(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 December 2014 (18.12.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/200997 A3

(51) International Patent Classification:
C12Q 1/02 (2006.01) *C12N 5/02* (2006.01)
C12N 5/00 (2006.01)

(21) International Application Number:
PCT/US2014/041703

(22) International Filing Date:
10 June 2014 (10.06.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/833,052 10 June 2013 (10.06.2013) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

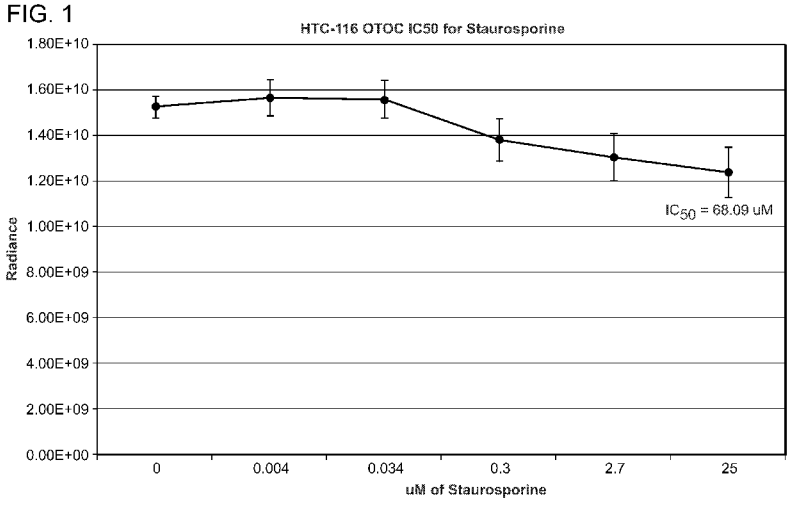
— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
26 February 2015

(54) Title: METHOD FOR PREPARING THREE-DIMENSIONAL, ORGANOTYPIC CELL CULTURES AND USES THEREOF

FIG. 1



(57) Abstract: The present invention is directed to methods for generating isolated, unencapsulated, three-dimensional, cell culture products comprising a naturally-derived cell matrix distributed throughout the product and having dimensions suitable for use in *in vitro* applications including histological applications and imaging.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/02, C12N 5/00, C12N 5/02 (2014.01)
CPC - C12Q 1/04, C12N 5/0031, C12N 2531/00, A61K 38/00, A61K 35/12
According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/02, C12N 5/00, C12N 5/02 (2014.01)
CPC: C12Q 1/04, C12N 5/0031, C12N 2531/00, A61K 38/00, A61K 35/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/29, 435/383, 435/325, 435/403, 435/375, 435/378

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: Tissue, model, unencapsulated, isolated, suspen*, encapsulated, cell culture product, matrix, matural matrix, collagen, transplant, three-dimensional, cell culture, diameter, 5 mm, 1.5 mm, width, 3 mm, vessel, culture vessel, bioculture, oganotypic, plurality, culture chamber

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0193421 A1 (KRUSE et al.) 14 August 2008 (14.08.2008) abstract, para [0011]-[0013], [0028]-[0030], [0039], [0040], [0080], [0090]	1-4
Y	US 7,115,257 B1 (TAO et al.) 03 October 2006 (03.10.2006) abstract, col 15, ln 60-64	1-41
Y	US 2007/0116680 A1 (STEGEMANN et al.) 24 May 2007 (24.05.2007) claims 3, 18, para [0002], [0021], [0023], [0029], [0032], [0033], [0035], [0040], [0044], [0047], [0048], [0053], [0054], [0055]	5-41
Y	WO 2006/022671 A1 (MIZUNO et al.) 02 March 2006 (02.03.2006) pg 12, ln 1-9, pg 18, ln 14-19, pg 26, ln 2-16	6, 7
Y	US 2011/0053185 A1 (RUSTGI et al.) 03 March 2011 (03.03.2011) Claim 29, abstract, para [0041], [0047], [0048], [0085], [0089], [0092]	13, 14, 23-34
Y	US 2005/0260745 A1 (DOMANSKY et al.) 24 November 2005 (24.11.2005) para [0012], [0013], [0044], [0045], [0053], [0065], [0067], [0068], [0070], [0072], [0078]	35-41

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 December 2014 (10.12.2014)	19 DEC 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-4, directed to an isolated, unencapsulated, three-dimensional, cell culture product.

Group II, claims 5-41, directed to a method for producing an isolated, unencapsulated, three-dimensional oganotypic cell culture product.

********** See Supplemental Sheet to continue **********

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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Continuation of Box No. III, Observations where unity of invention is lacking:

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I has the special technical feature of a cell culture product having dimensions ranging from about 1-10 mm in diameter (length), and about 1-5 mm in width (thickness), that is not required by Group II.

Group II has the special technical feature of a method for producing a cell culture product, the method comprising the steps of: a. harvesting one or more cells from an in vitro culture; b. resuspending the one or more cells with a naturally derived gel matrix under conditions sufficient to form a liquid cell suspension; c. dispensing at least a portion of the liquid cell suspension directly into a hydrophobic solution under conditions sufficient to enable the liquid cell suspension to form a gelled three-dimensional cell matrix within the hydrophobic solution; d. isolating the three-dimensional cell matrix from the hydrophobic solution; and e. culturing the three-dimensional cell matrix in a growth medium under conditions sufficient for promoting proliferation of the cells within the three dimensional cell matrix, thereby producing an unencapsulated, three dimensional organotypic culture, that is not required by Group I.

Common technical features:
Group I (a cell culture product) is related to Group II (a method for making a cell culture product), and share the common technical feature of an isolated, unencapsulated, three-dimensional, cell culture product comprising a naturally-derived cell matrix distributed throughout the product. However, these shared technical features do not represent a contribution over prior art, because these shared technical features are obviated by US 2008/0193421 A1 to Kruse et al., (hereinafter Kruse), in view of US 7,115,257 B1 to Tao et al., (hereinafter Tao). Kruse teaches an isolated, three-dimensional, cell culture product (abstract, "The invention relates to methods for producing three-dimensional multicellular tissue and organ culture systems using multicellular mammalian cell aggregates", para [0011], "The adult stem cells obtained from such sources can be readily isolated and maintained in a stable long-time culture"), comprising a naturally-derived [e. g. Biological material] cell matrix distributed throughout the product (para [0028], "the tissue or organ culture system according to the invention comprises a physiologically compatible matrix or a physiologically compatible carrier system for the cells. It is especially preferable if the matrix or the carrier system can be degraded in the body.", para [0029] "A special embodiment concerns sterilized acellular, that is, cell-free, biological material, e.g., small intestine submucosa (SIS) or a natural collagen - or chitin/chitosan matrix.", para [0030] "The matrix or the carrier system preferably has a defined form ...The form can ... be advantageous for the preservation of a certain three-dimensional cell arrangement and/or for the supplying of the cells on or in the matrix"),
Kruse does not expressly teach that the cell aggragetes are unencapsulated, however, therapeutic use of both encapsulated and uncapsulated cells, along with encapsulation of cells is known in the art, such as taught by Tao (abstract, 'ARPE-19 cells were evaluated as a platform cell line for encapsulated and unencapsulated cell-based delivery technology.", col 15, ln 60-64 "Encapsulated cell therapy is based on the concept of isolating cells from the recipient host's immune system by surrounding the cells with a semipermeable biocompatible material before implantation within the host."). Kruse further teaches culturing of cell aggragtes (para [0012] "These adult stem cells can be stimulated in a simple manner to differentiate without the addition of special growth factors or differentiation factors in that they are cultivated under spatial conditions that ensure a three-dimensional contact of the cells. In a preferred embodiment these conditions are the cultivation in hanging drops", para [0013] "Under these conditions three-dimensional cell compounds or cell aggregates spontaneously develop that have been referred to as "organoid bodies"...These organoid bodies can be transferred into suspension cultures or adhesion cultures and further cultivated."). An artisan of ordinary skill would have readily appreciated that Kruse's cell aggregates and embryoid bodies are not encapsulated as understood in the art: they spontaneously form in drops of medium, and can be transferred to other culture modes, and the matrix in which they are cultured is preferably degraded in the body. Thus, it would be obvious to make unencapsulated 3-D cell cultures based on the cell cultures of Kruse.

Therefore, Groups I and II lack unity of invention under PCT Rule 13.