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(71) Demandeur/Applicant:
SPIBER TECHNOLOGIES AB, SE

(72) Inventeurs/Inventors:
HEDHAMMAR, MY, SE;
WIDHE, MONA, SE;
JOHANSSON, ULRIKA, SE

(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : CELLULES INTEGREES
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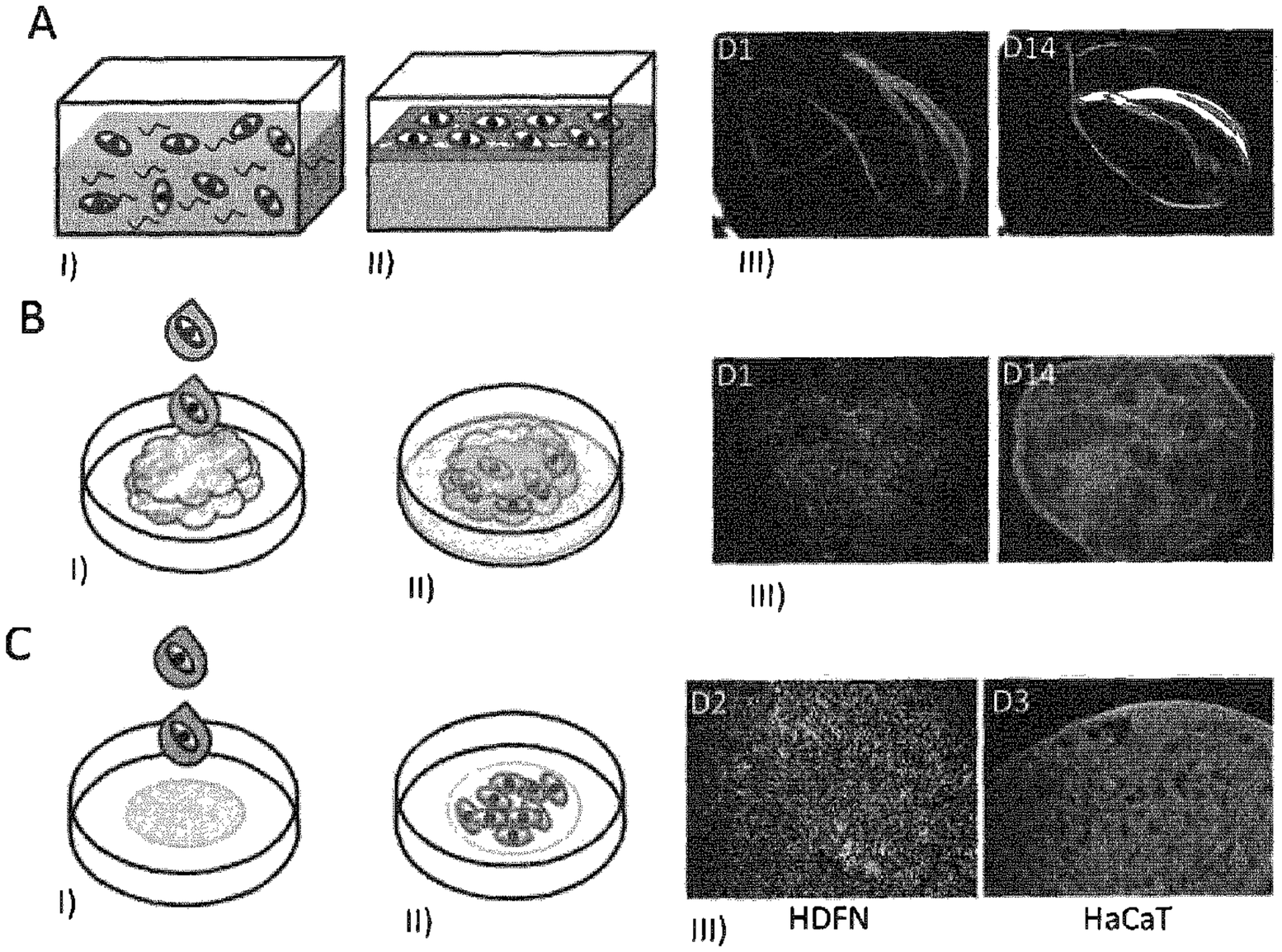
Fig 3



(57) Abrégé/Abstract:
A cell scaffold material is manufactured by providing an aqueous solution of a silk protein capable of assembling into a water-insoluble macrostructure. The silk protein is mixed with eukaryotic cells, and the silk protein is assembled into a water-insoluble macrostructure in the presence of the cells, thereby forming a scaffold material for cultivating the cells. The cells can be grown integrated with the scaffold material under conditions suitable for cell culture.

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(71) Applicant: **SPIBER TECHNOLOGIES AB** [SE/SE]; KTH Avd Proteinteknologi Alba Nova Univers Centrum, 106 91 Stockholm (SE).

(72) Inventor: **HEDHAMMAR, My**; Odengatan 40, 113 51 Stockholm (SE).

(74) Agent: **AWAPATENT AB**; Box 45086, 104 30 Stockholm (SE).

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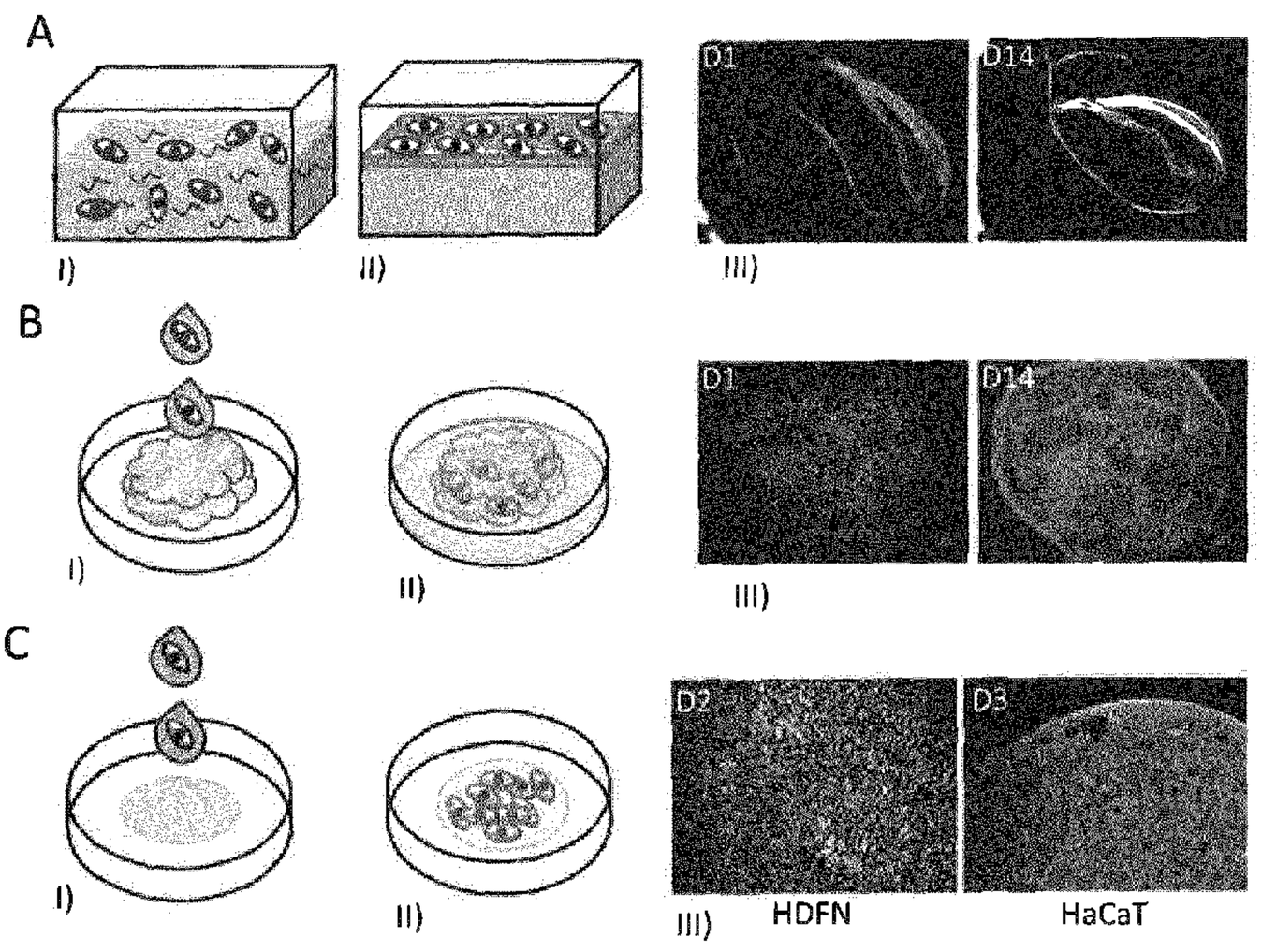

(57) Abstract: A cell scaffold material is manufactured by providing an aqueous solution of a silk protein capable of assembling into a water-insoluble macrostructure. The silk protein is mixed with eukaryotic cells, and the silk protein is assembled into a water-insoluble macrostructure in the presence of the cells, thereby forming a scaffold material for cultivating the cells. The cells can be grown integrated with the scaffold material under conditions suitable for cell culture.

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CLAIMS

1. A method for the cultivation of eukaryotic cells, comprising the steps:

(a) providing an aqueous solution of a silk protein capable of assembling into a water-insoluble macrostructure, wherein the silk protein optionally contains a cell-binding motif;

(b) preparing an aqueous mixture of a sample of the eukaryotic cells with the silk protein, wherein thesilk protein remains dissolved in the aqueous mixture;

(c) allowing the silk protein to assemble into a water-insoluble macrostructure in the presence of the eukaryotic cells, thereby forming a scaffold material for cultivating the eukaryotic cells; and

(d) maintaining the eukaryotic cells within the scaffold material under conditions suitable for cell culture.

2. A method according to claim 1, wherein the macrostructure is brought into a shape selected from fiber, foam, film, fiber mesh, capsules and nets, preferably fiber or foam.

3. A method according to any one of claims 1-2, wherein the eukaryotic cells are selected from mammalian cells, preferably selected from primary cells and cell lines, such as endothelical cells, fibroblasts, keratinocytes, skeletal muscle satellite cells, skeletal muscle myoblasts, Schwann cells, pancreatic β-cells, pancreatic islet cells, hepatocytes and glioma-forming cells; and stem cells, such as mesenchymal stem cells; or a combination of at least two different mammalian cell types.

4. A method according to any one of claims 1-3, wherein the silk protein is a fibroin, such as a silkworm fibroin.

5. A method according to any one of claims 1-3, wherein the silk protein is a spider silk protein.

6. A method according to claim 5, wherein the spider silk protein is comprising, or consisting of, the protein moieties **REP** and **CT**, wherein

REP is a repetitive fragment of from 70 to 300 amino acid residues, selected from the group consisting of **L(AG)$_n$L**, **L(AG)$_n$AL**, **L(GA)$_n$L**, and **L(GA)$_n$GL**, wherein

n is an integer from 2 to 10;

each individual **A** segment is an amino acid sequence of from 8 to 18 amino acid residues, wherein from 0 to 3 of the amino acid residues are not Ala, and the remaining amino acid residues are Ala;

each individual **G** segment is an amino acid sequence of from 12 to 30 amino acid residues, wherein at least 40% of the amino acid residues are Gly; and

each individual **L** segment is a linker amino acid sequence of from 0 to 30 amino acid residues; and

CT is a fragment of from 70 to 120 amino acid residues, having at least 70% identity to SEQ ID NO: 3 or SEQ ID NO: 68;

and wherein the optional cell-binding motif is arranged either terminally in the spider silk protein, or between the moieties, or within any of the moieties, preferably terminally in the spider silk protein.

7. A method according to any one of claims 1-6, wherein the silk protein contains a cell-binding motif, such as a cell-binding motif selected from RGD, IKVAV (SEQ ID NO: 10), YIGSR (SEQ ID NO: 11), EPDIM (SEQ ID NO: 12), NKDIL (SEQ ID NO: 13), GRKRK (SEQ ID NO: 14), KYGAASIKVAVSADR (SEQ ID NO: 15), NGEPRGDTYRAY (SEQ ID NO: 16), PQVTRGDVFTM (SEQ ID NO: 17), AVTGRGDSPASS (SEQ ID NO: 18), TGRGDSPA (SEQ ID NO: 19), CTGRGDSPAC (SEQ ID NO: 20) and FN_{cc} (SEQ ID NO: 9); and preferably from FN_{cc}, GRKRK, IKVAV, RGD and CTGRGDSPAC, more preferably FN_{cc} and CTGRGDSPAC;

wherein FN_{cc} is $C^1X^1X^2RGDX^3X^4X^5C^2$;

wherein each of X^1, X^2, X^3, X^4 and X^5 are independently selected from natural amino acid residues other than cysteine; and C^1 and C^2 are connected via a disulphide bond.

8. A process for manufacturing a cell culture product comprising (i) a scaffold material for cultivating eukaryotic cells; and (ii) eukaryotic cells, which are growing integrated with the scaffold material, comprising the steps:

(a) providing an aqueous solution of a silk protein capable of assembling into a water-insoluble macrostructure, wherein the silk protein optionally contains a cell-binding motif;

(b) preparing an aqueous mixture of a sample of the eukaryotic cells with the silk protein, wherein the silk protein remains dissolved in the aqueous mixture; and

(c) allowing the silk protein to assemble into a water-insoluble macrostructure in the presence of the eukaryotic cells, thereby forming the scaffold material for cultivating the eukaryotic cells.

9. A process for manufacturing a cell culture product according to claim 8;
wherein the macrostructure is as defined in claim 2; and/or
wherein the eukaryotic cells are as defined in claim 3; and/or
wherein the silk protein is as defined in any one of claims 4-7.

10. A cell culture product comprising (i) a scaffold material for cultivating eukaryotic cells, which is a water-insoluble macrostructure of a silk protein capable of assembling into a water-insoluble macrostructure, wherein the silk protein optionally contains a cell-binding motif; and (ii) eukaryotic cells, which are growing integrated with the scaffold material.

11. A cell culture product according to claim 10, obtainable or obtained by the process according to any one of claims 8-9.

12. Use of a silk protein capable of assembling into a water-insoluble macrostructure in the formation of a scaffold material for cultivating eukaryotic cells in the presence of said cells; wherein the scaffold material is a water-insoluble macrostructure of the silk protein; and wherein the silk protein optionally contains a cell-binding motif.

13. Use according to claim 12, wherein the macrostructure is as defined in claim 2; and/or
wherein the eukaryotic cells are as defined in claim 3; and/or
wherein the silk protein is as defined in any one of claims 4-7.