The present inventors describe for the first time the physical and biocompatibility properties of plant derived CNF hydrogel. Plant cellulose is extensively used in the paper and textile industry and is abundant naturally. The native cellulose nanofiber hydrogel is opaque. Chemical modification of cellulose pulp prior to mechanical disintegration gives rise to optically transparent hydrogels.

The present invention is based on experimental studies on hydrogels composed of cellulose nanofibers (CNF), which are dispersed in aqueous environment. The nanofibers are highly hydrophilic due to hydroxyl functionalities of cellulose polymers and partly covered with hemicellulose polysaccharides.

Accordingly the present invention provides as a first aspect a cell culture or cell delivery composition comprising cellulose nanofibers or a derivative thereof, wherein the cellulose nanofiber in a form of a hydrogel or membrane.

A significant advantage of the present invention is that cells can be maintained (and proliferated) on or in the biomaterial media without animal or human based chemicals originating outside the cells. The cells are evenly dispersed on or in the media/matrix containing cellulose nanofibers or a derivative thereof. Cells divide on or in the media, start to proliferate and the cell clusters start to grow spontaneously without the accumulation of cells on the bottom of the cell culture platform. The homogenous dividing of the cells in the cellulose nanofibers or a derivative thereof is a prerequisite for the biomaterial to function as 3D cell culture media.

Further advantages of the present invention include: cellulose nanofibers and/or derivatives thereof are inert and give no fluorescent background. The media comprising cellulose nanofibers or a derivative thereof can be injected. Injectability is explained by the rheological properties. The injection can be performed so that the cells stay stable inside matrix and they are homogeneously distributed in the matrix after injection.

SUMMARY

In accordance with a first aspect, there is provided a cell culture or cell delivery composition, characterized in that the composition comprises plant derived, mechanically disintegrated cellulose nanofibers and/or derivatives thereof, in a form of a hydrogel or membrane in wet state.

In accordance with another aspect, there is provided a cell culture or cell delivery matrix, characterized in that the matrix comprises living cells and a composition as disclosed herein, forming a hydrogel and the cells are present in said matrix in a three-dimensional or two-dimensional arrangement.

In accordance with another aspect, there is provided a method for producing a composition as disclosed herein, characterized in that said method comprises the steps of
- providing plant derived, mechanically disintegrated cellulose nanofibers and/or derivatives thereof,
- mixing together said cellulose nanofibers and/or derivatives thereof with water.

In accordance with another aspect, there is provided a method for removing plant based cellulose nanofibers and/or derivatives thereof from a cell culture material, characterized in that the method comprises the steps of
- providing a material comprising cell culture medium and cells;
- diluting said material with aqueous or non-aqueous liquid;
- optionally centrifuging the material to sediment the cells and cell aggregates;
- removing the cellulose nanofibers by decantation.

In accordance with another aspect, there is provided a method for removing plant based cellulose nanofibers and/or derivatives thereof from a cell culture material, characterized in that the method comprises the steps of
- providing a material comprising cell culture medium and cells;
-contacting the cell culture material with a degrading enzyme;
- optionally centrifuging the material to sediment the cells and cell aggregates;
- removing the cellulose nanofibers by decantation.

In accordance with another aspect, there is provided microbiological use of plant derived, mechanically disintegrated cellulose nanofibers and/or derivatives thereof or the composition as disclosed herein for laboratory and/or industrial purposes as a medium or a compound of a media for maintaining cell *in vitro*.

In accordance with another aspect, there is provided use of the composition as disclosed herein for immobilizing cells or enzymes.

In accordance with another aspect, there is provided a method of culturing cells, characterized in that the method comprises the steps of

- providing cells;
- contacting the cells with a composition as disclosed herein to form a matrix;
- culturing the cells within said matrix in a three-dimensional or two-dimensional arrangement.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts cryo-TEM images of cellulose nanofiber hydrogels. Native CNF is on the left side (A) and transparent CNF is on the right side (B).

Figure 2 depicts viability of HepG2 cells in commercial cell culture materials [MaxGel™ (Sigma-Aldrich), HydroMatrix™ (Sigma-Aldrich) and PuraMatrix™ (3DM Inc.)], in two different cellulose nanofiber materials (native CNF and transparent CNF) and in CNF in which fibronectin (FN) was added. In proliferation AB assay, cells

CLAIMS

1. A cell culture or cell delivery composition, **characterized** in that the composition comprises plant derived, mechanically disintegrated cellulose nanofibers and/or derivatives thereof, in a form of a hydrogel or membrane in wet state.

2. The composition according to claim 1, **characterized** in that the diameter of cellulose nanofibers or nanofiber bundles in the cellulose nanofibers and/or derivatives thereof is less than 1 µm, preferably less than 200 nm, more preferably less than 100 nm.

3. The composition according to claim 1 or 2, **characterized** in that the cellulose nanofibers and/or derivatives thereof comprise chemically or physically modified derivates of a cellulose nanofiber or nanofiber bundles.

4. The composition according to any one of claims 1 - 3, **characterized** in that the mechanically disintegrated cellulose nanofibers are acid-base pretreated.

5. The composition according to any one of the claims 1 - 4, **characterized** in that the composition further comprises additives selected from the group consisting of extra cellular matrix components, serum, growth factors and proteins.

6. A cell culture or cell delivery matrix, **characterized** in that the matrix comprises living cells and a composition according to any one of the claims 1 – 5, forming a hydrogel and the cells are present in said matrix in a three-dimensional or two-dimensional arrangement.

7. The matrix according to claim 6, **characterized** in that the cells are eukaryotic cells or prokaryotic cells.

8. A method for producing a composition according to any one of the claims 1 – 5, **characterized** in that said method comprises the steps of
   - providing plant derived, mechanically disintegrated cellulose nanofibers and/or derivatives thereof,
   - mixing together said cellulose nanofibers and/or derivatives thereof with water.

9. The method according to claim 8, **characterized** in that the method further comprises combining the mixture with a suitable medicament.

10. The method according to claim 8 or 9, **characterized** in that the mechanically disintegrated cellulose nanofibers are acid-base pretreated.

11. A method for removing plant based cellulose nanofibers and/or derivatives thereof from a cell culture material, **characterized** in that the method comprises the steps of
- providing a material comprising cell culture medium and cells;
- diluting said material with aqueous or non-aqueous liquid;
- optionally centrifuging the material to sediment the cells and cell aggregates;
- removing the cellulose nanofibers by decantation.

12. A method for removing plant based cellulose nanofibers and/or derivatives thereof from a cell culture material, **characterized** in that the method comprises the steps of
- providing a material comprising cell culture medium and cells;
- contacting the cell culture material with a degrading enzyme;
- optionally centrifuging the material to sediment the cells and cell aggregates;
- removing the cellulose nanofibers by decantation.

13. Microbiological use of plant derived, mechanically disintegrated cellulose nanofibers and/or derivatives thereof or the composition according to any one of claims 1 - 5 for laboratory and/or industrial purposes as a medium or a compound of a media for maintaining cells *in vitro*.

14. Use of the composition according to any one of claims 1 - 5 for immobilizing cells or enzymes.

15. A method of culturing cells, **characterized** in that the method comprises the steps of
- providing cells;
- contacting the cells with a composition according to any of claims 1 to 5 to form a matrix;
- culturing the cells within said matrix in a three-dimensional or two-dimensional arrangement.

16. The method according to claim 15, **characterized** in that before the culture the matrix is transferred or placed to an environment for cell culture or for cell delivery.

17. The composition, method or use according to one or more of the preceding claims, wherein the cells are eukaryotic cells.

18. The composition, method or use according to one or more of the preceding claims, wherein the cells are prokaryotic cells.

19. The composition, method or use according to one or more of the preceding claims, wherein the cells are stem cells.