

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.

In another aspect, the present disclosure refers to a method for storing eukaryotic cells, the method comprising

- 35 -providing eukaryotic cells,
-providing nanofibrillar cellulose,
-providing cell storage medium,
-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
40 system of any of the preceding claims,
wherein the eukaryotic cells are at an undifferentiated state and/or paused state.

2a

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 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 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.

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 eukaryotic cells are mammalian cells.

3.2. The cell system of claim 1, wherein the cell storage medium comprises zwitterionic buffering agent, ~~such as 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.~~

4.3. The cell system of any of claims 1-3 or 2, wherein the cell storage medium comprises one or more osmotic agent(s) selected from, ~~such as~~ glucose, glucose-based polymers, starch, dextran, gelatin, albumin, amino acids, ~~such as glutamine~~, polypeptides, glycerol, ~~and/or~~ combinations thereof, ~~preferably wherein the osmotic pressure of the medium is in the range of 250–350 mOsm/kg.~~

5.4. The cell system of any of the preceding claims, wherein the cells are stem cells, ~~such as mesenchymal stem cells.~~

6. The cell system of claim 5, wherein the stem cells are mesenchymal stem cells.

5.7. The cell system of any of the preceding claims, wherein the nanofibrillar cellulose is in a form of separate bodies, ~~such as beads.~~

8.6. 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.

97. 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).~~

5 10. The cell system of claim 9, wherein the concentration of the nanofibrillar cellulose in the hydrogel is in the range selected from the group consisting of 0.2–5% (w/w), 0.4–2% (w/w), or 0.8–1.5% (w/w).

10 118. 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.~~

15 12. The cell system of claim 11, wherein the oxidized nanofibrillar cellulose is TEMPO oxidized nanofibrillar cellulose.

20 139. A method for storing eukaryotic cells, the method comprising
 -providing eukaryotic cells, ~~such as mammalian cells, preferably stem cells, such as mesenchymal stem cells.~~
 -providing nanofibrillar cellulose,
 -providing cell storage medium,
 -combining the cells, the nanofibrillar cellulose and the cell storage medium, and
 25 -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,
wherein the eukaryotic cells are at an undifferentiated state and/or paused state.

30 14. The method of claim 13, wherein the eukaryotic cells are mammalian cells.

15. The method of claim 14, wherein the mammalian cells are stem cells.

35 16. The method of claim 15, wherein the stem cells are mesenchymal stem cells.

170. The method of any of the claim 13-169, comprising storing the cell system at a temperature in the range of 15–25°C, ~~such as 18–23°C~~.

- 5 181. The method of any of the claims 13-179-10, 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~~.

- 10 1912. An *in vitro* method for providing eukaryotic cells, the method comprising
 -providing the cell system of any of the claims 1–128,
 -releasing the cells from the hydrogel, ~~such as by enzymatically digesting the hydrogel or by diluting the hydrogel~~, to provide the cells.

- 15 20. The method of claim 19, wherein the step of releasing the cells from the hydrogel comprises enzymatically digesting the hydrogel or diluting the hydrogel.

- 20 2113. The method of claim 2012, 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~~.

- 25 2214. Use of the cells system of any of the claims 1–128 for transporting cells.

2315. The cell system of any of the claims 1–128 for use in therapy.