

Cell system and method for storing cells

Field of the application

- 5 The present application relates to cell systems comprising nanofibrillar cellulose for storing cells. The present applications also relate to methods for storing cells at hypothermic temperatures. The present application also relates to uses of the cell systems.

10 Background

Stem cells, such as human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs), are self-renewing pluripotent cells that are able to differentiate into many cell types in the body. They hold great promises e.g.
15 for cell therapy, drug research and tissue engineering. Further, it is envisioned that in the future human induced pluripotent stem cells, multipotent cells and other undifferentiated cells will be proliferated and directed to differentiate into specific lineages so as to develop differentiated cells or tissues which can be transplanted into human bodies for therapeutic purposes. Human pluripotent
20 stem cells and the differentiated cells that may be derived from them are also powerful scientific tools for studying human cellular and developmental systems.

The medical applications of cell-based therapies are rapidly increasing.
25 Therefore it is desired to develop systems that enable the storage and transportation of cell therapy products from manufacture site to site of use. Freezing and thawing the cells may damage the cells, lower the amount of viable cells, and is time consuming, complex and expensive. There is need for simpler and inexpensive methods for storing cells for a shorter term, for
30 example during transportation.

Summary

In the present disclosure it was found out that cells, especially stem cells, can
35 be stored in a nanofibrillar cellulose hydrogel at hypothermic temperatures for several days. This can be used for stabilizing the cells for transportation. Herein it is disclosed how to use the nanofibrillar cellulose hydrogel as a matrix

for the cells. The matrix provides support for the cells, but it also provides cytoprotective properties, *i.e.* conditions where the cells remain stable and viable.

- 5 In general hypothermic storage may induce adverse effects to the cells, for example hypoxic stress, ischemia and reperfusion injury, disruptions in membrane potential (redox balance), cellular ionic imbalances such as disturbance of Na^+ , Ca^{2+} , and Fe^{2+} homeostasis, generation of reactive oxygen species, collapse of cytoskeleton, and ultrastructural damage. Especially
10 increased hypothermic storage intervals may result in the activation of necrotic or apoptosis cell death pathways. The solutions presented in this disclosure aim to prevent or alleviate these negative effects.

- 15 In one aspect, the present disclosure refers to a cell system comprising eukaryotic cells, such as mammalian cells, at an undifferentiated state and/or paused state in a hydrogel comprising nanofibrillar cellulose in cell storage medium at a temperature in the range of 1–25°C.

- 20 The present disclosure provides a cell system comprising eukaryotic cells in a hydrogel comprising nanofibrillar cellulose in cell storage medium at a temperature in the range of 0–25°C.

- In another aspect, the present disclosure also provides a method for storing eukaryotic cells, the method comprising
25 -providing eukaryotic cells,
-providing nanofibrillar cellulose,
-providing cell storage medium,
-combining the cells, the nanofibrillar cellulose and the cell storage medium to form a cell system, and
30 -storing the cell system at a temperature in the range of 0–25°C.

- The temperatures used for storing and/or transporting the cells are hypothermic temperatures, which may include ambient temperatures. Hypothermic temperatures as used herein may refer to temperatures which are lower than a
35 body temperature or a temperature used for culturing cells. Hypothermic temperature may refer to a temperature below 37°C, or 30°C or below, or 25°C or below, or even lower temperature. However temperatures below 0°C are not required, which prevents damaging the cells by freezing. As no freezing is carried out, the process is simpler and does not require specific freezing
40 equipment, thawing or for example use of liquid nitrogen. Also no cryoprotectants or lyoprotectants are needed. The cells and the materials are not dried, such as lyophilized, so they do not need to be rehydrated. When

using the hypothermic temperatures the cells remain viable and may be used immediately after releasing from the matrix, or already when bound to the matrix, for example to enable quality control testing. The cells may enter pause state, which helps maintaining them in the cell system, especially maintaining them at a desired state.

In another aspect, the present disclosure also provides an *in vitro* method for providing eukaryotic cells, the method comprising

- providing the cell system as disclosed herein,
- releasing the cells from the hydrogel, such as by enzymatically digesting the hydrogel or by diluting the hydrogel, to provide the cells.

The present disclosure also provides a method for providing eukaryotic cells, the method comprising

- providing the cell system, and
- extracting the cells from the hydrogel, such as by enzymatically digesting the hydrogel or by diluting the hydrogel, to provide the cells.

In another aspect, the present disclosure provides for a use of the cells system as disclosed herein for transporting cells.

In another aspect, the present disclosure provides for the cell system as disclosed herein for use in therapy.

The main embodiments are characterized in the independent claims. Various embodiments are disclosed in the dependent claims. The embodiments and examples recited in the claims and the specification are mutually freely combinable unless otherwise explicitly stated.

The formed cell system enables obtaining a scalable, reproducible and cost-effective cell storage system, wherefrom the cells may be easily released and harvested.

The formed cell system also enables providing a composition for storing cells in an undifferentiated state, *i.e.* to prevent spontaneous or induced differentiation of cells, especially stem cells. Also the proliferation of the cells may be paused or substantially decreased. For example maintaining stem cells in pluripotent or multipotent state is demanding and requires careful control of storing conditions, materials and handling of the cells.

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The nanofibrillar cellulose hydrogel provides a hydrophilic matrix, which is non-toxic, biocompatible and also biodegradable. The matrix can be degraded enzymatically, for example by adding cellulase. On the other hand the hydrogel is stable at physiological conditions, and does not need to be crosslinked by using additional agents. It is also possible to dilute the hydrogel, for example to obtain a dispersion which is no longer in a gel state, which may enable releasing and harvesting the cells, for example by centrifuging or filtering. Use of reactive agents can be avoided, which agents could affect the cells. Cells, especially challenging and/or sensitive cells, can be stored and transported in

Claims:

1. A cell system comprising eukaryotic cells, such as mammalian cells, at an undifferentiated state and/or paused state in a hydrogel comprising nanofibrillar cellulose in cell storage medium at a temperature in the range of 1–25°C.
2. The cell system of claim 1 wherein the cell storage medium comprises zwitterionic buffering agent, such as 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
3. The cell system of claim 1 or 2, wherein the cell storage medium comprises one or more osmotic agent(s), such as glucose, glucose-based polymers, starch, dextran, gelatin, albumin, amino acids, such as glutamine, polypeptides, glycerol, or combinations thereof, preferably wherein the osmotic pressure of the medium is in the range of 250–350 mOsm/kg.
4. The cell system of any of the preceding claims, wherein the cells are stem cells, such as mesenchymal stem cells.
5. The cell system of any of the preceding claims, wherein the nanofibrillar cellulose is in a form of separate bodies, such as beads.
6. The cell system of any of the preceding claims, wherein the nanofibrillar cellulose, when dispersed in water, provides yield stress in the range of 1–50 Pa, such as in the range of 3–15 Pa, determined by rotational rheometer at a consistency of 0.5% (w/w) by weight in aqueous medium at 20°C±1°C, and/or has an average diameter of a fibril of 200 nm or less.
7. The cell system of any of the preceding claims, wherein the concentration of the nanofibrillar cellulose in the hydrogel is in the range of 0.1–10%, such as 0.2–5% (w/w), 0.4–2% (w/w), or 0.8–1.5% (w/w).
8. The cell system of any of the preceding claims, wherein the nanofibrillar cellulose is selected from anionically modified nanofibrillar cellulose, cationically modified nanofibrillar cellulose, unmodified nanofibrillar cellulose,

and oxidized nanofibrillar cellulose, such as TEMPO oxidized nanofibrillar cellulose.

9. A method for storing eukaryotic cells, the method comprising
- 5 -providing eukaryotic cells, such as mammalian cells, preferably stem cells, such as mesenchymal stem cells.
- providing nanofibrillar cellulose,
- providing cell storage medium,
- 10 -combining the cells, the nanofibrillar cellulose and the cell storage medium, and
- storing the cell system at a temperature in the range of 1–25°C to form the cell system of any of the preceding claims.
10. The method of claim 9, comprising storing the cell system at a
- 15 temperature in the range of 15–25°C, such as 18–23°C.
11. The method of any of the claims 9–10, comprising storing the cells in the cell system for at least 24 hours, or at least 56 hours, such as 24–56, 24–72 or 24–168 hours.
- 20 12. An *in vitro* method for providing eukaryotic cells, the method comprising
- providing the cell system of any of the claims 1–8,
- releasing the cells from the hydrogel, such as by enzymatically digesting the hydrogel or by diluting the hydrogel, to provide the cells.
- 25 13. The method of claim 12, comprising digesting the hydrogel enzymatically with one or more cellulase(s), preferably by using a dose of 500–1500 µg enzyme/mg gel, more preferably 1000–1300 µg/mg, such as for 1–4 hours, preferably 1.5–2.5 hours.
- 30 14. Use of the cells system of any of the claims 1–8 for transporting cells.
15. The cell system of any of the claims 1–8 for use in therapy.