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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/145653 A3

(51) International Patent Classification:
C12N 5/00 (2006.0 1) *C12N 5/02* (2006.0 1)
C12N 5/071 (2010.01)

(21) International Application Number:
PCT/US2014/030456

(22) International Filing Date:
17 March 2014 (17.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/788,285 15 March 2013 (15.03.2013) US

(71) Applicant: **WAKE FOREST UNIVERSITY HEALTH SCIENCES** [US/US]; 391 Technology Way, Suite 199, Winston-Salem, North Carolina 27101 (US).

(72) Inventor: **Bitar, Khalil;** 634 Gramercy Street, Winston-Salem, North Carolina 27104 (US).

(74) Agent: **ENGELLENNER J., Thomas;** Pepper Hamilton LLP, 125 High Street, 19th Floor - High Street Tower, Boston, Massachusetts 021 10 (US).

(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))*

[Continued on next page]

(54) Title: NEURAL PROGENITOR CELL DIFFERENTIATION

(57) Abstract: Differentiation and stability of neural stem cells can be enhanced by *in vitro* or *in vivo* culturing with one or more extracellular matrix (ECM) compositions, such as collagen I, IV, laminin and/or a heparan sulfate proteoglycan. In one aspect of the invention, adult mammalian enteric neuronal progenitor cells can be induced to differentiate on various substrates derived from components or combinations of neural ECM compositions. Collagen I and IV supported neuronal differentiation and extensive glial differentiation individually and in combination. Addition of laminin or heparan sulfate to collagen substrates unexpectedly improved neuronal differentiation, increasing neuron number, branching of neuronal processes, and initiation of neuronal network formation. In another aspect, neuronal subtype differentiation was affected by varying ECM compositions in hydrogels overlaid on intestinal smooth muscle sheets. The matrix compositions of the present invention can be used to tissue engineer transplantable innervated GI smooth muscle constructs to remedy aganglionic disorders.

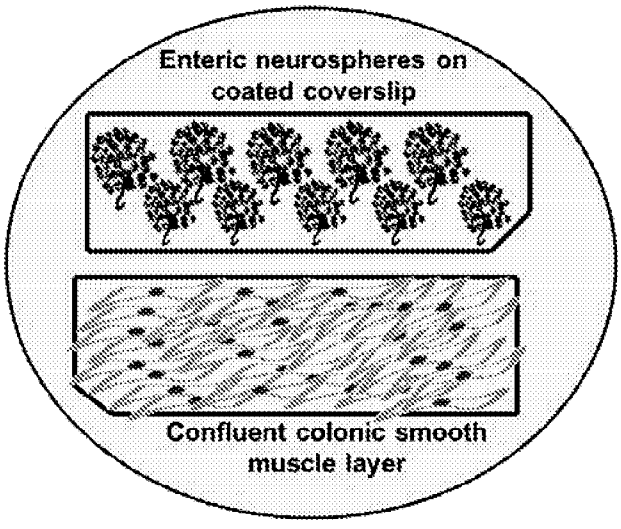

FIG. 2

WO 2014/145653 A3

w o 2014/145653 A3

(88) Date of publication of the international search report:

13 November 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/30456

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 5/00, 5/071, 5/02 (2014.01)
USPC - 435/377, 375, 395, 325, 383, 41
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): C12N 5/00, 5/071, 5/02, 5/07, 5/0775, 5/074, 5/08, 5/02; A61K 35/44, 35/12; G01N 33/50 (2014.01)
USPC: 435/377, 375, 395, 325, 383, 41, 378, 380, 373, 283.1, 7.1, 7.21; 424/93.7; 548/365.7, 248; CPC Classification(s): C12N 5/0602

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 practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-CB, DE-A, DE-T, DE-U, GB-A, FR-A); Google; GoogleScholar; ProQuest; 'biasing', 'neural', 'stem cell', 'smooth muscle cells'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	GEISBAUER, C.L., et al. Transplantation Of Enteric Cells Into The Rodent Stomach With Basic Fibroblast Growth Factor. Journal of Cell Science & Therapy. 10 March 2011, Vol. 2; pp.1-6. Abstract; page 1, right column, second paragraph; page 2, left column, second paragraph; page 2, left column, fourth paragraph; page 3, right column, third paragraph; page 5, left column, third paragraph; page 5, left column, fourth paragraph; page 4, figure 3; page 4, figure 3A; page 4, figure 3C	16, 17, 20-22, 25, 26 ---------- 1-15, 18, 19, 23, 24
Y	RAGHAVAN, S., et al. Bioengineered Three-Dimensional Physiological Model Of Colonic Longitudinal Smooth Muscle In Vitro. Tissue Engineering: Part C: Methods. October 2010, Vol. 16; pp. 999-1009. Abstract, methods; page 1002, figure 1; page 1003, figure 2D	1-15
Y	WARD, S.M., et al. Interstitial Cells Of Cajal Mediate Cholinergic Neurotransmission From Enteric Motor Neurons. The Journal of Neuroscience. 15 February 2000, Vol. 20; pp. 1393-1403. page 1393, left column, first paragraph; page 1402, right column, second paragraph	2-5
Y	HANSEN, R.R., et al. Characterization Of Collagen Thin Flims For Von Willebrand Factor Binding And Platelet Adhesion. Langmuir. 03 October 2011, Vol. 27; pp. 13648-13658. Abstract	5

☒ 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
04 August 2014 (04.08.2014)

Date of mailing of the international search report
29 AUG 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:
Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/30456

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BAGYANSZKI, M., et al. Diabetes-Related Alterations In The Enteric Nervous System And Its Microenvironment. World Journal of Diabetes. 15 May 2012, Vol. 3; pp. 80-93. Abstract	6-8
Y	US 2011/015101 1 A1 (FLYNN, L) June 23, 2011; paragraphs [0010], [0028], [0091]	7, 8, 10, 18, 19, 23
Y	FUJIMIYA, M., et al. Peptidergic Regulation Of Gastrointestinal Motility In Rodents. Peptides. October 2000, Vol. 21; pp. 1565-1582. Abstract	9-1 1
Y	DAHM, L.M., et al. Substance P Responsiveness Of Smooth Muscle Cells Is Regulated By The Integrin Ligand, Thrombospondin. Proc. Natl. Acad. Sci. USA. 06 February 1996, Vol. 93; pp. 1276-1281. page 1277, left column, third paragraph; page 1278, left column, fourth paragraph	11
Y	US 2006/0153815 A1 (SEYDA, A., et al.) July 13, 2006; paragraphs [0053], [0056], [0085]; figure 1	14, 15
Y	TULLA, M., et al. Selective Binding Of Collagen Subtypes By Integrin Alpha 1-1, Alpha 2-1, and Alpha 10-1 Domains. The Journal Of Biological Chemistry. 25 September 2001, Vol. 276; pp. 48206-48212. page 48208, figure 2; page 48209, figure 3	24
P, X	RAGHAVAN, S., et al. The Influence Of Extracellular Matrix Composition On The Differentiation Of Neuronal Subtypes In Tissue Engineered Innervated Intestinal Smooth Muscle Sheets. Biomaterials. 11 June 2014, Vol. 35; pp. 7429-7440. entire document	1-12, 14-20, 22, 23, 25