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(54) Title: PROCESS FOR DETACHING CONFLUENT CELLS FROM TWO DIMENSIONAL MICROSUPPORTS AND ITS APPLICATION FOR PREPARING TRANSPLANTS

(57) Abstract: The present invention relates to a process for detaching confluent anchoredependent cells (ADCs') from two-dimensional micros supports (2D-MS) onto which these ADCs' are cultivated, particularly ADCs' which can be cultivated in a state suitable for transplantation, such as ADCs' derived from pancreatic cells. The invention further relates to a process for preparing reconstituted organoids from such detached confluent ADCs', which can be immortalized, according to the invention. The invention also relates to the use of organoids obtained by the process according to the invention for screening cell activity modulator or as therapeutic transplant for their administration in patients in need of such treatment. Finally, the present invention relates to a method for preventing or treating diabetes wherein pancreatic cells-derived organoids according to the invention are transplanted in a patient in need of such treatment.



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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB2004/000930

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/06

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)

C12N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	A.G.SMITH ET AL.: "Successful Sub-Culture of Human Dermal Fibroblasts and Intact HACAT Monolayers from Hydrogel Carriers" EUR CELLS MATERIALS, vol. 2002, no. Suppl.2, 2002, page 91, XP002286802 the whole document	1-22,27
Y	----- "Now introducing: Virtually two dimensional ..." [Online] November 2001 (2001-11), NUNC BRAND PRODUCTS "FLYER", XP002286805 Retrieved from the Internet: URL:http://www.nuncbrand.com/page.asp?ID=238&lang=GB> [retrieved on 2004-06-30] the whole document ----- -/--	1-22,27

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

° 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 document but published on or after the international filing date

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB2004/000930

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GEBB C ET AL: "Harvesting and subculturing cells growing on denatured-collagen coated microcarriers (Cytodex 3)." DEVELOPMENTS IN BIOLOGICAL STANDARDIZATION. SWITZERLAND 1983, vol. 55, 1983, pages 57-65, XP001182316 ISSN: 0301-5149 abstract	1-22,27
A	----- CRUZ H J ET AL: "Cell-dislodging methods under serum-free conditions" APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 47, no. 5, 1997, pages 482-488, XP002286803 ISSN: 0175-7598 Introduction; Table 1	
A	----- EMING SABINE A ET AL: "Regulation of the spatial organization of mesenchymal connective tissue: Effects of cell-associated versus released isoforms of platelet-derived growth factor" AMERICAN JOURNAL OF PATHOLOGY, vol. 154, no. 1, January 1999 (1999-01), pages 281-289, XP002286804 ISSN: 0002-9440 Page 283, left column "Grafting"	
A	----- OLIVARES ELIZABETH ET AL: "Functional integrity of dispersed islet cells attached to a two-dimensional microsupport" INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE, vol. 6, no. 3, September 2000 (2000-09), pages 313-314, XP009033028 ISSN: 1107-3756 the whole document	12,13
Y	----- GRUBER H E ET AL: "Human disc cells in monolayer vs 3D culture: cell shape, division and matrix formation." BMC MUSCULOSKELETAL DISORDERS [ELECTRONIC RESOURCE]. 23 OCT 2000, vol. 1, 23 October 2000 (2000-10-23), page 1, XP002383862 ISSN: 1471-2474 abstract ----- -/--	23-26, 28-30

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB2004/000930

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STEINER M G ET AL: "Characterization of novel cell lines from pleomorphic adenomas of the parotid gland established in a collagen gel system." THE LARYNGOSCOPE. MAY 1997, vol. 107, no. 5, May 1997 (1997-05), pages 654-660, XP008065008 ISSN: 0023-852X abstract	23-26, 28-30
A	----- GMYR V ET AL: "HUMAN PANCREATIC DUCTAL CELLS: LARGE-SCALE ISOLATION AND EXPANSION" CELL TRANSPLANTATION, ELSEVIER SCIENCE, US, vol. 10, no. 1, 2001, pages 109-121, XP001010271 ISSN: 0963-6897 the whole document -----	23-26, 28-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2004/000930

Box 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 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 additional sheet

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 an additional fee, this Authority did not invite payment of any additional fee.
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.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-22 and 27

Methods to detach enzymatically, using eg trypsin or dispase II, a confluent anchorage-dependent cell (ADC) monolayer from the flat microsupport onto which said ADC's are cultured, and methods for preparing isolated organoids comprising said detachment method.

2. claims: 23-26 and 28-30

Uses of the detached monolayers or organoids as an in vitro model system, as bioreactors, for the production of biological material, as a transplant.
