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(54) **Title:** METHODS FOR STIMULATING THE PROLIFERATION AND DIFFERENTIATION OF EUKARYOTIC CELLS

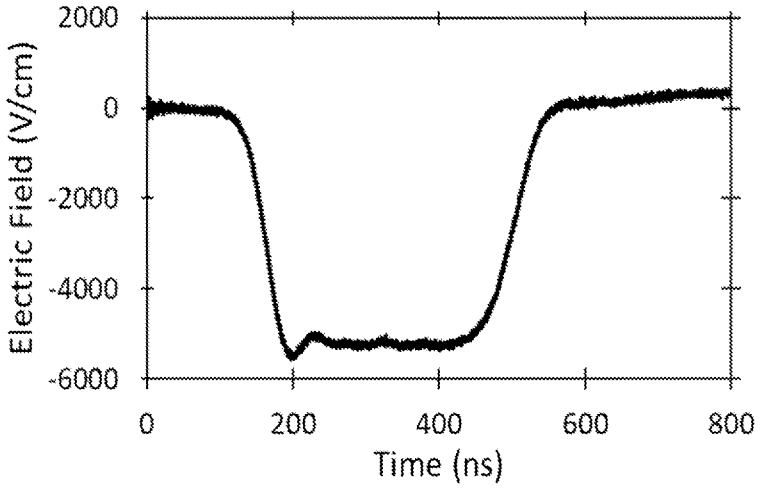

FIG. 1

(57) **Abstract:** The present disclosure relates to methods of stimulating cell proliferation, promoting differentiation of cells, regenerating cells, promoting nodule formation, and promoting myotube formation. The methods include applying one or more pulses of electricity to cells, each pulse of electricity having a duration of between about 10 nanoseconds and about 1,000 nanoseconds, wherein said pulses of electricity are applied under conditions effective to stimulate cell proliferation, promote differentiation of cells, regenerate cells, promote nodule formation, and promote myotube formation.

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[Continued on next page]

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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:
15 October 2020 (15.10.2020)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/013030

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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

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:

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 fees, this Authority did not invite payment of additional fees.

3. [X] 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.:

1-12, 14, 16-18, 21-32, 34, 36-38

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

[X] 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/US2020/013030

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61N1/32 C12M1/42 C12N13/00
ADD. A61N1/36

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)
A61N C12R C12M 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 practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NING T ET AL: "Nanosecond pulsed electric fields enhance chondrogenesis of mesenchymal stem cells (MSCS) partially via phosphorylation of P38-MAPK", OSTEOARTHRITIS AND CARTILAGE, vol. 24, 2016, XP029466990, ISSN: 1063-4584, DOI: 10.1016/J.JOCA.2016.01.333 the whole document ----- -/--	1,2, 6-10,14, 16-18, 21-32, 34,36-38

[X] 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

"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
26 August 2020	07/09/2020

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANDREW L. FRELINGER ET AL: "Modification of Pulsed Electric Field Conditions Results in Distinct Activation Profiles of Platelet-Rich Plasma", PLOS ONE, vol. 11, no. 8, 24 August 2016 (2016-08-24), page e0160933, XP055343048, DOI: 10.1371/journal.pone.0160933 page 5/17 - page 13/17; figures 1B, 3A-3E	1,3-12, 14, 21-32, 34,36-38
X	ALLEN L. GARNER ET AL: "Design, characterization and experimental validation of a compact, flexible pulsed power architecture for ex vivo platelet activation", PLOS ONE, vol. 12, no. 7, 26 July 2017 (2017-07-26), page e0181214, XP055697099, DOI: 10.1371/journal.pone.0181214 cited in the application page 4/27 - page 23/27; figures 6-10, 16-18	1,6-11, 14, 21-32, 34,36-38
X	EING C ET AL: "Effects of nanosecond pulsed electric field exposure on arabidopsis thaliana", IEEE TRANSACTIONS ON DIELECTRICS AND ELECTRICAL INSULATION, IEEE SERVICE CENTER, PISCATAWAY, NJ, US, vol. 16, no. 5, October 2009 (2009-10), pages 1322-1328, XP011278893, ISSN: 1070-9878, DOI: 10.1109/TDEI.2009.5293945 page 1323, left-hand column - page 1327, left-hand column, last paragraph; figure 2; tables 1-2	1,2, 6-10,12, 14,17, 18, 21-32, 34,36-38
X	M STACEY ET AL: "Differential effects in cells exposed to ultra-short, high intensity electric fields: cell survival, DNA damage, and cell cycle analysis", MUTATION RESEARCH. GENETIC TOXICOLOGY AND ENVIRONMENTAL MUTAGENESIS, vol. 542, no. 1-2, December 2003 (2003-12) , pages 65-75, XP055723761, NL ISSN: 1383-5718, DOI: 10.1016/j.mrgentox.2003.08.006 abstract; p. 67, "2.2 Exposure of cells to nsPEF", p. 70, "3.3. Exposure of cells to nsPEF causes DNA damage measured by the comet assay" - p. 71, "3.6. nsPEF exposure causes changes in cell cycle parameters"	21-32, 34,36-38

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BAI FAN ET AL: "Nanosecond pulsed electric fields trigger cell differentiation inChlamydomonas reinhardtii", BBA - BIOMEMBRANES, ELSEVIER, AMSTERDAM, NL, vol. 1859, no. 5, 6 January 2017 (2017-01-06), pages 651-661, XP029949407, ISSN: 0005-2736, DOI: 10.1016/J.BBAMEM.2017.01.007 Abstract; p. 652-653, "2.2 Nanosecond pulsed electric fields (nsPEFs)"; Table 1 -----	21-32, 34,36-38

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-20

   Method of STIMULATING CELL PROLIFERATION by applying electrical pulses having a duration of 10-1000 nanoseconds to cells.

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2. claims: 21-40

   Method of PROMOTING DIFFERENTIATION OF CELLS by applying electrical pulses having a duration of 10-1000 nanoseconds to cells.

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3. claims: 41-60

   Method of REGENERATING CELLS by applying electrical pulses having a duration of 10-1000 nanoseconds to cells.

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4. claims: 61-81

   Method of PROMOTING NODULE FORMATION by applying electrical pulses having a duration of 10-1000 nanoseconds to cells.

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5. claims: 82-101

   Method of PROMOTING MYOTUBE FORMATION by applying electrical pulses having a duration of 10-1000 nanoseconds to cells.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 13, 15, 19, 20, 33, 35, 39, 40(completely); 1-12, 14, 16-18, 21-32, 34, 36-38(partially)

The subject-matter of claims 1-20 relates to a method for treatment of the human or animal body by therapy (Rule 39.1(iv) PCT), since it covers also in vivo treatment of cells by electrical "nanopulses". In particular, claims 13, 15, 19, 20 relate to in vivo treatment and to the application of an additional agent with therapeutic effect. Hence, claims 13, 15, 19, 20 have not been searched at all, since they relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the subject-matter of these claims (Article 34(4)(a)(i) PCT). As a matter of courtesy and service for the applicant, the subject-matter of claims 1-11, 12, 14, 16-18 has nevertheless at least partially been searched on the basis of the alleged device/system features, i.e. the corresponding pulse generation device/system (pulse duration, electric field strength and pulse repetition rate, number of pulses, pulse rise/fall time). It is to be noted that also the subject-matter of claims 21-101 relates to a method for treatment of the human or animal body by therapy (Rule 39.1(iv) PCT), since it covers also in vivo treatment of cells by electrical "nanopulses". Hence, claims 21-101 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT.