservation fluid increased by at least about 100000 times.

196. A cryopreservation composition as described in clause 184 or any other clause wherein

10 said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- 20 - an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- 25 - an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- 30 - an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,

- an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- 5 - an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- an assemblage of enhanced cryogenic viability melanocyte cells,
- 10 - an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures.

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197. A cryopreservation composition as described in clause 193 or any other clause wherein

15 said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- 20 - an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- 25 - an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- 30 - an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells

- an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- 5 - an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- 10 - an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- 15 - an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures.

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20 198. A cryopreservation composition as described in clause 184 wherein said sized droplet cryopreservation fluid mixed with said biological cell structures comprises a cryopreservation fluid containing a substance having a substantially uniform maximum size of droplets while contained within said cryopreservation fluid.

199. A cryopreservation composition as described in clause 198 wherein said cryopreservation fluid containing a substance having a substantially uniform maximum size of droplets while contained within said cryopreservation fluid comprises a substance selected from a group consisting of:

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- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 1000nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 900nm,

- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 700nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 500nm,
- 5 - a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 300nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 100nm,
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 70nm,
- 10 - a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 50nm, and
- a substance contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 30nm.

15 200. A cryopreservation composition as described in clause 198 or any other clause wherein said cryopreservation fluid containing a substance having a substantially uniform maximum size of droplets while contained within said cryopreservation fluid comprises a cryopreservation fluid containing a lipid selected from a group consisting of:

- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 1000nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 900nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 700nm,
- 20 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 500nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 300nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 100nm,

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- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 70nm,
- a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 50nm, and

5 - a lipid contained within said cryopreservation fluid having no substantial number of droplets larger than at least about 30nm.

201. A cryopreservation composition as described in clause 184 wherein said sized droplet cryopreservation fluid mixed with said biological cell structures comprises a substance contained within said cryopreservation fluid having a droplet size with a skewed size distribution that favors smaller size droplets.

10 202. A cryopreservation composition as described in clause 201 or any other clause wherein said substance contained within said cryopreservation fluid having a droplet size with a skewed size distribution that favors smaller size droplets comprises a lipid selected from a group consisting of:

- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 1000nm,
- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 900nm,
- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 700nm,
- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 500nm,
- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 300nm,

20 25 - a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 100nm,

- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 70nm,
- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 50nm, and

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- a lipid contained within said cryopreservation fluid having a mode drop size of less than at least about 30nm.

203. A cryopreservation composition comprising:

- an assemblage of biological cell structures;

5 - a foundation medium; and

-a cryopreservation fluid component selected from a group consisting of: sea buckthorn lipids, saturated fatty acids, unsaturated fatty acids, lauric acid, myristic acid, palmitic acid, stearic acid, arachadonic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachadonic acid, lecithin, triglycerides, spermaceti, bees wax, carnuba wax, sphingomyelins, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, cholic acid, oleic acid, β -sitosterol, β -amyrin, γ -carotene, α -amyrin, β -carotene, lycopene, lutein, tocopherol, ubiquinol, tocotrienols, eugenol, phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, ceramide phosphorylcholine, ceramine phosphorylglycerol, digalactosyldiacylglycerol, monogalactosymonoacylglycerol, 16:1 fatty acids, 18:1 fatty acids, 18:2 fatty acids, 18:3 fatty acids, natural rubber, and gutta-percha.

204. A cryopreservation composition comprising:

- an assemblage of biological cell structures;

20 - a foundation medium;

- a lipid cryoprotectant; and

- a cryopreservation fluid component selected from a group consisting of: thiols, ascorbic acid, polypropenols, superoxide dismutase, catalase, peroxidase, lipoic acid, uric acid, hydrophobic substances (non-lipid), silicones, fluorocarbons, glutathione, melatonin, peroxiredoxins, resveratrol, phytic acid, flavonoids, vitamin A, vitamin E, vitamin D2, vitamin K1, lipid soluble substances, and lipid soluble vitamins.

205. A cryopreservation composition as described in clause 203 or 204 or any other clause

wherein said assemblage of biological cell structures comprises an enhanced post-cryogenic viability assemblage of biological cell structures.

206. A cryopreservation composition as described in clause 205 or any other clause wherein
said enhanced post-cryogenic viability assemblage of biological cell structures comprises
a relatively high post-cryogenic viability assemblage of biological cell structures.

207. A cryopreservation composition as described in clause 206 or any other clause wherein
5 said relatively high post-cryogenic viability assemblage of biological cell structures
comprises an assemblage of biological cell structures selected from a group consisting of:
- an at least about 5% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,
10 - an at least about 10% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment,
- an at least about 15% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
15 thermal treatment,
- an at least about 20% higher post-cryogenic viability assemblage of biological cell
structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment, and
- an at least about 25% higher post-cryogenic viability assemblage of biological cell
20 structures over traditional post-cryogenic cell structures of a similar character and
thermal treatment.

208. A cryopreservation composition as described in clause 206 or any other clause wherein
said relatively high post-cryogenic viability assemblage of biological cell structures
comprises an assemblage of biological cell structures selected from a group consisting of:
25 - an assemblage of biological cell structures having not less than about 80% of the pre-
cryogenic viability for said biological cell structures,
- an assemblage of biological cell structures having not less than about 70% of the pre-
cryogenic viability for said biological cell structures,
- an assemblage of biological cell structures having not less than about 60% of the pre-
30 cryogenic viability for said biological cell structures,

- an assemblage of biological cell structures having not less than about 50% of the pre-cryogenic viability for said biological cell structures,
- an assemblage of biological cell structures having not less than about 40% of the pre-cryogenic viability for said biological cell structures,
- 5 - an assemblage of biological cell structures having not less than about 30% of the pre-cryogenic viability for said biological cell structures, and
- an assemblage of biological cell structures having not less than about 20% of the pre-cryogenic viability for said biological cell structures.

209. A cryopreservation composition as described in clause 205 or any other clause wherein
10 said cryopreservation fluid comprises a substantially increased surface energy
cryopreservation fluid.

210. A cryopreservation composition as described in clause 209 or any other clause wherein
said substantially increased surface energy cryopreservation fluid comprises a
substantially increased surface energy cryopreservation fluid selected from a group
15 consisting of:

- a cryopreservation fluid containing a substance having its surface energy increased by
at least about 100 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by
at least about 270 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by
at least about 320 j/kg surface energy,
- 20 - a cryopreservation fluid containing a substance having its surface energy increased by
at least about 650 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by
at least about 1050 j/kg surface energy,
- a cryopreservation fluid containing a substance having its surface energy increased by
at least about 3600 j/kg surface energy, and
- a cryopreservation fluid containing a substance having its surface energy increased by
at least about 5200 j/kg surface energy.

25 30 211. A cryopreservation composition as described in clause 209 or any other clause wherein
said substantially increased surface energy cryopreservation fluid comprises a

substantially increased surface energy cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 200 j/kg surface energy,
- 5 - a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 420 j/kg surface energy,
- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 760 j/kg surface energy,
- 10 - a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 1200 j/kg surface energy,
- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 3800 j/kg surface energy, and
- a cryopreservation fluid having a surface energy of a substance in said cryopreservation fluid increased to at least about 5400 j/kg surface energy.

15 212. A cryopreservation composition as described in clause 209 or any other clause wherein said substantially increased surface energy cryopreservation fluid comprises a substantially increased surface energy cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having its surface energy increased by 20 at least about 30%,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 50%,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 100%,
- 25 - a cryopreservation fluid containing a substance having its surface energy increased by at least about 3 times,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 5 times,
- a cryopreservation fluid containing a substance having its surface energy increased by at least about 10 times,

- a cryopreservation fluid containing a substance having its surface energy increased by at least about 30 times, and

- a cryopreservation fluid containing a substance having its surface energy increased by at least about 50 times.

5 213. A cryopreservation composition as described in clause 205 or any other clause wherein said cryopreservation composition comprises a drop transformed cryopreservation fluid.

214. A cryopreservation composition as described in clause 213 or any other clause wherein said drop transformed cryopreservation fluid comprises a drop transformed cryopreservation fluid selected from a group consisting of:

10 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 30 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100 times,

15 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 500 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 1000 times,

20 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 5000 times,

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 10000 times,

25 - a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 50000 times, and

- a cryopreservation fluid containing a substance having its number of drops in said cryopreservation fluid increased by at least about 100000 times.

215. A cryopreservation composition as described in clause 205 or any other clause wherein said cryopreservation composition comprises a larger drop rended cryopreservation fluid.

216. A cryopreservation composition as described in clause 215 or any other clause wherein
said larger drop rended cryopreservation fluid comprises a larger drop rended
cryopreservation fluid selected from a group consisting of:

- a cryopreservation fluid containing a substance having no substantial number of
5 droplets with a diameter larger than about 1000nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 900nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 700nm,
- a cryopreservation fluid containing a substance having no substantial number of
10 droplets with a diameter larger than about 500nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 300nm,
- a cryopreservation fluid containing a substance having no substantial number of
15 droplets with a diameter larger than about 100nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 70nm,
- a cryopreservation fluid containing a substance having no substantial number of
droplets with a diameter larger than about 50nm, and
- a cryopreservation fluid containing a substance having no substantial number of
20 droplets with a diameter larger than about 30nm.

217. A cryopreservation composition as described in clause 205 or any other clause wherein
said cryopreservation composition comprises a cryopreservation fluid that has been
frozen by reducing its temperature below the freezing point of water.

25 218. A cryopreservation composition as described in clause CSa657.06 or any other clause
wherein said cryopreservation composition comprises a cryopreservation fluid that has
been thawed by increasing its temperature above the freezing point of water.

219. A cryopreservation composition as described in clause 203 or 204 or any other clause
wherein said assemblage of biological cell structures comprises an assemblage of
30 biological cell structures selected from a group consisting of:
- an assemblage of enhanced cryogenic viability blood cell structures,

- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- 5 - an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- 10 - an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- 15 - an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- 20 - an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- 25 - an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,
- an assemblage of enhanced cryogenic viability keratinocyte cells,
- an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- 30 - an assemblage of enhanced cryogenic viability oocyte cells, and
- an assemblage of enhanced cryogenic viability sperm cell structures.

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220. A cryopreservation composition as described in clause 205 or any other clause wherein said assemblage of biological cell structures comprises an assemblage of biological cell structures selected from a group consisting of:

- 5 - an assemblage of enhanced cryogenic viability blood cell structures,
- an assemblage of enhanced cryogenic viability stem cell structures,
- an assemblage of enhanced cryogenic viability skin cells,
- an assemblage of enhanced cryogenic viability embryonic stem cells,
- an assemblage of enhanced cryogenic viability neural stem cells,
- 10 - an assemblage of enhanced cryogenic viability umbilical cord blood cells,
- an assemblage of enhanced cryogenic viability epithelial stem cells
- an assemblage of enhanced cryogenic viability cardiac stem cells
- an assemblage of enhanced cryogenic viability muscle stem cells
- an assemblage of enhanced cryogenic viability connective stem cells
- 15 - an assemblage of enhanced cryogenic viability epithelial cells
- an assemblage of enhanced cryogenic viability cardiac cells
- an assemblage of enhanced cryogenic viability of muscle cells
- an assemblage of enhanced cryogenic viability connective cells
- an assemblage of enhanced cryogenic viability nerve cells
- 20 - an assemblage of enhanced cryogenic viability histological sample cells,
- an assemblage of enhanced cryogenic viability plant seed cells,
- an assemblage of enhanced cryogenic viability plant shoot cells,
- an assemblage of enhanced cryogenic viability ovarian tissue cell structures,
- an assemblage of enhanced cryogenic viability testicular tissue cell structures,
- 25 - an assemblage of enhanced cryogenic viability embryo cells,
- an assemblage of enhanced cryogenic viability tumorous tissue cell structures,
- an assemblage of enhanced cryogenic viability yeast cells,
- an assemblage of enhanced cryogenic viability bacterial cells,
- an assemblage of enhanced cryogenic viability algal cells,
- 30 - an assemblage of enhanced cryogenic viability fungal cells,
- an assemblage of enhanced cryogenic viability mesenchymal cells,

- an assemblage of enhanced cryogenic viability keratinocyte cells,
- an assemblage of enhanced cryogenic viability melanocyte cells,
- an assemblage of enhanced cryogenic viability hepatocyte cells,
- an assemblage of enhanced cryogenic viability oocyte cells, and
- 5 - an assemblage of enhanced cryogenic viability sperm cell structures.
- .

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves both cryopreservation techniques and processes as 10 well as substances and devices to accomplish the appropriate cryopreservation. In this application, the cryopreservation devices are often disclosed as part of the explanation of the process. The invention includes both methods and apparatus with apparatus shown to be the inherent equipment needed to achieve the various steps explained and visa versa. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this 15 disclosure.

The discussion included in this application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully enumerate all generic aspects of 20 the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in device- or substance-oriented terminology, each element of the device or substance implicitly 25 performs a function. Both method and process claims should be understood as included to address the functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims that may be included in this or any subsequent patent application.

It should also be understood that a variety of changes may be made without departing from the 30 essence of the invention. Such changes are also implicitly included in the description. They still fall within the scope of this invention. A broad disclosure encompassing both the explicit

embodiment(s) shown, the great variety of implicit alternative embodiments, and the broad methods or processes and the like are encompassed by this disclosure and may be relied upon when drafting the claims for any subsequent patent application. It should be understood that language changes and broader or more detailed claiming may be accomplished at a later date.

5 With this understanding, the reader should be aware that this disclosure is to be understood to support a patent covering numerous aspects of the invention both independently and as an overall system.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. Additionally, when used or implied, an element is to be understood as encompassing individual as well as plural structures that may or may not be physically associated. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms -- even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of the act of "mixing" should be understood to encompass disclosure of a "mixer" -- whether explicitly discussed or not -- and, conversely, were there effectively disclosure of a "mixer, such a disclosure should be understood to encompass disclosure of the act of "mixing" and even a "means for mixing." Such changes and alternative terms are to be understood to be explicitly included in the description. Further, each such means (whether explicitly so described or not) should be understood as encompassing all elements that can perform the given function, and all descriptions of elements that perform a described function should be understood as a non-limiting example of means for performing that function.

In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with a broadly supporting interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary,
5 second edition.

U.S.PATENTS

| Patent Number | Kind Code | Issue Date | Name of Patentee or Applicant of cited Document |
|---------------|-----------|------------|---|
| 7622143 | B2 | 2009-11-24 | Herickhoff et al. |
| 8202558 | B2 | 2012-06-19 | Herickhoff et al. |
| 7820425 | B2 | 2010-10-26 | Schenk |
| 7838210 | B2 | 2010-11-23 | Ludwig et al. |
| 7892725 | XX | 2011-02-22 | Graham et al. |

U.S.PATENT APPLICATION PUBLICATIONS

| Publication Number | Kind Code | Publication Date | Name of Patentee or Applicant of cited Document |
|--------------------|-----------|------------------|---|
| 20110086336 | A1 | 2011-04-14 | Herickhoff et al. |
| 20060222724 | A1 | 2006-10-05 | Herickhoff et al. |
| 20090123906 | A1 | 2009-05-14 | Herickhoff |
| 20100311036 | | 2010-06-09 | He |

FOREIGN PATENT DOCUMENTS

| Foreign Document Number | Country Code | Kind Code | Publication Date | Name of Patentee or Applicant of cited Document |
|-------------------------|--------------|-----------|------------------|---|
| 2009109550 | WO | A1 | 2009-09-11 | Sanofi Pasteur |

5 NON-PATENT LITERATURE DOCUMENTS

| |
|--|
| Tcholakova, Slavka et al; "Role of Surfactant Type and Concentration for the Mean Drop Size during Emulsification in Turbulent Flow;" <i>Langmuir</i> 2004, 20, 7444-7458 |
| Adiga, K.C.; "Droplet Breakup Energies and Formation of Ultra-fine Mist"; NanoMist Systems, LLC; The Navy Technology Center for Safety and Survivability; Chemistry Division, Naval Research Laboratory; Washington D.C.; 14 pages |
| Seidel Jr., George; "Modifying oocytes and embryos to improve their cryopreservation;" <i>Theriogenology</i> 65 (2006) 228-235; Animal Reproduction and Biotechnology Laboratory, Colorado State University |
| Smith, Lloyd M et al; "Stability of Milk Fat Emulsions. I. Preparation of Model Oil-in-Water Emulsions and Evaluation of Their Stability;" Department of Food Science and Technology; University of California, Davis; <i>Journal of Dairy Science</i> ; Vol. 58 No. 9; 1249-1252. |
| Pillet, Elodie et al; "Liposomes as an alternative to egg yolk in stallion freezing extender;" <i>Theriogenology</i> 77 (2012) 268-279. |
| Zeron, Y, et al; "The effect of liposomes on thermotropic membrane phase transitions of bovine spermatozoa and oocytes: implications for reducing chilling sensitivity;" <i>Cryobiology</i> 45 (2002) 143-152. |
| Barnes, D. and G. Sato (1980) Methods for growth of cultured cells in serum-free medium. <i>Anal. Biochem.</i> 102:255-270. |

| |
|--|
| Foote, R.H., C.C. Brockett and M. T. Kaproth (2002). Motility and fertility of bull sperm in 5 whole milk extender containing antioxidants. <i>An Repro Sci</i> 71:13-23 |
| Pickett, B.W. and Amann, R.P. (1992). Cryopreservation of Semen, ch 83 in <i>Equine Reproduction</i> A. McKinnon and J. L. Voss, eds. pp. 769-789 |
| Thompson, J. et al. Rate-controlled cryopreservation and thawing of mammalian cells, 2011 Protocol Exchange |
| Shalfer, M, Pharmacological considerations in cryopreservation. In Karow AM (ed). <i>Organ Preservation for Transplantation</i> . 2nd edition. New York: Marcel Dekker 1981: 177-212 and modified and expanded by biocor.umn.edu/listing-of-protective-agents |
| D. Barnes and G. Sato 1980, <i>Analytical Biochemistry</i> 102, p 255 |
| Provisional Application Number 61/685,224, filed 14 March 2012, entitled Lipophilic protection of cryopreserved cells. |

Thus, the applicant(s) should be understood to have support to claim and make a statement of invention to at least: i) each of the cryopreservation substances and devices as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even 5 implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or 10 components disclosed, viii) the resulting products produced by such systems or components, ix) each system, method, and element shown or described as now applied to any specific field or devices mentioned, x) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, xi) an apparatus for performing the methods described herein comprising means for performing the steps, xii) the various combinations and 15 permutations of each of the elements disclosed, xiii) each potentially dependent claim or concept as a dependency on each and every one of the independent claims or concepts presented, and xiv) all inventions described herein.

With regard to claims whether now or later presented for examination, it should be understood that for practical reasons and so as to avoid great expansion of the examination burden, the applicant may at any time present only initial claims or perhaps only initial claims with only initial dependencies. The office and any third persons interested in potential scope of this or

5 subsequent applications should understand that broader claims may be presented at a later date in this case, in a case claiming the benefit of this case, or in any continuation in spite of any preliminary amendments, other amendments, claim language, or arguments presented, thus throughout the pendency of any case there is no intention to disclaim or surrender any potential subject matter. It should be understood that if or when broader claims are presented, such may

10 require that any relevant prior art that may have been considered at any prior time may need to be re-visited since it is possible that to the extent any amendments, claim language, or arguments presented in this or any subsequent application are considered as made to avoid such prior art, such reasons may be eliminated by later presented claims or the like. Both the examiner and any person otherwise interested in existing or later potential coverage, or considering if there has at

15 any time been any possibility of an indication of disclaimer or surrender of potential coverage, should be aware that no such surrender or disclaimer is ever intended or ever exists in this or any subsequent application. Limitations such as arose in *Hakim v. Cannon Avent Group, PLC*, 479 F.3d 1313 (Fed. Cir 2007), or the like are expressly not intended in this or any subsequent related matter. In addition, support should be understood to exist to the degree required under new

20 matter laws -- including but not limited to European Patent Convention Article 123(2) and United States Patent Law 35 USC 132 or other such laws-- to permit the addition of any of the various dependencies or other elements presented under one independent claim or concept as dependencies or elements under any other independent claim or concept. In drafting any claims at any time whether in this application or in any subsequent application, it should also be

25 understood that the applicant has intended to capture as full and broad a scope of coverage as legally available. To the extent that insubstantial substitutes are made, to the extent that the applicant did not in fact draft any claim so as to literally encompass any particular embodiment, and to the extent otherwise applicable, the applicant should not be understood to have in any way intended to or actually relinquished such coverage as the applicant simply may not have been

30 able to anticipate all eventualities; one skilled in the art, should not be reasonably expected to have drafted a claim that would have literally encompassed such alternative embodiments.

Further, if or when used, the use of the transitional phrase “comprising” is used to maintain the “open-end” claims herein, according to traditional claim interpretation. Thus, unless the context requires otherwise, it should be understood that the term “comprise” or variations such as “comprises” or “comprising”, are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible. The use of the phrase, “or any other claim” is used to provide support for any claim to be dependent on any other claim, such as another dependent claim, another independent claim, a previously listed claim, a subsequently listed claim, and the like. As one clarifying example, if a claim were dependent “on claim 20 or any other claim” or the like, it could be re-drafted as dependent on claim 1, claim 15, or even claim 25 (if such were to exist) if desired and still fall with the disclosure. It should be understood that this phrase also provides support for any combination of elements in the claims and even incorporates any desired proper antecedent basis for certain claim combinations such as with combinations of method, apparatus, process, and the like claims.

Claims:

1. A process to enhance the cryopreservation of biological cells comprising the steps of:

- assembling a cellular collection containing a plurality of biological cells;
- establishing a lipid containing cryopreservation fluid to be incorporated with said biological cells in cryopreservation;
- adding at least 270 J/kg surface energy to the lipids in said lipid containing cryopreservation fluid to create a lipid surface energy increased cryopreservation fluid by the multiplication in the number of discrete lipid droplets in the solution without changing the total concentration of lipids;
- mixing said lipid surface energy increased cryopreservation fluid and said biological cells to form an energy increased fluidic cryopreservation composite;
- removing thermal energy from said fluidic cryopreservation composite; and
- freezing said fluidic cryopreservation composite by reducing the temperature of said fluidic cryopreservation composite below the freezing point of water.

2. The process as described in claim 1, wherein said step of adding at least 270 J/kg surface energy to the lipids in said lipid containing cryopreservation fluid to create a lipid surface energy increased cryopreservation fluid comprises the step of adding at least 650 J/kg lipid surface energy to the lipids in said cryopreservation fluid.

3. The process as described in claim 1 or 2, further comprising the step of drop transforming at least one lipid in said cryopreservation fluid into droplets numbering at least 50 times the number of initial drops of said lipid in said cryopreservation fluid.

4. The process as described in claim 1 or 2, further comprising the step of drop transforming at least one lipid in said cryopreservation fluid into droplets numbering at least 50000 times the number of initial drops of said lipid in said cryopreservation fluid.

5. The process as described in any one of claims 1 to 4, wherein adding at least 270 J/kg surface energy to the lipids in said lipid containing cryopreservation fluid renders the formation of smaller droplets of said lipids in said cryopreservation fluid.
6. The process as described in claim 5, wherein said cryopreservation fluid contains no substantial number of droplets having a diameter of about 1000nm or larger than 1000nm of the lipids in said cryopreservation fluid.
7. The process as described in claim 6, wherein said cryopreservation fluid comprises no substantial number of droplets having a diameter larger of about 200nm or larger than 200nm of the lipids in said cryopreservation fluid.
8. The process as described in claim 6 or 7, wherein said step of assembling a cellular collection containing a plurality of biological cells comprises a step selected from a group consisting of the steps of:
 - assembling a cellular collection containing a plurality of blood cells,
 - assembling a cellular collection containing a plurality of stem cells,
 - assembling a cellular collection containing a plurality of skin cells,
 - assembling a cellular collection containing a plurality of embryonic stem cells,
 - assembling a cellular collection containing a plurality of neural stem cells,
 - assembling a cellular collection containing a plurality of epithelial stem cells,
 - assembling a cellular collection containing a plurality of cardiac stem cells,
 - assembling a cellular collection containing a plurality of muscle stem cells,
 - assembling a cellular collection containing a plurality of connective stem cells,
 - assembling a cellular collection containing a plurality of epithelial cells,
 - assembling a cellular collection containing a plurality of cardiac cells,
 - assembling a cellular collection containing a plurality of muscle cells,
 - assembling a cellular collection containing a plurality of connective cells,
 - assembling a cellular collection containing a plurality of nerve cells,
 - assembling a cellular collection containing a plurality of umbilical cord blood cells,

- assembling a cellular collection containing a plurality of histological sample cells,
- assembling a cellular collection containing a plurality of plant seed cells,
- assembling a cellular collection containing a plurality of plant shoot cells,
- assembling a cellular collection containing a plurality of ovarian tissue cells,
- assembling a cellular collection containing a plurality of testicular tissue cells,
- assembling a cellular collection containing a plurality of embryo cells,
- assembling a cellular collection containing a plurality of tumorous tissue cells,
- assembling a cellular collection containing a plurality of yeast cells,
- assembling a cellular collection containing a plurality of bacterial cells,
- assembling a cellular collection containing a plurality of algal cells,
- assembling a cellular collection containing a plurality of fungal cells,
- assembling a cellular collection containing a plurality of mesenchymal cells,
- assembling a cellular collection containing a plurality of keratinocyte cells,
- assembling a cellular collection containing a plurality of melanocyte cells,
- assembling a cellular collection containing a plurality of hepatocyte cells,
- assembling a cellular collection containing a plurality of oocyte cells, and
- assembling a cellular collection containing a plurality of sperm cells.

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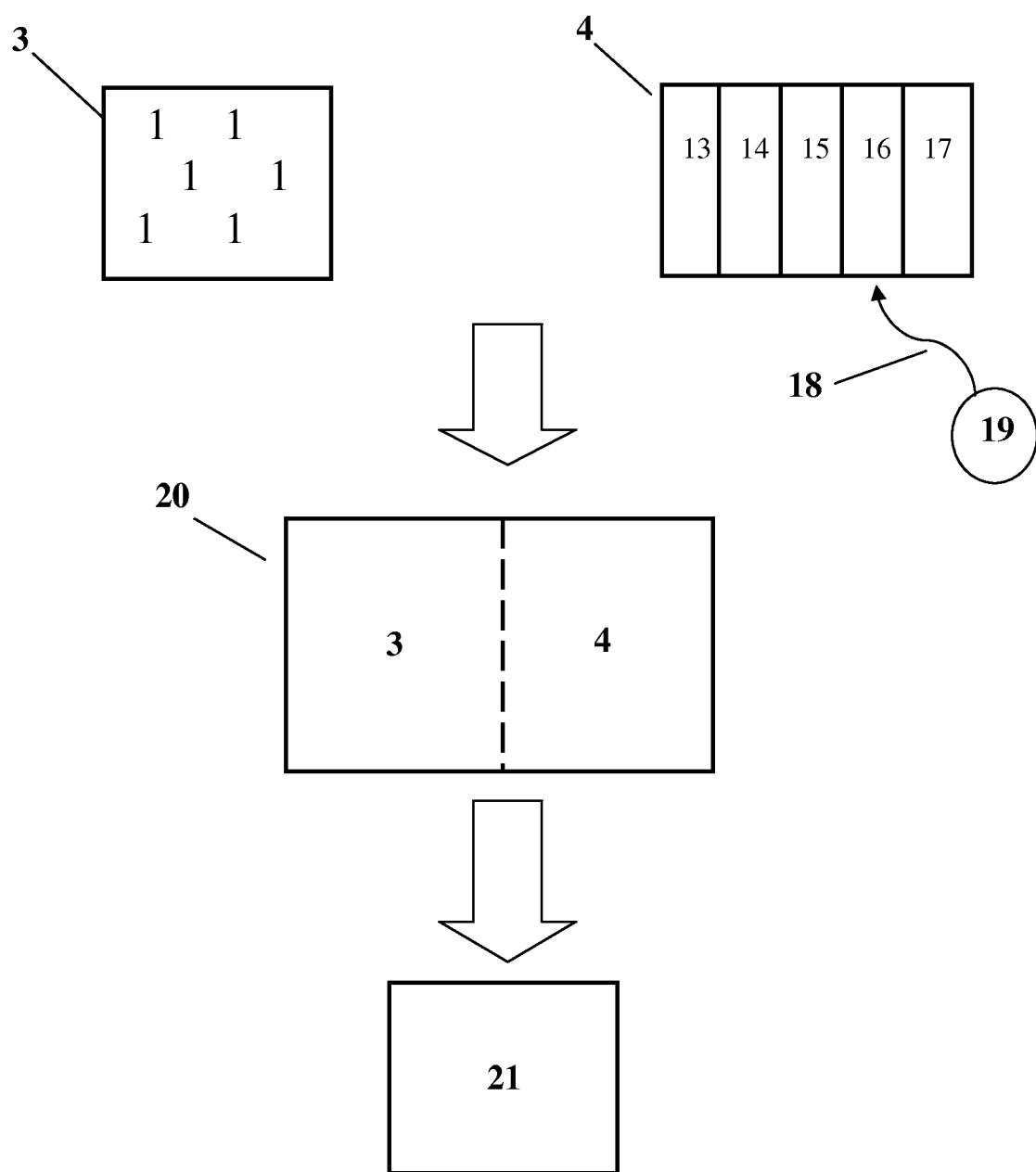


Fig. 1

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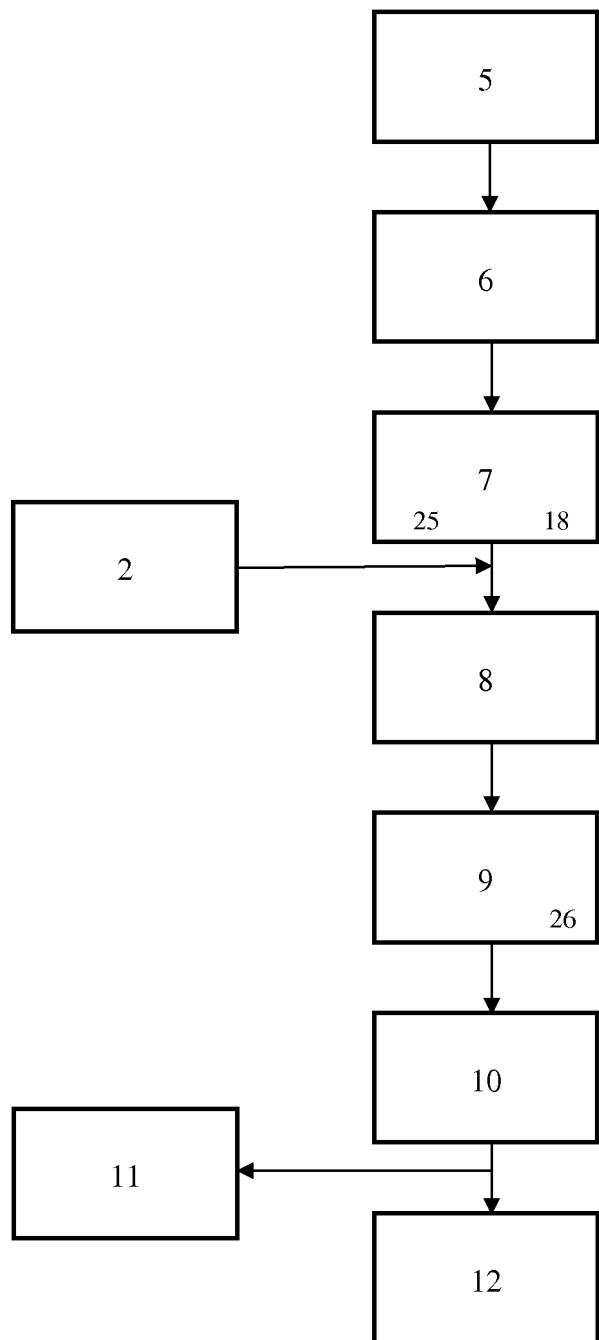


Fig. 2

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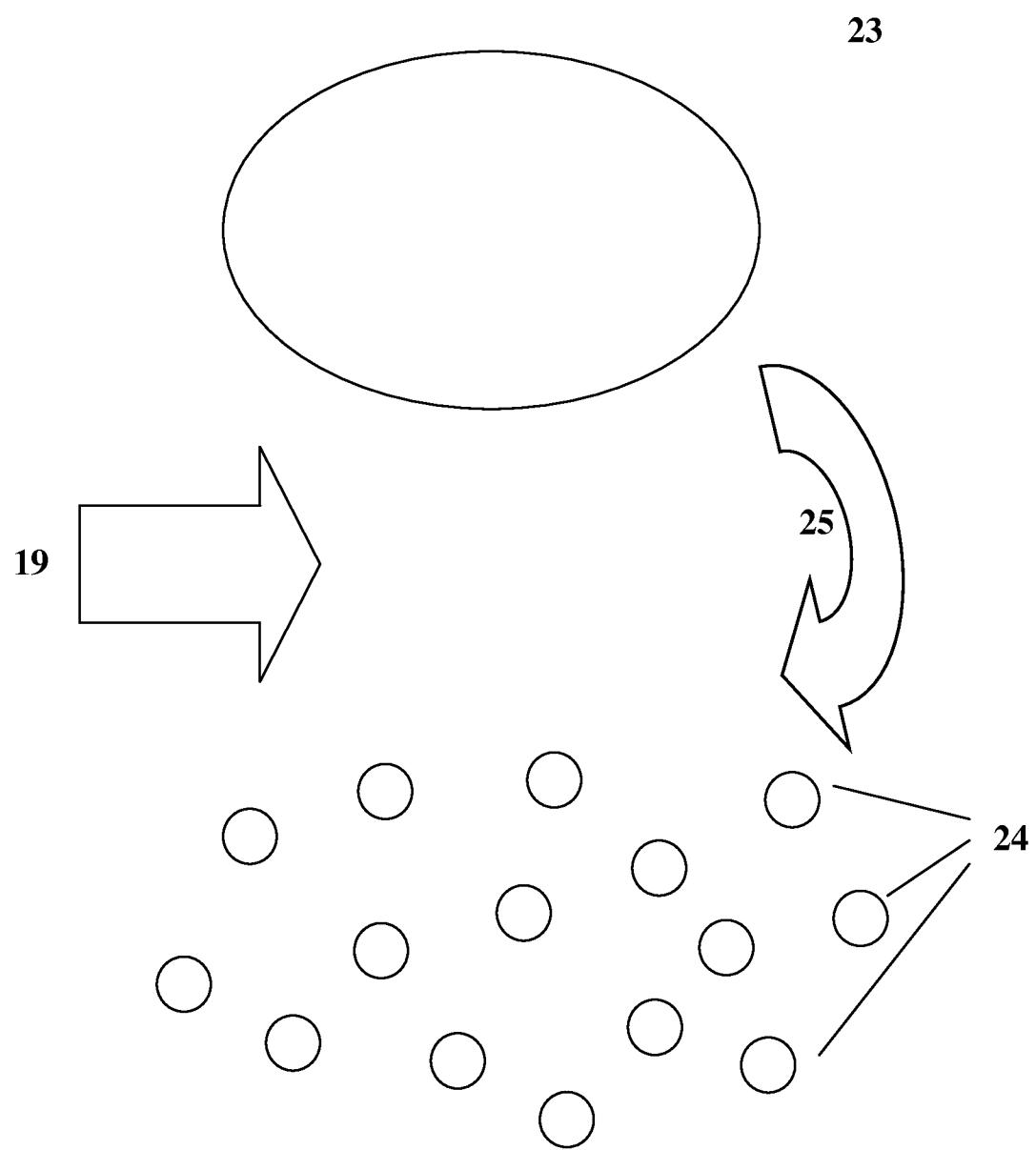


Fig. 3

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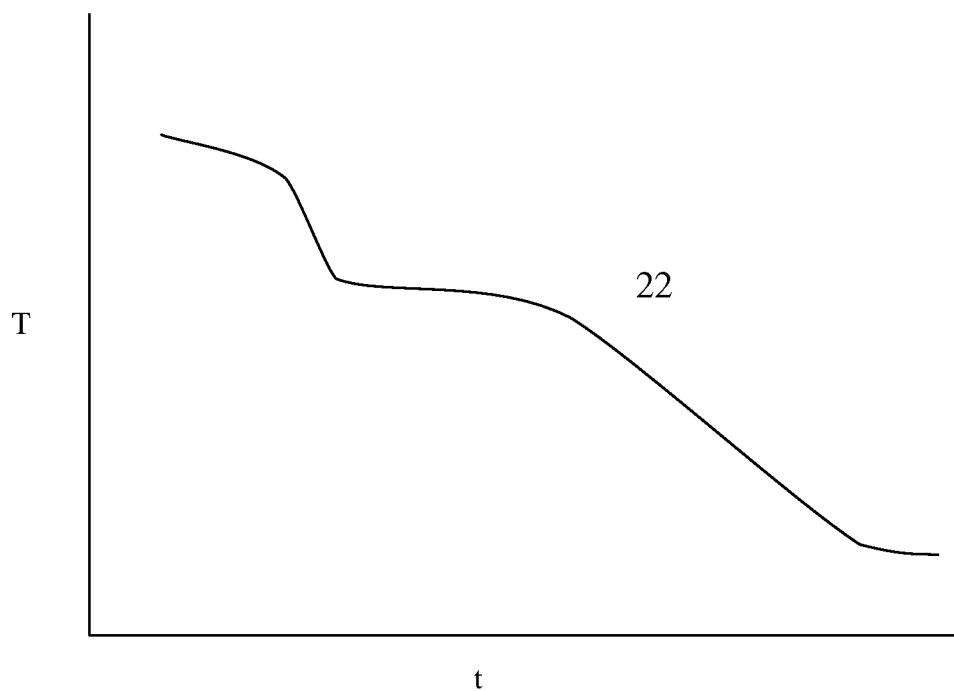


Fig. 4

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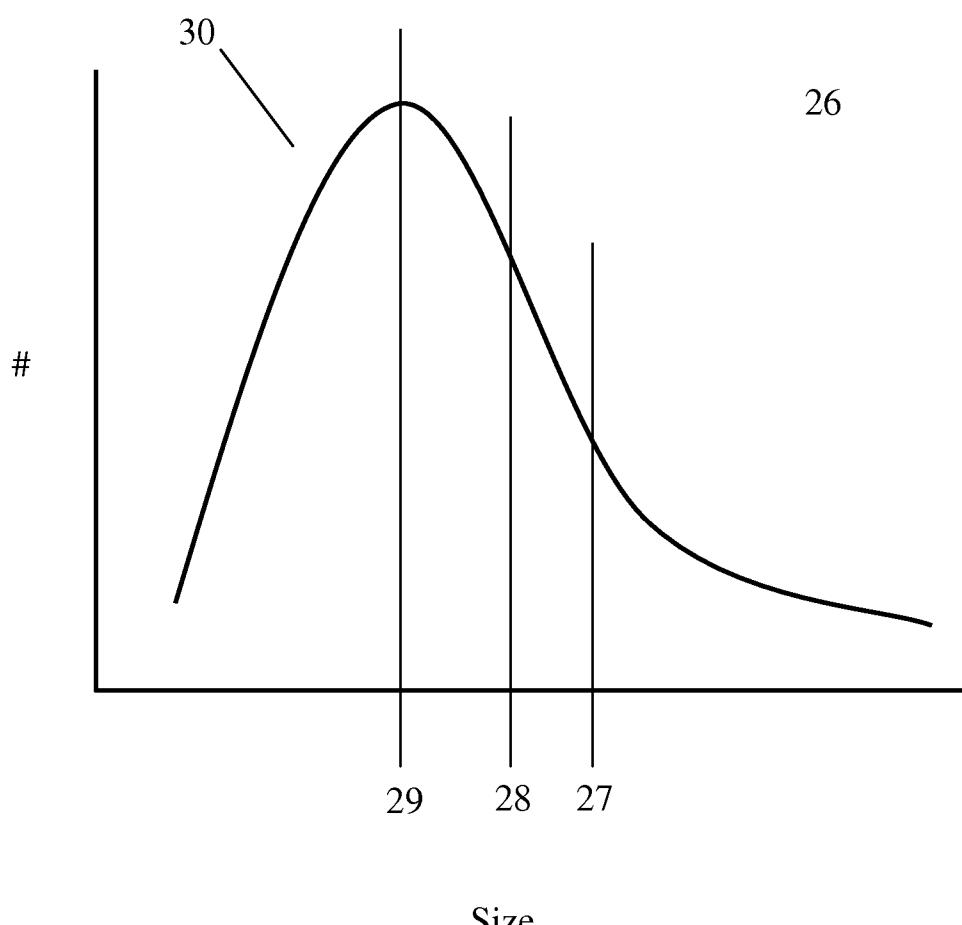


Fig. 5

