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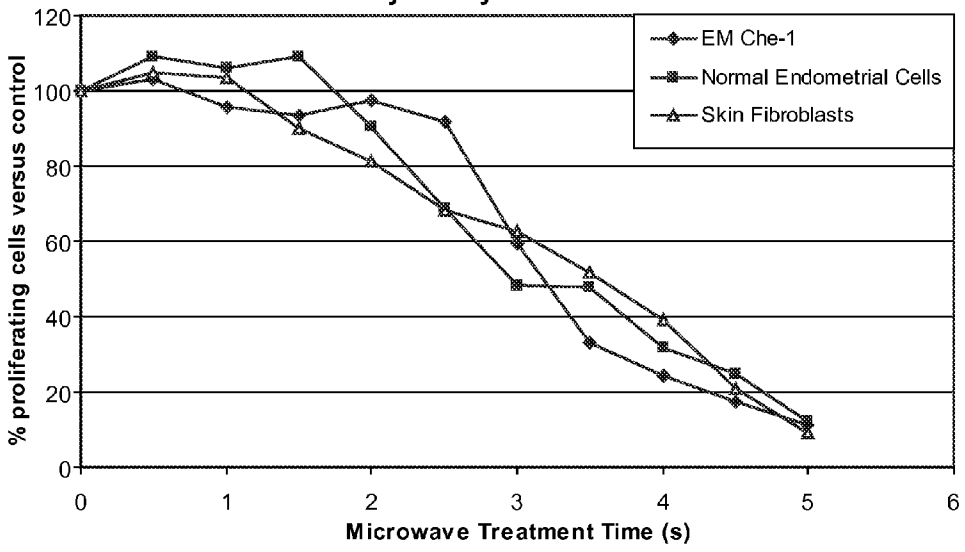
(54) **Title:** EFFECT OF RADIOFREQUENCY FIELDS ON CELL GROWTH AND METHODS FOR LAPAROSCOPIC TREATMENT

FIG. 1 **Proliferation Curves 8h after MicrowaveTreatment by Nymax System**



(57) **Abstract:** The invention relates to the use of a radiofrequency/microwave treatment device and its effect on cell growth and survival, and in a more particular embodiment on endometriotic cells. In another aspect, the invention relates to the use of radiofrequency/microwave energy to effect cell growth in a desired population while substantially avoiding the deleterious heating and destructive effects on neighboring or nearby cells. Using the devices, specific treatment regimens and controlling energy output to cells and tissue can effect specific cells and diseases of humans, for example, those involving uncontrolled or deleterious cell growth.

WO 2009/063083 A1

EFFECT OF RADIOFREQUENCY FIELDS ON CELL GROWTH AND METHODS FOR LAPAROSCOPIC TREATMENT

This application claims priority benefit to United States Provisional application number 60/996,377, filed November 14, 2007, the entire contents of which are incorporated herein by reference.

Field of the Invention

[0001] The invention relates to devices and methods for the treatment of tissue and the effect of energy transfer on cells. In one particular aspect, the invention relates to the use of a radiofrequency/microwave treatment device and its effect on cell growth and survival, and in a more particular embodiment on endometriotic cells. In another aspect, the invention relates to the use of radiofrequency/microwave energy to effect cell growth in a desired population while substantially avoiding the deleterious heating and destructive effects on neighboring or nearby cells. Thus, in one aspect the invention relates to the control of the energy treatment to cells and tissue. As shown below, short, focused treatments on cells result in a significant decrease in the proliferation rate of the cells and/or a degradation in cellular DNA. Based upon the effect on certain cells and tissues, a treatment can be designed or controlled to avoid or substantially avoid thermal destruction of surrounding cells. The device and methods of treatment can be used in a number of disease conditions, including, without limitation, endometriosis.

Relevance of the Invention and Description of Related Art

[0002] Various methods exists for the induction of toxic effects on live cells through exposure to radiofrequency (RF) energy. For the most part, the RF fields used in these treatments heat the cells, eventually resulting in cell death or damage. For several reasons, including heat transfer between cells and within tissue, the effect is generally over a relatively large area and not limited to specific cells.

[0003] As one type of cell proliferation disease, endometriosis is a common medical condition characterized by growth of tissue like endometrium, the lining of the uterus, beyond or outside the uterus. The disease affects an estimated 90 million women (usually around 30 to 40 years of age who have never been pregnant) around the world. In other rare cases, endometriosis has also been found in skin, lung, eye, diaphragm, and brain tissue

[0004] Treatment is typically via surgery, and alternatives, especially laparoscopic or minimally invasive methods, are desired. However, typical RF devices and treatments are not specific, have side effects, and do not employ a controlled effect on cell growth.

Brief Summary of the Invention

[0005] The invention, in one aspect, satisfies the need for improved methods for treating proliferating or damaged cells. In other aspects, the methods can be used to cause cell damage and induce repair in a specific set of cells or tissue.

Thus, a variety of cell treatment methods and regimens can be included in the scope of the invention. In part, these treatments are based upon the recognition of certain effects on the treated cells, effects that can be controlled and limited in size and extent within tissue. In another aspect, the methods of the invention relate to measuring the effect of radiofrequency/microwave treatments on cells and tissue. In another aspect, the invention relates to treatments that decrease cell growth or proliferation.

[0006] More particularly, the present findings indicate that treatment of endometriotic cells, stromal cells, and fibroblasts by radiofrequency/microwave (RF/EMFs) fields inhibits their proliferation and colony-forming capacity. Accordingly, RF/MW treatment can be an important tool and method for a variety of cell and tissue treating methods, including laparoscopic treatment of endometriotic lesions, treatment of aged or damaged skin, and similar treatments of humans and animals.

[0007] Throughout this disclosure, applicants refer to journal articles, patent documents, published references, web pages, and other sources of information. One skilled in the art can use the entire contents of any of the cited sources of information to make and use aspects of this invention. Each and every cited source of information is specifically incorporated herein by reference in its entirety. Portions of these sources may be included in this document as allowed or required. However, the meaning of any term or phrase specifically defined or explained in this disclosure shall not be modified by the content of any of the

sources. The description and examples that follow are merely exemplary of the scope of this invention and content of this disclosure and do not limit the scope of the invention. In fact, one skilled in the art can devise and construct numerous modifications to the examples listed below without departing from the scope of this invention.

Brief Description of the Drawings

[0008] Figure 1 depicts a graph of the % of proliferating cells measured 8 hours after treatment with various time periods of MW energy. Endometrial (EM Che-1) cells are compared to normal endometrial cells and skin fibroblasts.

[0009] Figure 2 depicts a graph of the % of proliferating cells measured 72 hours after treatment with various time periods of MW energy. Again, endometrial (EM Che-1) cells are compared to normal endometrial cells and skin fibroblasts. The percentage of proliferating cells present after a 3 or more second treatment is very low, indicating this 3-5 second treatment suffices to inhibit proliferation under the conditions.

[0010] Figures 3, 4 and 5 show cultured cells after control treatment, 2 second MW treatment (2s) and 5 second MW treatment (5s).

[0011] Figures 6, 7 and 8 show cultured cells after control treatment, 2 second MW treatment (2s) and 5 second MW treatment (5s).

[0012] Figures 9A and 9B show the colony-forming capacity in control (9A) versus MW treated (9B) cells.

[0013] Figure 10 shows gel electrophoresis separated genomic DNA from cells of

control treatment, cells treated with MW for 1 sec (1s), 2 sec (2s), and 3 sec (3s). Marker DNA (M) is shown at left. A marked degradation of the DNA can be seen in the 2s and 3s bands in particular.

Detailed Description of Exemplary Embodiments

[0014] In one aspect the invention involves the use of RF/MW energy on various cell types, including edometriotic and fibroblast cells. The endometriosis cell line EM.Che-1, normal endometrial stromal cells, and human skin fibroblasts are cultured in DMEM medium with 10%FCS, L-glutamine and antibiotics. For RF/MW treatment, a Nymax device (device as described in pending application PCT/EP2007/059486 or US 11/882,453) is used for cell treatments. The cells are grown in 96 micro-well plates and exposed to desired energy field (2450 MHz) for various time periods. Progression of cell growth over hours/days, colony-forming capacity over time, and the occurrence of DNA fragmentation are evaluated. Various methods can be used to measure these characteristics or effects in treated cells, including BrDU incorporation assays, tri-dimensional cell growth procedure in MATRIGEL membranes, and by electrophoresis of cellular DNA.

[0015] After treatment, the growth curve of the treated EM.Che-1, stromal cells and skin fibroblasts after being cultured for 3h, 24h and 49 hours, shows a significant decrease in the proliferation rate. The growth inhibition of the EM.Che-1 cells was stronger compared with that of the stromal cells and skin

fibroblasts. In addition, colony-forming capacity of EM.Che-1 cells decreased by 42%±3 after treatment (3 sec.), which is evaluated approximately 8 days after treatment and compared to controls.

[0016] The treatment methods described here can be incorporated in numerous human and animal treatment regimens, including the treatment of endometriosis as described in Chapron, et al. (Hum Reproduct. 14: 329-332 (1999)), and the treatment of skin blemishes and wrinkles as described in PCT/EP2007/059486 or US 11/882,453. Other conditions involving the selective destruction of proliferating cells, such a cancers, can also benefit from the methods described here.

Examples

[0017] Endometriotic cell culture model (1)

[0018] A human cell line, designated Em Che-1, is obtained from the biopsy of the peritoneal nodule from a 35 year old patient. The cells are grown in DMEM + 10% FCS + L-glutamine + antibiotics over a basement membrane substrate (MATRIGEL™ Basement Membrane Matrix; BD Biosciences). The cells exhibit a stromal-like, adherent cell morphology and have the capacity to form colonies in matrix. The cells resemble an aberrant karyotype, but have consistent expression of cytokeratin 8, 9, 18 marker proteins, estrogen receptor (ER), and progesterone receptor (PR). The cells are shown in Figures 4 and 5.

[0019] Endometriotic cell culture model (2)

[0020] Cultured autologous endometrial stromal cells (shown in Figures 7 and 8) are maintained in DMEM medium supplemented with 10% FCS, L- glutamine, and antibiotics.

[0021] Human skin fibroblast cells (Figures 3 and 6) can also be used in MW treatments and used for controls as compared to the proliferating endometrial cells.

[0022] A cell proliferation rate can be established using assays known in the art, for example the TACS™ XTT cell proliferation assay (Trevigen, Inc). Similarly, colony-forming capacity can be determined by culturing in MATRIGEL™ (BD Biosciences), and cellular DNA fragmentation can be evaluated using standard gel electrophoresis of isolated genomic DNA.

[0023] RF/MW exposure system

[0024] An RF/MW treatment device (as described in pending application PCT/EP2007/059486 or US 11/882,453) is used to allow very accurate energy transfer through a bipolar, handheld probe. Cells can be maintained to sub-confluence in 96-well plates, exposed to 2.45 GHz with average power of 10W for various times, for example ranging from about 1 sec to about 12.5 sec. Typical energy delivery can be between about 5 and about 125 Joules.

[0025] Results

[0026] Effect of RF/MW on cell growth

[0027] After exposure to RF for different times (0 to 5 sec), the growth curves (after 8 hrs and 76 hrs, Figures 1 and 2) of EM.Che-1 endometriotic cells,

autologous endometrial stromal cells, and human skin fibroblasts showed a significant decrease of the proliferation rate. The photomicrographs of the cells in Figures 3-8 show the same results. Colony-forming capacity is compared in the photomicrographs of Figures 9A and 9B, where the MW treated endometriotic cells (9B) show a marked reduction in number of colonies 8 days after the 25 to 125 Joule treatment as compared to the untreated endometriotic cells. Genomic cell DNA degradation can be measured by isolating genomic DNA using conventional methods and separating the DNA by gel electrophoresis. Typical results are depicted in the comparison of bands in the gel of Figure 10.

[0028] Laparoscopic Treatment

[0029] Laparoscopic treatment devices, such as those available in the art (*see, for example,*Karl Storz, Thubingen, Germany) can be used to visualize the effect on cells during and after treatment to monitor optimal treatment regimens. The device can be used in conjunction with the RF/MW treatment device noted above. A fluorescence probe to indicate endometriotic cells can also be added to the treatment, so that specific treatment sites can be visualized.

[0030] The examples presented above and the contents of the application define and describe examples of only some of the many methods, treatment regimens, human treatments, and cell measurement processes encompassed by the invention. Additional products, devices, and methods can be produced or used according to the invention. None of the examples and no part of the description

should be taken as a limitation on the scope of the invention as a whole or of the meaning of the following claims.

CLAIMS

What is claimed is:

1. A method of treating cells to prevent cell proliferation comprising treating the cells to RF/MW energy at one of more of: about 2.45 GHz with average power of 10W for a desired period of time between 1 sec and 15 sec; or between about 5 and about 125 Joules, and measuring one or more of: decrease in cell growth or proliferation over time; capacity for colony-forming; or genomic DNA degradation.

2. A method for determining a cell growth inhibiting RF/MW treatment for cells, comprising applying RF/MW energy from a bipolar device to the cells, and measuring one or more of: decrease in cell growth or proliferation over time; capacity for colony-forming; or genomic DNA degradation.

3. A method of treating endometriosis comprising providing a RF/MW emitting device and delivering from about 5 to about 125 Joules, whereby the cells capacity for growth or colony formation is decreased.

4. The method of claim 3, wherein the emitting device is inserted into the body using minimally invasive techniques.

5. A MW emitting device for treating endometriosis or cell proliferating disease, comprising a handheld bipolar tip for emitting MW incorporated into a laparoscopic surgical wand, the device capable of delivering about 25 to about 125 Joules, or about 2.54 GHz at about 10W for 1 to 15 seconds.

6. The device of claim 5, configured for the laparoscopic treatment of endometriosis in humans.

7. A method as claimed in claim 1 for the treatment of endometriosis in a patient in need thereof, wherein a device to deliver RF/MW energy is configured

for laparoscopic surgery in a human, and whereby the treatment results in cell damage to proliferating cells associated with the endometriosis.

8. A method as in one of claims 1-4 or 7, further comprising treating cells or tissue with a fluorescent or autofluorescent compound, whereby the treatment area or cells are indicated by fluorescence.

FIG. 1 Proliferation Curves 8h after MicrowaveTreatment by Nymax System

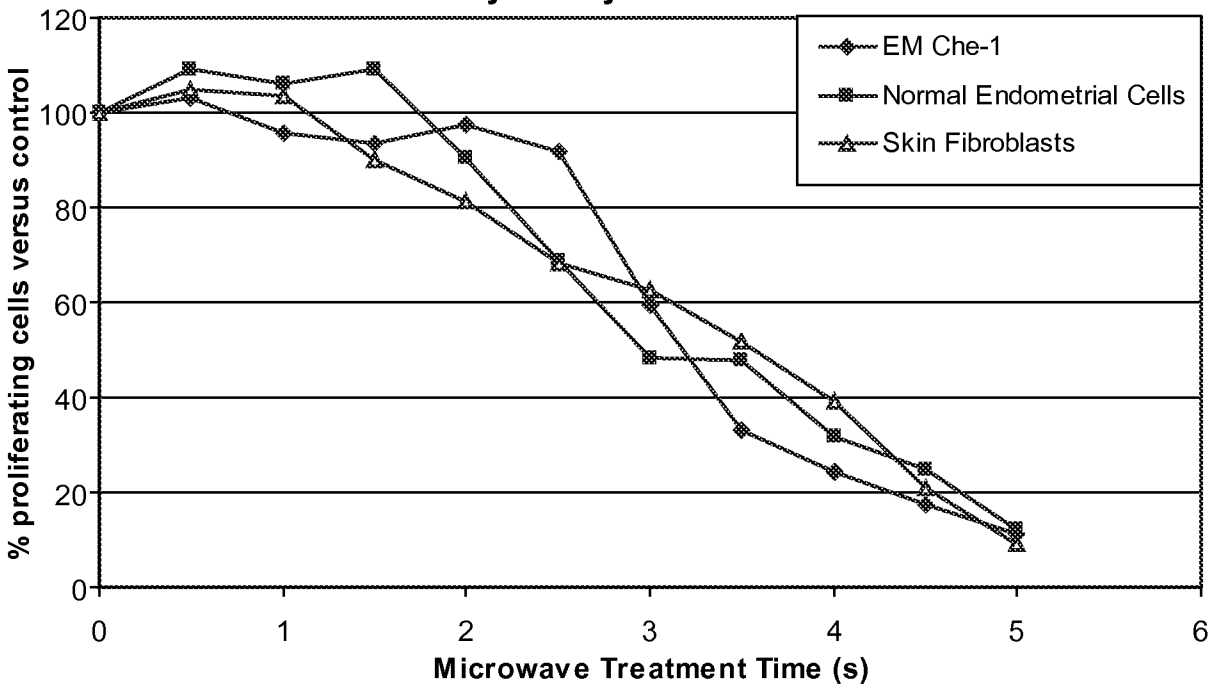


FIG. 2 Proliferation Curves 76h after MicrowaveTreatment by Nymax System

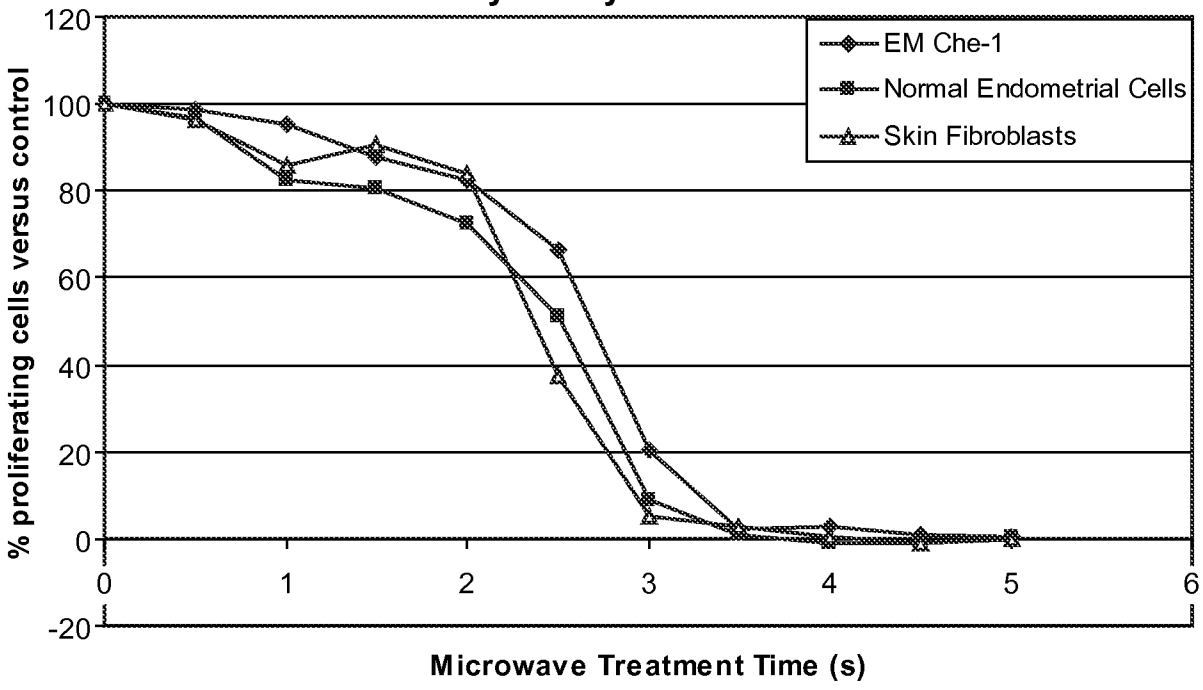


FIG. 3

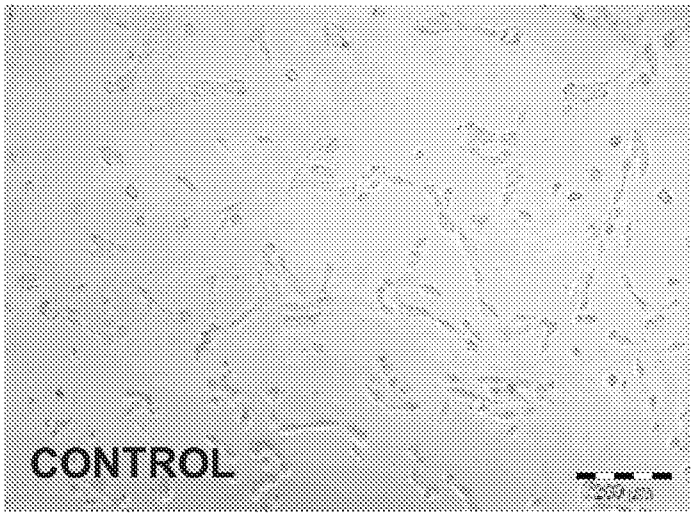


FIG. 4

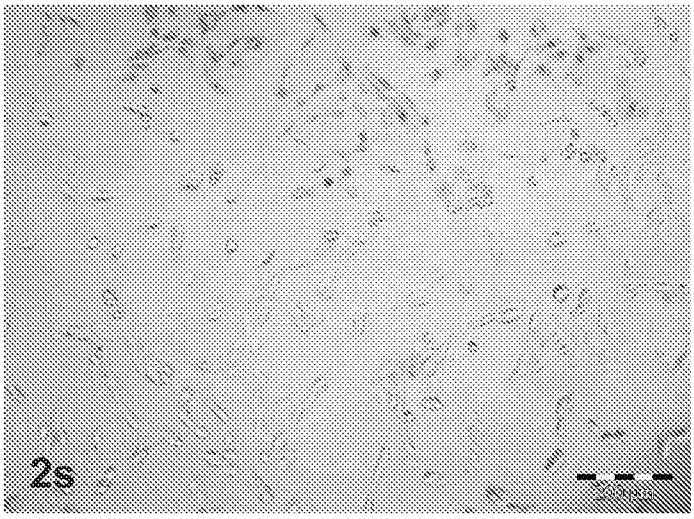


FIG. 5

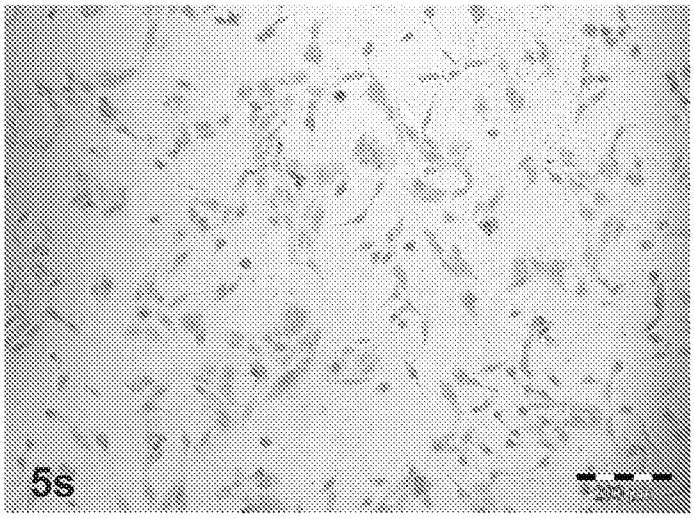


FIG. 6

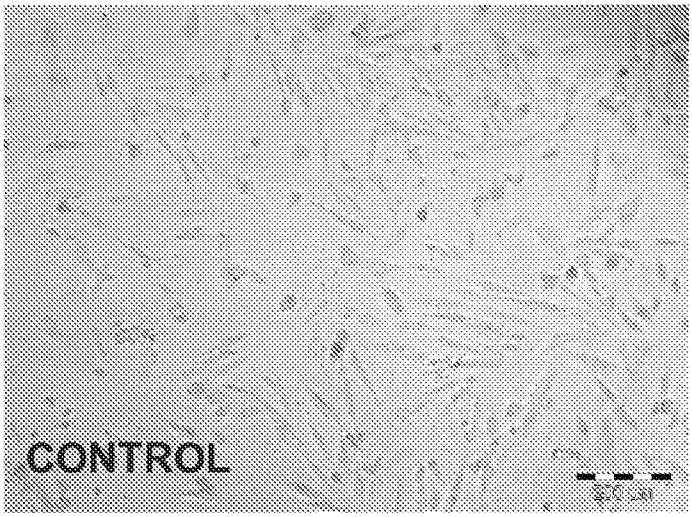


FIG. 7

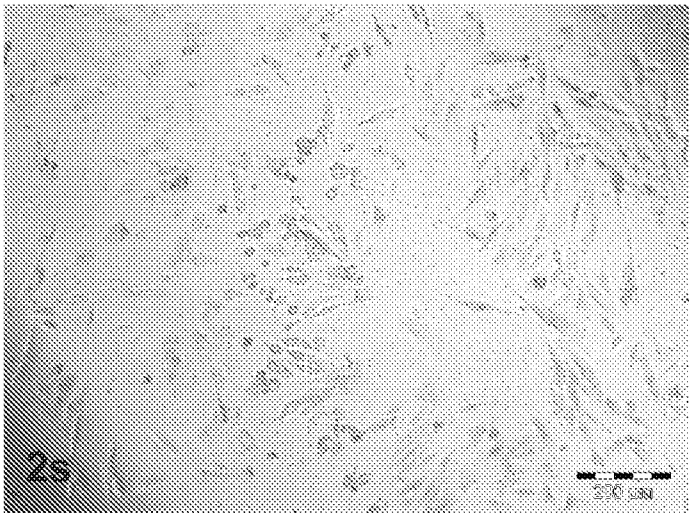
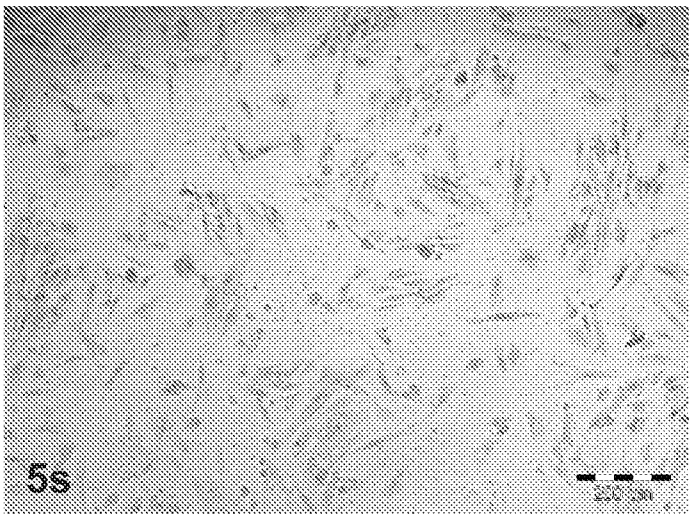
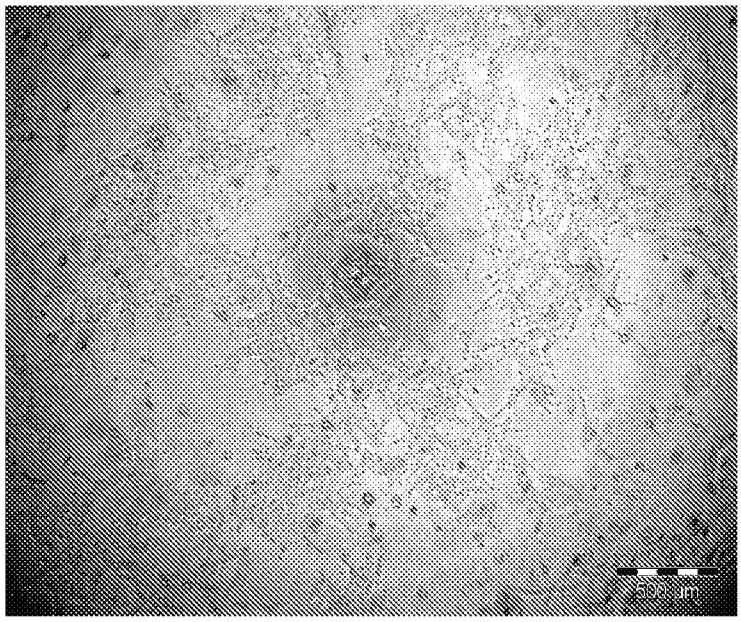


FIG. 8



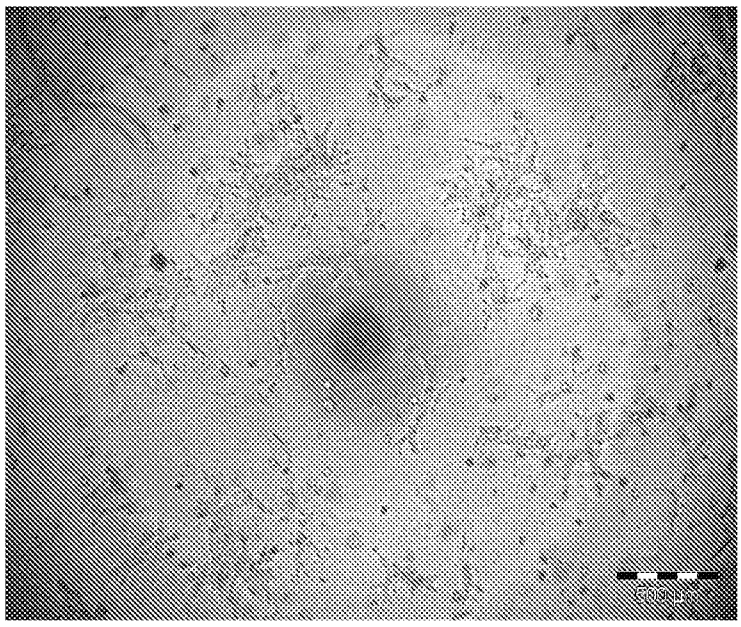
EM.Che -1 cells cultured during 8 days

FIG. 9A



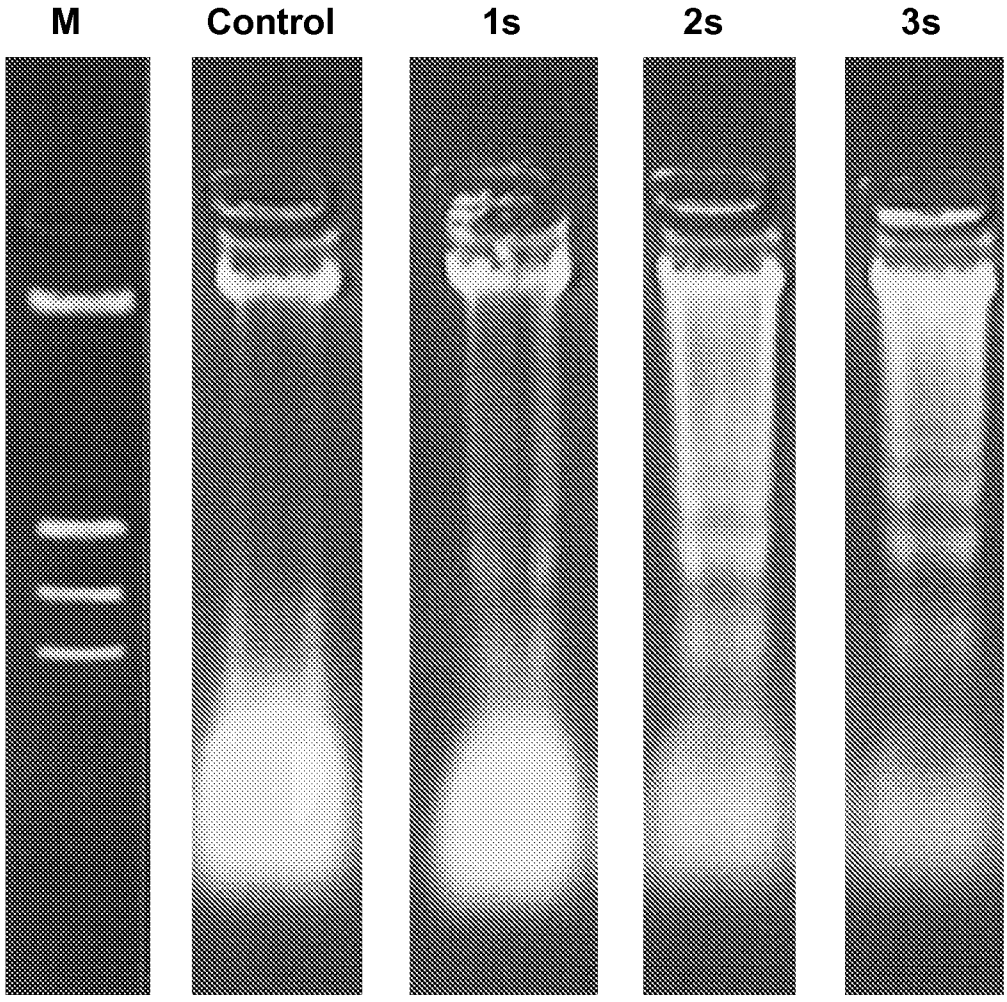
Control (no treatment)

FIG. 9B



Nymax treatment (3s)

FIG. 10



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/065620

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61B18/18
ADD. A61N5/02 A61N5/04

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)
A61B A61N

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, 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	BOUQUET DE JOLINIERE ET AL: "37: Effect of Radiofrequency Fields on Endometriotic Cell Growth In Vitro: A Prospective Study for Laparoscopic Treatment" JOURNAL OF MINIMALLY INVASIVE GYNECOLOGY, ELSEVIER, NL, vol. 14, no. 6, 1 November 2007 (2007-11-01), pages S14-S15, XP022325220 ISSN: 1553-4650 the whole document -/--	2,5,6

[X] Further documents are listed in the continuation of Box C.

[X] 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 document 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
9 March 2009	19/03/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Grochol, Jana

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.
	-& WO 2008/028980 A (RENEWAVE MEDICAL SYSTEMS SA [CH]; LUKOWIAK MARC [FR]; LAVIGNE CHRISTOP) 13 March 2008 (2008-03-13) page 3, line 26 - line 29 page 6, line 3 - line 26 page 9, line 2 - line 3 figure 1 claims 3,17,18	
X	GB 2 387 544 A (MICROSULIS PLC [GB]; MICROSULIS LTD [GB] MICROSULIS LTD [GB]) 22 October 2003 (2003-10-22) page 1, line 7 - line 12 page 4, line 13 - line 15 page 4, line 22 - line 25 claim 1	5,6
A	WO 99/53853 A (VIDACARE INC [US]; EDWARDS STUART D [US]) 28 October 1999 (1999-10-28)	5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/065620

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.: 1,3,4,7,8
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

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:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable 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. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers 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 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

Information on patent family members

International application No
PCT/EP2008/065620

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008028980 A	13-03-2008	US 2008065059 A1	13-03-2008
GB 2387544 A	22-10-2003	AU 2003267607 A1	04-05-2004
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		WO 2004033039 A1	22-04-2004
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		US 2006151485 A1	13-07-2006
		ZA 200503071 A	27-09-2006
WO 9953853 A	28-10-1999	AU 3660699 A	08-11-1999
		US 6002968 A	14-12-1999

专利名称(译)	射频场对细胞生长的影响及腹腔镜治疗方法		
公开(公告)号	EP2227164A1	公开(公告)日	2010-09-15
申请号	EP2008850745	申请日	2008-11-14
申请(专利权)人(译)	RENEWAVE医疗系统SA		
当前申请(专利权)人(译)	RENEWAVE医疗系统SA		
[标]发明人	GOGUSEV JEAN BOUQUET DE JOLINIERE JEAN		
发明人	GOGUSEV, JEAN BOUQUET DE JOLINIERE, JEAN		
IPC分类号	A61B18/18 A61N5/02 A61N5/04		
CPC分类号	A61N1/40 A61B18/18 A61B18/1815 A61N5/02 A61N5/045		
优先权	60/996377 2007-11-14 US		
外部链接	Espacenet		

摘要(译)

本发明涉及射频/微波治疗装置的用途及其对细胞生长和存活的影响，并且在更具体的实施方案中涉及子宫内膜异位细胞。另一方面，本发明涉及射频/微波能量在所需人群中实现细胞生长同时基本上避免对邻近或附近细胞的有害加热和破坏作用的用途。使用这些装置，特定的治疗方案和控制细胞和组织的能量输出可以影响人的特定细胞和疾病，例如涉及不受控制或有害细胞生长的细胞和疾病。