PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	18.08.2015
Applicant's or agent's file reference P150287WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP2015/064524	20.05.2015	20.05.2014

International Patent Classification (IPC) or both national classification and IPC
C12M1/00(2006.01)i

Applicant
THE UNIVERSITY OF TOKYO

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/JP	Date of completion of this opinion	Authorized officer
Facsimile No.		Telephone No.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2015/064524

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into _____ , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☐ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☐ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2015/064524

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-25	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	1-25	NO
Industrial applicability (IA)	Claims	1-25	YES
	Claims		NO

2. Citations and explanations:

○ This opinion is presented on the basis of the disclosures in the following documents cited in the ISR.

Document 1: LEE, K. H., et al., small, 2009, 5(11), pp. 1264-1268

Document 2: 尾上 弘晃ら, 生物工学会誌 25 April 2014, 92(4), pp. 161-165 (ONOE, Hiroaki, et al., Seibutsu Kogaku Kaishi)

Document 3: WO 2011/046105 A1 (THE UNIVERSITY OF TOKYO) 21 April 2011

Document 4: ONOE, H., et al., Nature Materials, 2013, 12, pp. 584-590

A. The invention as in claims 1-25 does not involve an inventive step in the light of documents 1-4.

Document 1 indicates that hollow microfibers having a layer that contains cells are fabricated by respectively forming by injection (i) a laminar flow of a medium used in cell culturing or a calcium chloride solution, (ii) a laminar flow of an alginate solution containing cells that cover the outside of the laminar

flow in (1) above, and (iii) a laminar flow of a calcium chloride solution that covers the outside of the laminar flow in (ii) above, and gelating the alginate solution together with the cells, and discloses as a specific experimental example the fabrication of hollow microfibers and the culturing of cells using vascular endothelial cells (entire text, in particular, fig. 1-4).

When the invention as in claims 1-25 is compared with the invention disclosed in document 1 here, the two differ in that an outer shell layer is formed in the former, whereas the latter does not include wording related to an outer shell layer, and the former sets forth that a cell layer covers the internal circumference of a cell adhesion layer, whereas the latter forms a layer in a state where the cells are mixed together with the gel. Furthermore, the former also differs from the latter in terms of the components of the hydrogel that forms the cell adhesion layer and the outer shell layer, the type of cells used, and the details of the method of fabricating the microfibers.

The above-mentioned differences will be considered below.

Document 2 indicates that hydrogel fibers formed using laminar flows are disadvantageous in that cells are unable to adhere due to the characteristics of the calcium alginate gel, and therefore the proliferation and organization of the cells encapsulated in the gel are likely to be inhibited, and as a way of compensating for this defect, discloses the synthesis of a material having cell adhesiveness and high mechanical strength, such as a

gelatin derivative or a peptide-based supramolecular gel, and the manufacturing of microfibers using this material, and discloses the apparatus and method for fabricating microfibers in which fiber-shaped tissue formed using an extracellular matrix hydrogel, such as collagen or fibrin, is wrapped in an alginate gel or the like (section titled "linear building blocks" from page 163, sixth line from bottom of right column to page 165, left column, line 18, and fig. 4 in particular). Documents 3 and 4 indicate that, by fabricating microfibers in which micro gel fibers that contain various cells and that are formed using a hydrogel such as collagen or fibrin are wrapped in a high-strength hydrogel like alginate gel, the mechanical strength of the microfibers thus produced is improved, and the high-strength hydrogel portion can be removed as necessary, and also disclose the same apparatus and method as those disclosed in document 2 (document 3: entire text, in particular, the claims, the examples, and fig. 1, 13 and 28; document 4: entire text, in particular, the abstract, and fig. 1).

Thus, with respect to the hollow microfibers disclosed in document 1, it would not be especially difficult for a person skilled in the art to form an outer shell layer by wrapping the outside of the hollow microfibers in a high-strength hydrogel like alginate gel while disposing the hollow microfibers in fiber-shaped tissue formed using an extracellular matrix hydrogel such as collagen or fibrin in order to promote cell proliferation and organization while maintaining mechanical strength, and, in order to dispose the cells so as to come in contact with hollow portions filled with

Box No. V **Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

a culture solution, to form microfibers and attempt to form a cell adhesion layer and a cell layer by adding the cells to the laminar flow of the culture medium configured to form an environment, that is, the hollow portions, so that the cells make contact with the hydrogel without being mixed together with the hydrogel.

Furthermore, documents 2-4 disclose a variety of components of the hydrogel for forming the cell adhesion layer and outer shell layer, various types of cells used, and details concerning the method of fabricating the microfibers, and a person skilled in the art could, as appropriate, even make changes to the detailed fabrication method therefor as needed.