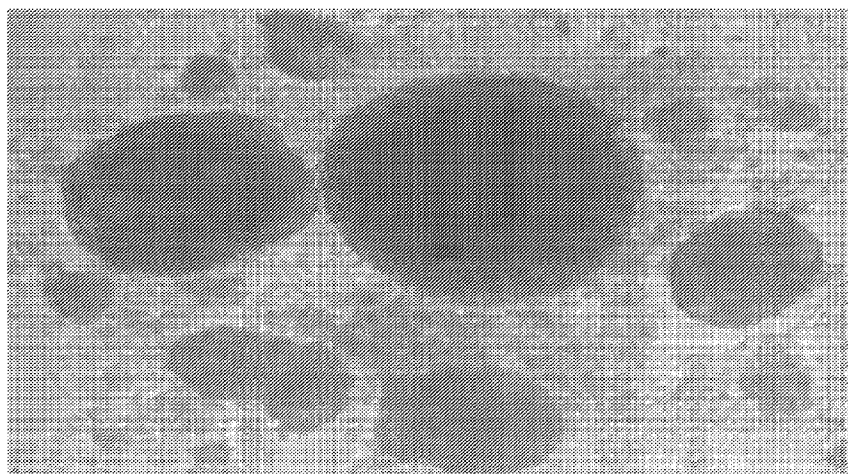




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(54) **Title:** CARDIOVASCULAR CELL CO-CULTURE MEDIUM AND METHOD OF GROWING MULTIPLE CARDIOVASCULAR CELL TYPES

FIG. 1



(57) **Abstract:** Described herein is a unique cell culture media that allows optimal growth of multiple cell types in the same system such as an organ-on-chip or organoid. Also described herein is use of the cell culture media in growing multiple cardiovascular cell types simultaneously in a single device or producing induced pluripotent stem cell (iPSC)-derived cardiac organoids containing iPSC derived-endothelial cells (iPSC-ECs) and iPSC derived- cardiomyocytes (iPSC-CMs). In some embodiments, a formulation for the cell culture media includes a base medium including glucose, a pH indicator, salts, amino acids, and vitamins; recombinant human albumin; L-ascorbic acid 2-phosphate; and at least one or more of insulin, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), a synthetic analogue of insulin-like growth factor (IGF)-I, heparin, or hydrocortisone.





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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/60137

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.: 24-26, 28-29, 32-44, 47-57
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:
---See Supplemental Box ---

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.:
1-19

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

International application No.

PCT/US 23/60137

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - INV. C12N 5/077, A61K 35/34 (2023.01)
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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)
 See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2019/0117701 A1 (ROHTO PHARMACEUTICAL CO.) 25 April 2019 (25.04.2019); abstract; para [0037], [0045], [0048], [0054], [0072], [0099], [0117], [0120], [0122], [0125]-[0128], [0191], [0200], [0210]	1-13, 19 ----- 14-18
Y	CORNING, RPMI 1640 Formulation. 2018 [online]. [Retrieved on 05 April 2023]. Retrieved from the internet: <URL: https://www.corning.com/catalog/cls/documents/formulations/CLS-CG-FM-031.pdf >; entire document	3-6, 8-11
Y	US 2016/0340652 A1 (THE USA, AS REPRESENTED BY THE SECRETARY, DEPT. OF HEALTH AND HUMAN SERVICES) 24 November 2016 (24.11.2016); abstract; para [0049]	14-18
Y	ERMAK et al., Quantitation of LONG@R3 IGF-I during production and purification of recombinant proteins from cell culture. 18 February 2019 [online]. [Retrieved on 06 April 2023]. Retrieved from the internet: <URL: http://www.cellsciences.com/PDF/Whitepaper_LongR3IGF1ELISA.pdf >; abstract	16-18

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"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

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Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/60137

Box No. III Observations where unity of invention is lacking

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 searched, the appropriate additional search fees must be paid.

Group I, claims 1-19, directed to a media formulation.

Group II, claims 20-23, 27, 30-31, 45-46, directed to a method of growing multiple cardiovascular cell types in a device, and producing induced pluripotent stem cell (iPSC)-derived cardiac organoids containing iPSC-derived endothelial cells (iPSC-ECs), iPSC-derived cardiac fibroblasts (iPSC-CFs), and iPSC-derived cardiomyocytes (iPSC-Cms).

The inventions listed as Groups I-II do not relate to a single special technical feature 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 media formulation, that is not required by Group II.

Group II has the special technical feature of a method of growing multiple cardiovascular cell types in a device, and producing induced pluripotent stem cell (iPSC)-derived cardiac organoids containing iPSC-derived endothelial cells (iPSC-ECs), iPSC-derived cardiac fibroblasts (iPSC-CFs), and iPSC-derived cardiomyocytes (iPSC-Cms), that is not required by Group I.

Common Technical Features:

Groups I-II share the common technical feature of:

a formulation comprising:

a base medium comprising glucose, a pH indicator, salts, amino acids, and vitamins;

recombinant human albumin;

L-ascorbic acid 2-phosphate; and

at least one or more of insulin, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), a synthetic analogue of insulin-like growth factor (IGF)-I, heparin, or hydrocortisone.

However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is anticipated by US 2019/0117701 A1 to Rohto Pharmaceutical Co. (hereinafter "Rohto").

Rohto discloses a formulation (abstract - "The present invention relates to a medium for culturing stem cells") comprising: a base medium comprising glucose (para [0099] - "The growth promoter of the invention may further contain, for example, a physiological isotonic solution [an isotonic solution containing physiological saline, any of the above-mentioned minimal essential media, glucose, or another adjuvant]"), a pH indicator (para [0048] - "Examples of the minor components include components generally used as medium components such as glutathione, hypoxanthine, lipoic acid, linolenic acid, phenol red"), salts, amino acids, and vitamins (para [0037] - "The minimal essential medium for stem cells preferably contains one or more types of saccharides, one or more types of inorganic salts, one or more types of amino acids, one or more types of vitamins"); recombinant human albumin (para [0117] - "A medium obtained by adding and dissolving separate components shown in the following Table 1 sterilized through a 0.22 m filter, 10 g/mL insulin (manufactured by Sigma-Aldrich Co. LLC.), 5 g/mL apotransferrin (manufactured by Sigma-Aldrich Co. LLC.), and oleic acid-conjugated recombinant human serum albumin"); L-ascorbic acid 2-phosphate (para [0045] - "Examples of the derived materials of ascorbic acid include ascorbic acid 2-phosphate"); and at least one or more of insulin (para [0072] - "Further, to the medium of the invention, insulin, transferrin, albumin, a lipid group, 2-mercaptoethanol, ethanolamine, sodium selenite, a nucleic acid, a nonessential amino acid, or the like may be added as needed"), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) (para [0120] - "a medium obtained by adding 2 ng/mL activin A (manufactured by Wako Pure Chemical Industries, Ltd.) and 5 ng/mL bFGF"), a synthetic analogue of insulin-like growth factor (IGF)-1 (para [0054] - "IGF-1 or the like that is a factor belonging to the insulin-like growth factor (IGF) family").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

Continuation of Item 4 above: claims 24-26, 28-29, 32-44, 47-57 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).