US010292755B2

(12) **United States Patent**
Arena et al.

(10) **Patent No.: US 10,292,755 B2**
(45) **Date of Patent: May 21, 2019**

(54) **HIGH FREQUENCY ELECTROPORATION FOR CANCER THERAPY**

(71) Applicant: **Virginia Tech Intellectual Properties, Inc.**, Blacksburg, VA (US)

(72) Inventors: **Christopher B. Arena**, Burlington, NC (US); **Rafael V. Davalos**, Blacksburg, VA (US); **Michael B. Sano**, Blacksburg, VA (US)

(73) Assignee: **VIRGINIA TECH INTELLECTUAL PROPERTIES, INC.**, Blacksburg, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 136 days.

(21) Appl. No.: **15/186,653**

(22) Filed: **Jun. 20, 2016**

(65) **Prior Publication Data**

US 2016/0287314 A1 Oct. 6, 2016

Related U.S. Application Data

(60) Division of application No. 13/332,133, filed on Dec. 20, 2011, which is a continuation-in-part of (Continued)

(51) **Int. Cl.**
A61B 18/12 (2006.01)
A61B 18/14 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC ***A61B 18/14*** (2013.01); ***A61N 1/327*** (2013.01); *A61B 2018/0016* (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC A61B 2018/00613
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

1,653,819 A 12/1927 Northcott et al.
3,730,238 A 5/1973 Butler
(Continued)

FOREIGN PATENT DOCUMENTS

AU 2002315095 A1 12/2002
AU 2003227960 A1 12/2003
(Continued)

OTHER PUBLICATIONS

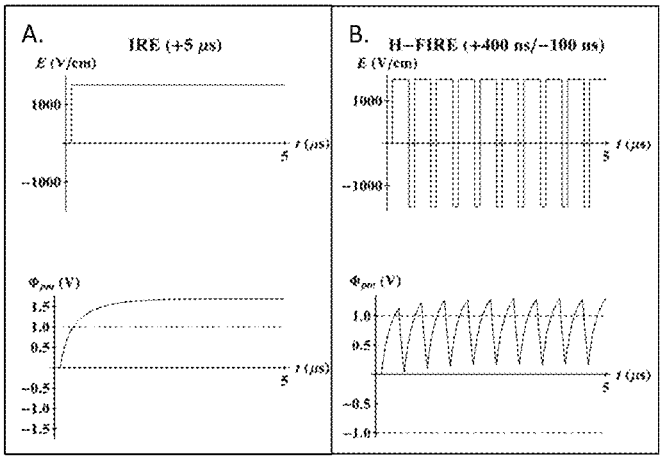
Co-Pending U.S. Appl. No. 14/686,380, filed Apr. 14, 2015 and Published as US 2015/0289923 on Oct. 15, 2015.
(Continued)

Primary Examiner — Daniel W Fowler
(74) *Attorney, Agent, or Firm* — New River Valley IP Law, PC; Michele L. Mayberry

(57) **ABSTRACT**

The present invention relates to the field of biomedical engineering and medical treatment of diseases and disorders. Methods, devices, and systems for in vivo treatment of cell proliferative disorders are provided. In embodiments, the methods comprise the delivery of high-frequency bursts of bipolar pulses to achieve the desired modality of cell death. More specifically, embodiments of the invention relate to a device and method for destroying aberrant cells, including tumor tissues, using high-frequency, bipolar electrical pulses having a burst width on the order of microseconds and duration of single polarity on the microsecond to nanosecond scale. In embodiments, the methods rely on conventional electroporation with adjuvant drugs or irreversible electroporation to cause cell death in treated tumors. The invention can be used to treat solid tumors, such as brain tumors.

16 Claims, 24 Drawing Sheets



Related U.S. Application Data

application No. 12/757,901, filed on Apr. 9, 2010, now Pat. No. 8,926,606.

(60) Provisional application No. 61/424,872, filed on Dec. 20, 2010, provisional application No. 61/285,618, filed on Dec. 11, 2009, provisional application No. 61/167,997, filed on Apr. 9, 2009.

(51) **Int. Cl.**
A61N 1/32 (2006.01)
A61B 18/00 (2006.01)

(52) **U.S. Cl.**
CPC *A61B 2018/00577* (2013.01); *A61B 2018/00613* (2013.01); *A61B 2018/00761* (2013.01); *A61B 2018/00767* (2013.01)

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,746,004	A		7/1973	Jankelson
3,871,359	A		3/1975	Pacela
4,016,886	A		4/1977	Doss et al.
4,037,341	A		7/1977	Odle et al.
4,216,860	A		8/1980	Heimann
4,226,246	A		10/1980	Fragnet
4,262,672	A		4/1981	Kief
4,267,047	A		5/1981	Henne et al.
4,278,092	A		7/1981	Borsanyi et al.
4,299,217	A		11/1981	Sagae et al.
4,311,148	A		1/1982	Courtney et al.
4,336,881	A		6/1982	Babb et al.
4,344,436	A		8/1982	Kubota
4,392,855	A		7/1983	Oreopoulos et al.
4,406,827	A		9/1983	Carim
4,407,943	A		10/1983	Cole et al.
4,416,276	A		11/1983	Newton et al.
4,447,235	A		5/1984	Clarke
4,469,098	A		9/1984	Davi
4,489,535	A		12/1984	Veltman
4,512,765	A		4/1985	Muto
4,580,572	A		4/1986	Granek et al.
4,636,199	A		1/1987	Victor
4,672,969	A		6/1987	Dew
4,676,258	A		6/1987	Inokuchi et al.
4,676,782	A		6/1987	Yamamoto et al.
4,687,471	A		8/1987	Twardowski et al.
4,716,896	A		1/1988	Ackerman
4,723,549	A		2/1988	Wholey et al.
D294,519	S		3/1988	Hardy
4,756,838	A		7/1988	Veltman
4,772,269	A		9/1988	Twardowski et al.
4,798,585	A		1/1989	Inoue et al.
4,810,963	A		3/1989	Blake-Coleman et al.
4,813,929	A		3/1989	Semrad
4,819,637	A		4/1989	Dormandy et al.
4,822,470	A	*	4/1989	Chang C12M 35/02 435/173.6
4,836,204	A		6/1989	Landymore et al.
4,840,172	A		6/1989	Augustine et al.
4,863,426	A		9/1989	Ferragamo et al.
4,885,003	A		12/1989	Hillstead
4,886,496	A		12/1989	Conoscenti et al.
4,886,502	A		12/1989	Poirier et al.
4,889,634	A		12/1989	El-Rashidy
4,907,601	A		3/1990	Frick
4,919,148	A		4/1990	Muccio
4,920,978	A		5/1990	Colvin
4,921,484	A		5/1990	Hillstead
4,946,793	A		8/1990	Marshall, III
4,976,709	A		12/1990	Sand
4,981,477	A		1/1991	Schon et al.
4,986,810	A		1/1991	Semrad
4,987,895	A		1/1991	Heimlich
5,019,034	A		5/1991	Weaver et al.
5,031,775	A		7/1991	Kane
5,052,391	A		10/1991	Silberstone et al.
5,053,013	A		10/1991	Ensminger et al.
5,058,605	A		10/1991	Slovak
5,071,558	A		12/1991	Itoh
5,098,843	A		3/1992	Calvin
5,122,137	A		6/1992	Lennox
5,134,070	A		7/1992	Casnig
5,137,517	A		8/1992	Loney et al.
5,141,499	A		8/1992	Zappacosta
D329,496	S		9/1992	Wotton
5,156,597	A		10/1992	Verreet et al.
5,173,158	A		12/1992	Schmukler
5,186,715	A		2/1993	Phillips et al.
5,186,800	A		2/1993	Dower
5,188,592	A		2/1993	Hakki
5,190,541	A		3/1993	Abele et al.
5,192,312	A		3/1993	Orton
5,193,537	A		3/1993	Freeman
5,209,723	A		5/1993	Twardowski et al.
5,215,530	A		6/1993	Hogan
5,224,933	A		7/1993	Bromander
5,227,730	A		7/1993	King et al.
5,242,415	A		9/1993	Kantrowitz et al.
5,273,525	A		12/1993	Hofmann
D343,687	S		1/1994	Houghton et al.
5,277,201	A		1/1994	Stern
5,279,564	A		1/1994	Taylor
5,281,213	A		1/1994	Milder
5,283,194	A		2/1994	Schmukler
5,290,263	A		3/1994	Wigness et al.
5,308,325	A		5/1994	Quinn et al.
5,308,338	A		5/1994	Helfrich
5,318,543	A		6/1994	Ross et al.
5,318,563	A		6/1994	Malis et al.
5,328,451	A		7/1994	Davis et al.
5,334,167	A		8/1994	Cocanower
5,348,554	A		9/1994	Imran et al.
D351,661	S		10/1994	Fischer
5,383,917	A		1/1995	Desai et al.
5,389,069	A		2/1995	Weaver
5,391,158	A		2/1995	Peters
5,403,311	A		4/1995	Abele et al.
5,405,320	A		4/1995	Twardowski et al.
5,425,752	A		6/1995	Vu Nguyen
5,439,440	A		8/1995	Hofmann
5,458,625	A		10/1995	Kendall
5,484,400	A		1/1996	Edwards et al.
5,484,401	A		1/1996	Rodriguez et al.
5,533,999	A		7/1996	Hood et al.
5,536,240	A		7/1996	Edwards et al.
5,536,267	A		7/1996	Edwards et al.
5,540,737	A		7/1996	Fenn
5,546,940	A		8/1996	Panescu et al.
5,562,720	A		10/1996	Stern et al.
5,575,811	A		11/1996	Reid et al.
D376,652	S		12/1996	Hunt et al.
5,582,588	A		12/1996	Sakurai et al.
5,586,982	A		12/1996	Abela
5,588,424	A		12/1996	Insler et al.
5,588,960	A		12/1996	Edwards et al.
5,599,294	A		2/1997	Edwards et al.
5,599,311	A		2/1997	Raulerson
5,616,126	A		4/1997	Malekmehr et al.
5,620,479	A		4/1997	Diederich
5,626,146	A		5/1997	Barber et al.
D380,272	S		6/1997	Partika et al.
5,634,899	A		6/1997	Shapland et al.
5,643,197	A		7/1997	Brucker et al.
5,645,855	A		7/1997	Lorenz
5,672,173	A		9/1997	Gough et al.
5,674,267	A		10/1997	Mir et al.
5,683,384	A		11/1997	Gough et al.
5,687,723	A		11/1997	Avitall
5,690,620	A		11/1997	Knott
5,697,905	A		12/1997	d'Ambrosio
5,700,252	A		12/1997	Klingenstein
5,702,359	A		12/1997	Hofmann et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,718,246 A 2/1998 Vona
5,720,921 A 2/1998 Meserol
5,735,847 A 4/1998 Gough et al.
5,752,939 A 5/1998 Makoto
5,778,894 A 7/1998 Dorogi et al.
5,782,882 A 7/1998 Lerman et al.
5,800,378 A 9/1998 Edwards et al.
5,800,484 A 9/1998 Gough et al.
5,807,272 A 9/1998 Kun et al.
5,807,306 A 9/1998 Shapland et al.
5,807,395 A 9/1998 Mulier et al.
5,810,742 A 9/1998 Pearlman
5,810,762 A 9/1998 Hofmann
5,830,184 A 11/1998 Basta
5,836,897 A 11/1998 Sakurai et al.
5,836,905 A 11/1998 Lemelson et al.
5,843,026 A 12/1998 Edwards et al.
5,843,182 A 12/1998 Goldstein
5,865,787 A 2/1999 Shapland et al.
5,868,708 A 2/1999 Hart et al.
5,873,849 A 2/1999 Bernard
5,904,648 A 5/1999 Amdt et al.
5,919,142 A 7/1999 Boone et al.
5,919,191 A 7/1999 Lennox et al.
5,921,982 A 7/1999 Lesh et al.
5,944,710 A 8/1999 Dev et al.
5,947,284 A 9/1999 Foster
5,947,889 A 9/1999 Hehrlein
5,951,546 A 9/1999 Lorentzen
5,954,745 A 9/1999 Gertler et al.
5,957,919 A 9/1999 Laufer
5,957,963 A 9/1999 Dobak
5,968,006 A 10/1999 Hofmann
5,983,131 A 11/1999 Weaver et al.
5,984,896 A 11/1999 Boyd
5,991,697 A 11/1999 Nelson et al.
5,999,847 A 12/1999 Elstrom
6,004,339 A 12/1999 Wijay
6,009,347 A 12/1999 Hofmann
6,009,877 A 1/2000 Edwards
6,010,613 A 1/2000 Walters et al.
6,016,452 A 1/2000 Kasevich
6,029,090 A 2/2000 Herbst
6,041,252 A 3/2000 Walker et al.
6,043,066 A 3/2000 Mangano et al.
6,050,994 A 4/2000 Sherman
6,055,453 A 4/2000 Hofmann et al.
6,059,780 A 5/2000 Gough et al.
6,066,134 A 5/2000 Eggers et al.
6,068,121 A 5/2000 McGlinch
6,068,650 A 5/2000 Hofmann et al.
6,071,281 A 6/2000 Burnside et al.
6,074,374 A 6/2000 Fulton
6,074,389 A 6/2000 Levine et al.
6,085,115 A 7/2000 Weaver et al.
6,090,016 A 7/2000 Kuo
6,090,105 A 7/2000 Zepeda et al.
6,090,106 A 7/2000 Goble et al.
D430,015 S 8/2000 Himbert et al.
6,096,035 A 8/2000 Sodhi et al.
6,102,885 A 8/2000 Bass
6,106,521 A 8/2000 Blewett et al.
6,109,270 A 8/2000 Mah et al.
6,110,192 A 8/2000 Ravenscroft et al.
6,113,593 A 9/2000 Tu et al.
6,116,330 A 9/2000 Salyer
6,122,599 A 9/2000 Mehta
6,123,701 A 9/2000 Nezhat
6,132,397 A 10/2000 Davis et al.
6,132,419 A 10/2000 Hofmann
6,134,460 A 10/2000 Chance
6,139,545 A 10/2000 Utley et al.
6,150,148 A 11/2000 Nanda et al.
6,159,163 A 12/2000 Strauss et al.
6,178,354 B1 1/2001 Gibson
D437,941 S 2/2001 Frattini
6,193,715 B1 2/2001 Wrublewski et al.
6,198,970 B1 3/2001 Freed et al.
6,200,314 B1 3/2001 Sherman
6,208,893 B1 3/2001 Hofmann
6,210,402 B1 4/2001 Olsen et al.
6,212,433 B1 4/2001 Behl
6,216,034 B1 4/2001 Hofmann et al.
6,219,577 B1 4/2001 Brown, III et al.
D442,697 S 5/2001 Hajianpour
6,233,490 B1 5/2001 Kasevich
6,235,023 B1 5/2001 Lee et al.
D443,360 S 6/2001 Haberland
6,241,702 B1 6/2001 Lundquist et al.
6,241,725 B1 6/2001 Cosman
D445,198 S 7/2001 Frattini
6,258,100 B1 7/2001 Alferness et al.
6,261,831 B1 7/2001 Agee
6,277,114 B1 8/2001 Bullivant et al.
6,278,895 B1 8/2001 Bernard
6,280,441 B1 8/2001 Ryan
6,283,988 B1 9/2001 Laufer et al.
6,283,989 B1 9/2001 Laufer et al.
6,284,140 B1 9/2001 Sommermeyer et al.
6,287,293 B1 9/2001 Jones et al.
6,287,304 B1 9/2001 Eggers et al.
6,296,636 B1 10/2001 Cheng et al.
6,298,726 B1 10/2001 Adachi et al.
6,299,633 B1 10/2001 Laufer
6,300,108 B1 10/2001 Rubinsky et al.
D450,391 S 11/2001 Hunt et al.
6,312,428 B1 11/2001 Eggers et al.
6,326,177 B1 12/2001 Schoenbach et al.
6,327,505 B1 12/2001 Medhkour et al.
6,328,689 B1 12/2001 Gonzalez et al.
6,347,247 B1 2/2002 Dev et al.
6,349,233 B1 2/2002 Adams
6,351,674 B2 2/2002 Silverstone
6,387,671 B1 5/2002 Rubinsky et al.
6,398,779 B1 6/2002 Buysse et al.
6,403,348 B1 6/2002 Rubinsky et al.
6,405,732 B1 6/2002 Edwards et al.
6,411,852 B1 6/2002 Danek et al.
6,419,674 B1 7/2002 Bowser et al.
6,428,802 B1 8/2002 Atala
6,443,952 B1 9/2002 Mulier et al.
6,463,331 B1 10/2002 Edwards
6,470,211 B1 10/2002 Ideker et al.
6,482,221 B1 11/2002 Hebert et al.
6,482,619 B1 11/2002 Rubinsky et al.
6,485,487 B1 11/2002 Sherman
6,488,673 B1 12/2002 Laufer et al.
6,488,678 B2 12/2002 Sherman
6,488,680 B1 12/2002 Francischelli et al.
6,491,706 B1 12/2002 Alferness et al.
6,493,589 B1 12/2002 Medhkour et al.
6,493,592 B1 12/2002 Leonard et al.
6,500,173 B2 12/2002 Underwood et al.
6,503,248 B1 1/2003 Levine
6,506,189 B1 1/2003 Rittman et al.
6,514,248 B1 2/2003 Eggers et al.
6,520,183 B2 2/2003 Amar
6,526,320 B2 2/2003 Mitchell
D471,640 S 3/2003 McMichael et al.
D471,641 S 3/2003 McMichael et al.
6,530,922 B2 3/2003 Cosman et al.
6,533,784 B2 3/2003 Truckai et al.
6,537,976 B1 3/2003 Gupta
6,558,378 B2 5/2003 Sherman et al.
6,562,604 B2 5/2003 Rubinsky et al.
6,569,162 B2 5/2003 He
6,575,969 B1 6/2003 Rittman et al.
6,589,161 B2 7/2003 Corcoran
6,592,594 B2 7/2003 Rimbaugh et al.
6,607,529 B1 8/2003 Jones et al.
6,610,054 B1 8/2003 Edwards et al.
6,611,706 B2 8/2003 Avrahami et al.
6,613,211 B1 9/2003 Mccormick et al.
6,616,657 B2 9/2003 Simpson et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,627,421 B1 9/2003 Unger et al.
D480,816 S 10/2003 McMichael et al.
6,634,363 B1 10/2003 Danek et al.
6,638,253 B2 10/2003 Breznock
6,653,091 B1 11/2003 Dunn et al.
6,666,858 B2 12/2003 Lafontaine
6,669,691 B1 12/2003 Taimisto
6,673,070 B2 1/2004 Edwards et al.
6,678,558 B1 1/2004 Dimmer et al.
6,689,096 B1 2/2004 Loubens et al.
6,692,493 B2 2/2004 Mcgovern et al.
6,694,979 B2 2/2004 Deem et al.
6,694,984 B2 2/2004 Habib
6,695,861 B1 2/2004 Rosenberg et al.
6,697,669 B2 2/2004 Dev et al.
6,697,670 B2 2/2004 Chomenky et al.
6,702,808 B1 3/2004 Kreindel
6,712,811 B2 3/2004 Underwood et al.
D489,973 S 5/2004 Root et al.
6,753,171 B2 6/2004 Karube et al.
6,761,716 B2 7/2004 Kadhiresan et al.
D495,807 S 9/2004 Agbodoe et al.
6,795,728 B2 9/2004 Chornenky et al.
6,801,804 B2 10/2004 Miller et al.
6,812,204 B1 11/2004 McHale et al.
6,837,886 B2 1/2005 Collins et al.
6,847,848 B2 1/2005 Sterzer et al.
6,860,847 B2 3/2005 Alferness et al.
6,865,416 B2 3/2005 Dev et al.
6,881,213 B2 4/2005 Ryan et al.
6,892,099 B2 5/2005 Jaafar et al.
6,895,267 B2 5/2005 Panescu et al.
6,905,480 B2 6/2005 McGuckin et al.
6,912,417 B1 6/2005 Bernard et al.
6,927,049 B2 8/2005 Rubinsky et al.
6,941,950 B2 9/2005 Wilson et al.
6,942,681 B2 9/2005 Johnson
6,958,062 B1 10/2005 Gough et al.
6,960,189 B2 11/2005 Bates et al.
6,962,587 B2 11/2005 Johnson et al.
6,972,013 B1 12/2005 Zhang et al.
6,972,014 B2 12/2005 Eum et al.
6,989,010 B2 1/2006 Francischelli et al.
6,994,689 B1 2/2006 Zadno-Azizi et al.
6,994,706 B2 2/2006 Chornenky et al.
7,011,094 B2 3/2006 Rapacki et al.
7,012,061 B1 3/2006 Reiss et al.
7,027,869 B2 4/2006 Danek et al.
7,036,510 B2 5/2006 Zgoda et al.
7,053,063 B2 5/2006 Rubinsky et al.
7,054,685 B2 5/2006 Dimmer et al.
7,063,698 B2 6/2006 Whayne et al.
7,087,040 B2 8/2006 McGuckin et al.
7,097,612 B2 8/2006 Bertolero et al.
7,100,616 B2 9/2006 Springmeyer
7,113,821 B1 9/2006 Sun et al.
7,130,697 B2 10/2006 Chornenky et al.
7,211,083 B2 5/2007 Chornenky et al.
7,232,437 B2 6/2007 Berman et al.
7,250,048 B2 7/2007 Francischelli et al.
D549,332 S 8/2007 Matsumoto et al.
7,257,450 B2 8/2007 Auth et al.
7,264,002 B2 9/2007 Danek et al.
7,267,676 B2 9/2007 Chornenky et al.
7,273,055 B2 9/2007 Danek et al.
7,291,146 B2 11/2007 Steinke et al.
7,331,940 B2 2/2008 Sommerich
7,331,949 B2 2/2008 Marisi
7,341,558 B2 3/2008 Torre et al.
7,344,533 B2 3/2008 Pearson et al.
D565,743 S 4/2008 Phillips et al.
D571,478 S 6/2008 Horacek
7,387,626 B2 6/2008 Edwards et al.
7,399,747 B1 7/2008 Clair et al.
D575,399 S 8/2008 Matsumoto et al.
D575,402 S 8/2008 Sandor
7,419,487 B2 9/2008 Johnson et al.
7,434,578 B2 10/2008 Dillard et al.
7,449,019 B2 11/2008 Uchida et al.
7,451,765 B2 11/2008 Adler
7,455,675 B2 11/2008 Schur et al.
7,476,203 B2 1/2009 DeVore et al.
7,520,877 B2 4/2009 Lee et al.
7,533,671 B2 5/2009 Gonzalez et al.
D595,422 S 6/2009 Mustapha
7,544,301 B2 6/2009 Shah et al.
7,549,984 B2 6/2009 Mathis
7,565,208 B2 7/2009 Harris et al.
7,571,729 B2 8/2009 Saadat et al.
7,632,291 B2 12/2009 Stephens et al.
7,655,004 B2 2/2010 Long
7,674,249 B2 3/2010 Ivorra et al.
7,680,543 B2 3/2010 Azure
D613,418 S 4/2010 Ryan et al.
7,718,409 B2 5/2010 Rubinsky et al.
7,722,606 B2 5/2010 Azure
7,742,795 B2 6/2010 Stone et al.
7,765,010 B2 7/2010 Chornenky et al.
7,771,401 B2 8/2010 Hekmat et al.
RE42,016 E 12/2010 Chornenky et al.
D630,321 S 1/2011 Hamilton
D631,154 S 1/2011 Hamilton
RE42,277 E 4/2011 Jaafar et al.
7,918,852 B2 4/2011 Tullis et al.
7,937,143 B2 5/2011 Demarais et al.
7,938,824 B2 5/2011 Chornenky et al.
7,951,582 B2 5/2011 Gazit et al.
7,955,827 B2 6/2011 Rubinsky et al.
RE42,835 E 10/2011 Chornenky et al.
D647,628 S 10/2011 Helfteren
8,048,067 B2 11/2011 Davalos et al.
RE43,009 E 12/2011 Chornenky et al.
8,109,926 B2 2/2012 Azure
8,114,070 B2 2/2012 Rubinsky et al.
8,162,918 B2 4/2012 Ivorra et al.
8,187,269 B2 5/2012 Shadduck et al.
8,221,411 B2 7/2012 Francischelli et al.
8,231,603 B2 7/2012 Hobbs et al.
8,240,468 B2 8/2012 Wilkinson et al.
8,251,986 B2 8/2012 Chornenky et al.
8,267,927 B2 9/2012 Dalal et al.
8,267,936 B2 9/2012 Hushka et al.
8,282,631 B2 10/2012 Davalos et al.
8,298,222 B2 10/2012 Rubinsky et al.
8,348,921 B2 1/2013 Ivorra et al.
8,361,066 B2 1/2013 Long et al.
D677,798 S 3/2013 Hart et al.
8,425,455 B2 4/2013 Nentwick
8,425,505 B2 4/2013 Long
8,454,594 B2 6/2013 Demarais et al.
8,465,484 B2 6/2013 Davalos et al.
8,511,317 B2 8/2013 Thapliyal et al.
8,518,031 B2 8/2013 Boyden et al.
8,562,588 B2 10/2013 Hobbs et al.
8,603,087 B2 12/2013 Rubinsky et al.
8,632,534 B2 1/2014 Pearson et al.
8,634,929 B2 1/2014 Chornenky et al.
8,647,338 B2 2/2014 Chornenky et al.
8,715,276 B2 5/2014 Thompson et al.
8,753,335 B2 6/2014 Moshe et al.
8,814,860 B2 8/2014 Davalos et al.
8,835,166 B2 9/2014 Phillips et al.
8,845,635 B2 9/2014 Daniel et al.
8,880,195 B2 11/2014 Azure
8,903,488 B2 12/2014 Callas et al.
8,906,006 B2 12/2014 Chornenky et al.
8,926,606 B2 1/2015 Davalos et al.
8,958,888 B2 2/2015 Chornenky et al.
8,968,542 B2 3/2015 Davalos et al.
8,992,517 B2 3/2015 Davalos et al.
9,005,189 B2 4/2015 Davalos et al.
9,078,665 B2 7/2015 Moss et al.
9,149,331 B2 10/2015 Deem et al.
9,173,704 B2 11/2015 Hobbs et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

9,198,733 B2 12/2015 Neal, II et al.
9,283,051 B2 3/2016 Garcia et al.
9,414,881 B2 8/2016 Callas et al.
9,598,691 B2 3/2017 Davalos
9,867,652 B2 1/2018 Sano et al.
10,117,707 B2 11/2018 Garcia et al.
10,154,874 B2 12/2018 Davalos et al.
2001/0039393 A1 11/2001 Mori et al.
2001/0044596 A1 11/2001 Jaafar
2001/0046706 A1 11/2001 Rubinsky et al.
2001/0047167 A1 11/2001 Heggeness
2001/0051366 A1 12/2001 Rubinsky et al.
2002/0002393 A1 1/2002 Mitchell
2002/0010491 A1 1/2002 Schoenbach et al.
2002/0022864 A1 2/2002 Mahvi et al.
2002/0040204 A1 4/2002 Dev et al.
2002/0049370 A1 4/2002 Laufer et al.
2002/0052601 A1 5/2002 Goldberg et al.
2002/0055731 A1 5/2002 Atala et al.
2002/0065541 A1 5/2002 Fredricks et al.
2002/0072742 A1 6/2002 Schaefer et al.
2002/0077314 A1 6/2002 Falk et al.
2002/0077676 A1 6/2002 Schroeppel et al.
2002/0082543 A1 6/2002 Park et al.
2002/0099323 A1 7/2002 Dev et al.
2002/0111615 A1 8/2002 Cosman et al.
2002/0112729 A1 8/2002 DeVore et al.
2002/0115208 A1 8/2002 Mitchell et al.
2002/0119437 A1 8/2002 Grooms et al.
2002/0133324 A1 9/2002 Weaver et al.
2002/0137121 A1 9/2002 Rubinsky et al.
2002/0138075 A1 9/2002 Edwards et al.
2002/0138117 A1 9/2002 Son
2002/0143365 A1 10/2002 Herbst
2002/0147462 A1 10/2002 Mair et al.
2002/0156472 A1 10/2002 Lee et al.
2002/0161361 A1 10/2002 Sherman et al.
2002/0183684 A1 12/2002 Dev et al.
2002/0183735 A1 12/2002 Edwards et al.
2002/0183740 A1 12/2002 Edwards et al.
2002/0188242 A1 12/2002 Wu
2002/0193784 A1 12/2002 McHale et al.
2002/0193831 A1 12/2002 Smith
2003/0009110 A1 1/2003 Tu et al.
2003/0016168 A1 1/2003 Jandrell
2003/0055220 A1 3/2003 Legrain
2003/0055420 A1 3/2003 Kadhiresan et al.
2003/0059945 A1 3/2003 Dzekunov et al.
2003/0060856 A1 3/2003 Chornenky et al.
2003/0078490 A1 4/2003 Damasco et al.
2003/0088189 A1 5/2003 Tu et al.
2003/0088199 A1 5/2003 Kawaji
2003/0096407 A1 5/2003 Atala et al.
2003/0105454 A1 6/2003 Cucin
2003/0109871 A1 6/2003 Johnson et al.
2003/0127090 A1 7/2003 Gifford et al.
2003/0130711 A1 7/2003 Pearson et al.
2003/0135242 A1 7/2003 Mongeon et al.
2003/0149451 A1 8/2003 Chomenky et al.
2003/0154988 A1 8/2003 DeVore et al.
2003/0159700 A1 8/2003 Laufer et al.
2003/0166181 A1 9/2003 Rubinsky et al.
2003/0170898 A1 9/2003 Gundersen et al.
2003/0194808 A1 10/2003 Rubinsky et al.
2003/0195385 A1 10/2003 DeVore
2003/0195406 A1 10/2003 Jenkins et al.
2003/0199050 A1 10/2003 Mangano et al.
2003/0208200 A1 11/2003 Palanker et al.
2003/0208236 A1 11/2003 Heil et al.
2003/0212394 A1 11/2003 Pearson et al.
2003/0212412 A1 11/2003 Dillard et al.
2003/0225360 A1 12/2003 Eppstein et al.
2003/0228344 A1 12/2003 Fields et al.
2004/0009459 A1 1/2004 Anderson et al.
2004/0019371 A1 1/2004 Jaafar et al.
2004/0055606 A1 3/2004 Hendricksen et al.
2004/0059328 A1 3/2004 Daniel et al.
2004/0059389 A1 3/2004 Chornenky et al.
2004/0068228 A1 4/2004 Cunningham
2004/0116965 A1 6/2004 Falkenberg
2004/0133194 A1 7/2004 Eum et al.
2004/0138715 A1 7/2004 Groeningen et al.
2004/0146877 A1 7/2004 Diss et al.
2004/0153057 A1 8/2004 Davison
2004/0176855 A1 9/2004 Badylak
2004/0193097 A1 9/2004 Hofmann et al.
2004/0199159 A1 10/2004 Lee et al.
2004/0200484 A1 10/2004 Springmeyer
2004/0206349 A1 10/2004 Alferness et al.
2004/0210248 A1 10/2004 Gordon et al.
2004/0230187 A1 11/2004 Lee et al.
2004/0236376 A1 11/2004 Miklavcic et al.
2004/0243107 A1 12/2004 Macoviak et al.
2004/0267189 A1 12/2004 Mavor et al.
2004/0267340 A1 12/2004 Cioanta et al.
2005/0010209 A1 1/2005 Lee et al.
2005/0010259 A1 1/2005 Gerber
2005/0013870 A1 1/2005 Freyman et al.
2005/0020965 A1 1/2005 Rioux et al.
2005/0043726 A1 2/2005 Mchale et al.
2005/0048651 A1 3/2005 Ryttsen et al.
2005/0049541 A1 3/2005 Behar et al.
2005/0061322 A1 3/2005 Freitag
2005/0066974 A1 3/2005 Fields et al.
2005/0143817 A1 6/2005 Hunter et al.
2005/0165393 A1 7/2005 Eppstein
2005/0171522 A1 8/2005 Christopherson
2005/0171523 A1 8/2005 Rubinsky et al.
2005/0171574 A1 8/2005 Rubinsky et al.
2005/0182462 A1 8/2005 Chornenky et al.
2005/0197619 A1 9/2005 Rule et al.
2005/0261672 A1 11/2005 Deem et al.
2005/0267407 A1 12/2005 Goldman
2005/0282284 A1 12/2005 Rubinsky et al.
2005/0288684 A1 12/2005 Aronson et al.
2005/0288702 A1 12/2005 McGurk et al.
2005/0288730 A1 12/2005 Deem et al.
2006/0004356 A1 1/2006 Bilski et al.
2006/0004400 A1 1/2006 McGurk et al.
2006/0009748 A1 1/2006 Mathis
2006/0015147 A1 1/2006 Persson et al.
2006/0020347 A1 1/2006 Barrett et al.
2006/0024359 A1 2/2006 Walker et al.
2006/0025760 A1 2/2006 Podhajsky
2006/0074413 A1 4/2006 Behzadian
2006/0079838 A1 4/2006 Walker et al.
2006/0079845 A1 4/2006 Howard et al.
2006/0079883 A1 4/2006 Elmouelhi et al.
2006/0085054 A1 4/2006 Zikorus et al.
2006/0089635 A1 4/2006 Young et al.
2006/0121610 A1 6/2006 Rubinsky et al.
2006/0142801 A1 6/2006 Demarais et al.
2006/0149123 A1 7/2006 Vidlund et al.
2006/0173490 A1 8/2006 Lafontaine et al.
2006/0182684 A1 8/2006 Beliveau
2006/0195146 A1 8/2006 Tracey et al.
2006/0212032 A1 9/2006 Daniel et al.
2006/0212078 A1 9/2006 Demarais et al.
2006/0217703 A1 9/2006 Chornenky et al.
2006/0224188 A1 10/2006 Libbus et al.
2006/0235474 A1 10/2006 Demarais
2006/0247619 A1 11/2006 Kaplan et al.
2006/0264752 A1 11/2006 Rubinsky et al.
2006/0264807 A1 11/2006 Westersten et al.
2006/0269531 A1 11/2006 Beebe et al.
2006/0276710 A1 12/2006 Krishnan
2006/0283462 A1 12/2006 Fields et al.
2006/0293713 A1 12/2006 Rubinsky et al.
2006/0293725 A1 12/2006 Rubinsky et al.
2006/0293730 A1 12/2006 Rubinsky et al.
2006/0293731 A1 12/2006 Rubinsky et al.
2006/0293734 A1 12/2006 Scott et al.
2007/0010805 A1 1/2007 Fedewa et al.
2007/0016183 A1 1/2007 Lee et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2007/0016185 A1 1/2007 Tullis et al.
2007/0021803 A1 1/2007 Deem et al.
2007/0025919 A1 2/2007 Deem et al.
2007/0043345 A1 2/2007 Davalos et al.
2007/0060989 A1 3/2007 Deem et al.
2007/0078391 A1 4/2007 Wortley et al.
2007/0088347 A1 4/2007 Young et al.
2007/0093789 A1 4/2007 Smith
2007/0096048 A1 5/2007 Clerc
2007/0118069 A1 5/2007 Persson et al.
2007/0129711 A1 6/2007 Altshuler et al.
2007/0129760 A1 6/2007 Demarais et al.
2007/0156135 A1 7/2007 Rubinsky et al.
2007/0191889 A1 8/2007 Lang
2007/0203486 A1 8/2007 Young
2007/0230757 A1 10/2007 Trachtenberg et al.
2007/0239099 A1 10/2007 Goldfarb et al.
2007/0244521 A1 10/2007 Bornzin et al.
2007/0287950 A1 12/2007 Kjeken et al.
2007/0295336 A1 12/2007 Nelson et al.
2007/0295337 A1 12/2007 Nelson et al.
2008/0015571 A1 1/2008 Rubinsky et al.
2008/0021371 A1 1/2008 Rubinsky et al.
2008/0027314 A1 1/2008 Miyazaki et al.
2008/0027343 A1 1/2008 Fields et al.
2008/0033340 A1 2/2008 Heller et al.
2008/0033417 A1 2/2008 Nields et al.
2008/0045880 A1 2/2008 Kjeken et al.
2008/0052786 A1 2/2008 Lin et al.
2008/0071262 A1 3/2008 Azure
2008/0097139 A1 4/2008 Clerc et al.
2008/0097422 A1 4/2008 Edwards et al.
2008/0103529 A1 5/2008 Schoenbach et al.
2008/0121375 A1 5/2008 Richason et al.
2008/0125772 A1 5/2008 Stone et al.
2008/0132826 A1 6/2008 Shadduck et al.
2008/0132884 A1 6/2008 Rubinsky et al.
2008/0132885 A1 6/2008 Rubinsky et al.
2008/0140064 A1 6/2008 Vegesna
2008/0146934 A1 6/2008 Czygan et al.
2008/0154259 A1 6/2008 Gough et al.
2008/0167649 A1 7/2008 Edwards et al.
2008/0171985 A1 7/2008 Karakoca
2008/0190434 A1 8/2008 Wai
2008/0200911 A1 8/2008 Long
2008/0200912 A1 8/2008 Long
2008/0208052 A1 8/2008 LePivert et al.
2008/0210243 A1 9/2008 Clayton et al.
2008/0214986 A1 9/2008 Ivorra et al.
2008/0236593 A1 10/2008 Nelson et al.
2008/0249503 A1 10/2008 Fields et al.
2008/0262489 A1 10/2008 Steinke
2008/0269586 A1 10/2008 Rubinsky et al.
2008/0269838 A1 10/2008 Brighton et al.
2008/0275465 A1 11/2008 Paul et al.
2008/0281319 A1 11/2008 Paul et al.
2008/0283065 A1 11/2008 Chang et al.
2008/0288038 A1 11/2008 Paul et al.
2008/0300589 A1 12/2008 Paul et al.
2008/0306427 A1 12/2008 Bailey
2008/0312599 A1 12/2008 Rosenberg
2009/0018206 A1 1/2009 Barkan et al.
2009/0024075 A1 1/2009 Schroeppel et al.
2009/0029407 A1 1/2009 Gazit et al.
2009/0038752 A1 2/2009 Weng et al.
2009/0062788 A1 3/2009 Long et al.
2009/0062792 A1 3/2009 Vakharia et al.
2009/0081272 A1 3/2009 Clarke et al.
2009/0105703 A1 4/2009 Shadduck
2009/0114226 A1 5/2009 Deem et al.
2009/0125009 A1 5/2009 Zikorus et al.
2009/0138014 A1 5/2009 Bonutti
2009/0143705 A1 6/2009 Danek et al.
2009/0157166 A1 6/2009 Singhal et al.
2009/0163904 A1 6/2009 Miller et al.
2009/0171280 A1 7/2009 Samuel et al.
2009/0177111 A1 7/2009 Miller et al.
2009/0186850 A1 7/2009 Kiribayashi et al.
2009/0192508 A1 7/2009 Laufer et al.
2009/0198231 A1 8/2009 Esser et al.
2009/0228001 A1 9/2009 Pacey
2009/0247933 A1 10/2009 Maor et al.
2009/0248012 A1 10/2009 Maor et al.
2009/0269317 A1 10/2009 Davalos
2009/0275827 A1 11/2009 Aiken et al.
2009/0281477 A1 11/2009 Mikus et al.
2009/0292342 A1 11/2009 Rubinsky et al.
2009/0301480 A1 12/2009 Elsakka et al.
2009/0306544 A1 12/2009 Ng et al.
2009/0306545 A1 12/2009 Elsakka et al.
2009/0318905 A1 12/2009 Bhargav et al.
2009/0326436 A1 12/2009 Rubinsky et al.
2009/0326570 A1 12/2009 Brown
2010/0004623 A1 1/2010 Hamilton et al.
2010/0023004 A1 1/2010 Francischelli et al.
2010/0030211 A1 2/2010 Davalos et al.
2010/0049190 A1 2/2010 Long et al.
2010/0057074 A1 3/2010 Roman et al.
2010/0069921 A1 3/2010 Miller et al.
2010/0087813 A1 4/2010 Long
2010/0130975 A1 5/2010 Long
2010/0147701 A1* 6/2010 Field A47L 11/4083 205/701
2010/0152725 A1 6/2010 Pearson et al.
2010/0160850 A1 6/2010 Ivorra et al.
2010/0168735 A1 7/2010 Deno et al.
2010/0174282 A1 7/2010 Demarais et al.
2010/0179530 A1 7/2010 Long et al.
2010/0196984 A1 8/2010 Rubinsky et al.
2010/0204560 A1 8/2010 Salahieh et al.
2010/0204638 A1 8/2010 Hobbs et al.
2010/0222677 A1 9/2010 Placek et al.
2010/0228234 A1 9/2010 Hyde et al.
2010/0228247 A1 9/2010 Paul et al.
2010/0241117 A1 9/2010 Paul et al.
2010/0249771 A1 9/2010 Pearson et al.
2010/0250209 A1 9/2010 Pearson et al.
2010/0255795 A1 10/2010 Rubinsky et al.
2010/0256628 A1 10/2010 Pearson et al.
2010/0256630 A1 10/2010 Hamilton, Jr. et al.
2010/0261994 A1 10/2010 Davalos et al.
2010/0286690 A1 11/2010 Paul et al.
2010/0298823 A1 11/2010 Cao et al.
2010/0331758 A1 12/2010 Davalos et al.
2011/0017207 A1 1/2011 Hendricksen et al.
2011/0034209 A1 2/2011 Rubinsky et al.
2011/0064671 A1 3/2011 Bynoe
2011/0106221 A1 5/2011 Robert et al.
2011/0112531 A1 5/2011 Landis et al.
2011/0118727 A1 5/2011 Fish et al.
2011/0118732 A1 5/2011 Rubinsky et al.
2011/0130834 A1 6/2011 Wilson et al.
2011/0144524 A1 6/2011 Fish et al.
2011/0144635 A1 6/2011 Harper et al.
2011/0144657 A1 6/2011 Fish et al.
2011/0152678 A1 6/2011 Aljuri et al.
2011/0202053 A1 8/2011 Moss et al.
2011/0217730 A1 9/2011 Gazit et al.
2011/0251607 A1 10/2011 Kruecker et al.
2011/0301587 A1 12/2011 Deem et al.
2012/0034131 A1 2/2012 Rubinsky et al.
2012/0059255 A1 3/2012 Paul et al.
2012/0071872 A1 3/2012 Rubinsky et al.
2012/0071874 A1 3/2012 Davalos et al.
2012/0085649 A1 4/2012 Sano et al.
2012/0089009 A1 4/2012 Omary et al.
2012/0090646 A1 4/2012 Tanaka et al.
2012/0095459 A1 4/2012 Callas et al.
2012/0109122 A1 5/2012 Arena et al.
2012/0130289 A1 5/2012 Demarais et al.
2012/0150172 A1 6/2012 Ortiz et al.
2012/0165813 A1 6/2012 Lee et al.
2012/0179091 A1 7/2012 Ivorra et al.
2012/0226218 A1 9/2012 Phillips et al.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2012/0226271	A1	9/2012	Callas et al.	
2012/0265186	A1	10/2012	Burger et al.	
2012/0277741	A1	11/2012	Davalos et al.	
2012/0303020	A1	11/2012	Chornenky et al.	
2012/0310236	A1	12/2012	Placek et al.	
2013/0090646	A1	4/2013	Moss et al.	
2013/0108667	A1	5/2013	Soikum et al.	
2013/0110106	A1	5/2013	Richardson	
2013/0184702	A1	7/2013	Neal, II et al.	
2013/0196441	A1	8/2013	Rubinsky et al.	
2013/0197425	A1	8/2013	Golberg et al.	
2013/0202766	A1	8/2013	Rubinsky et al.	
2013/0218157	A1	8/2013	Callas et al.	
2013/0253415	A1	9/2013	Sano et al.	
2013/0281968	A1	10/2013	Davalos et al.	
2013/0345697	A1	12/2013	Garcia et al.	
2013/0345779	A1	12/2013	Maor et al.	
2014/0039489	A1	2/2014	Davalos et al.	
2014/0046322	A1	2/2014	Callas et al.	
2014/0066913	A1*	3/2014	Sherman	A61B 18/1492 606/20
2014/0081255	A1	3/2014	Johnson et al.	
2014/0088578	A1	3/2014	Rubinsky et al.	
2014/0121663	A1	5/2014	Pearson et al.	
2014/0121728	A1	5/2014	Dhillon et al.	
2014/0163551	A1	6/2014	Maor et al.	
2014/0207133	A1	7/2014	Model et al.	
2014/0296844	A1	10/2014	Kevin et al.	
2014/0309579	A1	10/2014	Rubinsky et al.	
2014/0378964	A1	12/2014	Pearson	
2015/0088120	A1	3/2015	Garcia et al.	
2015/0088220	A1	3/2015	Callas et al.	
2015/0112333	A1	4/2015	Chorenky et al.	
2015/0126922	A1	5/2015	Willis	
2015/0164584	A1	6/2015	Davalos et al.	
2015/0173824	A1	6/2015	Davalos et al.	
2015/0201996	A1	7/2015	Rubinsky et al.	
2015/0265349	A1	9/2015	Moss et al.	
2015/0289923	A1	10/2015	Davalos et al.	
2015/0320488	A1	11/2015	Moshe et al.	
2015/0327944	A1	11/2015	Davalos et al.	
2016/0022957	A1	1/2016	Hobbs et al.	
2016/0066977	A1	3/2016	Neal et al.	
2016/0074114	A1	3/2016	Pearson et al.	
2016/0113708	A1	4/2016	Moss et al.	
2016/0143698	A1	5/2016	Garcia et al.	
2016/0235470	A1	8/2016	Callas et al.	
2016/0287313	A1	10/2016	Rubinsky et al.	
2016/0338761	A1	11/2016	Chornenky et al.	
2016/0354142	A1	12/2016	Pearson et al.	
2017/0035501	A1	2/2017	Chornenky et al.	
2017/0189579	A1	7/2017	Davalos	
2017/0209620	A1	7/2017	Davalos et al.	
2017/0266438	A1	9/2017	Sano	
2017/0360326	A1	12/2017	Davalos	
2018/0125565	A1	5/2018	Sano et al.	
2018/0161086	A1	6/2018	Davalos et al.	
2019/0029749	A1	1/2019	Garcia	
2019/0069945	A1	3/2019	Davalos et al.	

FOREIGN PATENT DOCUMENTS

AU	2005271471	A2	2/2006
AU	2006321570	A1	6/2007
AU	2006321574	A1	6/2007
AU	2006321918	A1	6/2007
CA	2297846	A1	2/1999
CA	2378110	A1	2/2001
CA	2445392	A1	11/2002
CA	2458676	A1	3/2003
CA	2487284	A1	12/2003
CA	2575792	A1	2/2006
CA	2631940	A1	6/2007
CA	2631946	A1	6/2007
CA	2632604	A1	6/2007
CA	2751462	A1	11/2010
CN	1525839	A	9/2004
CN	101534736	A	9/2009
CN	102238921	A	11/2011
CN	102421386	A	4/2012
DE	863111		1/1953
DE	4000893		7/1991
DE	60038026		2/2009
EP	0218275	A1	4/1987
EP	0339501	A2	11/1989
EP	0378132	A	7/1990
EP	0533511	A1	3/1993
EP	0998235	A1	5/2000
EP	0528891	B1	7/2000
EP	1196550	A2	4/2002
EP	1439792	A1	7/2004
EP	1442765	A1	8/2004
EP	1462065	A2	9/2004
EP	1061983	B1	11/2004
EP	1493397	A1	1/2005
EP	1506039	A1	2/2005
EP	0935482	B1	5/2005
EP	1011495	B1	11/2005
EP	1796568	A1	6/2007
EP	1207797	B1	2/2008
EP	1406685	B1	6/2008
EP	1424970	B1	12/2008
EP	2381829	A1	11/2011
EP	2413833	A1	2/2012
EP	1791485	B1	12/2014
EP	2373241	B1	1/2015
EP	1962710	B1	8/2015
EP	1962708	B1	9/2015
EP	1962945	B1	4/2016
ES	2300272		6/2008
ES	2315493		4/2009
JP	2001510702	A	8/2001
JP	2003505072	A	2/2003
JP	2003506064	A	2/2003
JP	2004203224	A	7/2004
JP	2004525726	A	8/2004
JP	2004303590	A	10/2004
JP	2005501596	A	1/2005
JP	2005526579	A	9/2005
JP	2008508946	A	3/2008
JP	4252316	B2	4/2009
JP	2009518130	A	5/2009
JP	2009518150	A	5/2009
JP	2009518151	A	5/2009
JP	2009532077	A	9/2009
JP	2010503496	A	2/2010
JP	2011137025		7/2011
JP	2011137025	A	7/2011
JP	2012510332	A	5/2012
JP	2012515018	A	7/2012
JP	2012521863	A	9/2012
KR	101034682	A	5/2011
WO	9104014		4/1991
WO	9634571		11/1996
WO	9639531	A	12/1996
WO	9810745		3/1998
WO	9814238	A	4/1998
WO	9901076		1/1999
WO	9904710		2/1999
WO	0020554	A	4/2000
WO	0107583	A	2/2001
WO	0107584	A	2/2001
WO	0107585	A	2/2001
WO	0110319	A	2/2001
WO	0148153	A	7/2001
WO	2001048153	A1	7/2001
WO	0170114	A1	9/2001
WO	0181533	A	11/2001
WO	02078527	A	10/2002
WO	02089686	A	11/2002
WO	02100459	A	12/2002
WO	2003020144	A1	3/2003
WO	2003047684	A2	6/2003

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO	03099382	A	12/2003
WO	2004037341	A2	5/2004
WO	2004080347	A2	9/2004
WO	2005065284	A	7/2005
WO	2006017666	A2	2/2006
WO	2006031541	A1	3/2006
WO	2006130194	A2	12/2006
WO	2007067628	A1	6/2007
WO	2007067937	A2	6/2007
WO	2007067938	A2	6/2007
WO	2007067939	A2	6/2007
WO	2007067940	A2	6/2007
WO	2007067941	A2	6/2007
WO	2007067943	A2	6/2007
WO	2007070361	A2	6/2007
WO	2007100727	A2	9/2007
WO	2007123690	A2	11/2007
WO	2008063195	A1	5/2008
WO	2008034103	A3	11/2008
WO	2009046176	A1	4/2009
WO	2007137303		7/2009
WO	2009134876	A	11/2009
WO	2009135070	A1	11/2009
WO	2009137800	A2	11/2009
WO	2010064154	A1	6/2010
WO	2010080974	A1	7/2010
WO	2010117806	A1	10/2010
WO	2010118387	A	10/2010
WO	2010132472	A1	11/2010
WO	2010151277	A	12/2010
WO	2011047387	A	4/2011
WO	2011062653	A1	5/2011
WO	2011072221	A1	6/2011
WO	2012051433	A2	4/2012
WO	2012071526	A	5/2012
WO	2012088149	A	6/2012
WO	2015175570	A1	11/2015
WO	2016100325	A1	6/2016
WO	2016164930	A1	10/2016

OTHER PUBLICATIONS

Co-pending U.S. Appl. No. 15/011,752, filed Feb. 1, 2016.

A.I. Daud et al., "Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma," Journal of Clinical Oncology, 26, pp. 5896-5903, 2008.

Agerholm-Larsen, B., et al., "Preclinical Validation of Electrochemotherapy as an Effective Treatment for Brain Tumors", Cancer Research 71: 3753-3762 (2011).

Alberts et al., "Molecular Biology of the Cell," 3rd edition, Garland Science, New York, 1994, 1 page.

Al-Sakere et al., "Tumor ablation with irreversible electroporation." PLoS ONE, Issue 11, e1135, 8 pages, 2007.

Amasha, et al., Quantitative Assessment of Impedance Tomography for Temperature Measurements in Microwave Hyperthermia, Clin. Phys. Physiol. Meas., 1998, Suppl. A, 49-53.

Andreason, Electroporation as a Technique for the Transfer of Macromolecules into Mammalian Cell Lines, J. Tiss. Cult. Meth., 15:56-62, 1993.

Appelbaum et al., "US Findings after Irreversible Electroporation Ablation: Radiologic-Pathologic Correlation," Radiology, 262, pp. 117-125, Jan. 1, 2012.

Arena et al. "High-Frequency Irreversible Electroporation (H-FIRE) for Non-thermal Ablation without Muscle Contraction." Biomed. Eng. Online, vol. 10, 20 pages (2011).

Arena, C.B., et al., "A three-dimensional in vitro tumor platform for modeling therapeutic irreversible electroporation." Biophysical Journal, 2012.103(9): p. 2033-2042.

Arena, Christopher B., et al., "Towards the development of latent heat storage electrodes for electroporation-based therapies", Applied Physics Letters, 101, 083902 (2012).

Arena, Christopher B., et al.,"Phase Change Electrodes for Reducing Joule Heating During Irreversible Electroporation". Proceedings of the ASME 2012 Summer Bioengineering Conference, SBC2012, Jun. 20-23, 2012, Fajardo, Puerto Rico.

Asami et al., "Dielectric properties of mouse lymphocytes and erythrocytes." Biochimica et Biophysica Acta (BBA)—Molecular Cell Research, 1010 (1989) pp. 49-55.

Bagla, S. and Papadouris, D., "Percutaneous Irreversible Electroporation of Surgically Unresectable Pancreatic Cancer: A Case Report" J. Vascular Int. Radiol. 23(1), 142-145 (2012).

Baker, et al., Calcium-Dependent Exocytosis in Bovine Adrenal Medullary Cells with Leaky Plasma Membranes, Nature, vol. 276, pp. 620-622, 1978.

Ball, C., K.R. Thomson, and H. Kavnoudias, "Irreversible electroporation: a new challenge in "out of-operating theater" anesthesia." Anesth Analg, 2010. 110(5): p. 1305-9.

Bancroft, et al., Design of a Flow Perfusion Bioreactor System for Bone Tissue-Engineering Applications, Tissue Engineering, vol. 9, No. 3, 2003, p. 549-554.

Baptista et al., "The Use of Whole Organ Decellularization for the Generation of a Vascularized Liver Organoid," Heptatology, vol. 53, No. 2, pp. 604-617 (2011).

Barber, Electrical Impedance Tomography Applied Potential Tomography, Advances in Biomedical Engineering, Beneken and Thevenin, eds., IOS Press, pp. 165-173, 1993.

Beebe, S.J., et al., "Diverse effects of nanosecond pulsed electric fields on cells and tissues", DNA and Cell Biology, 22(12): 785-796 (2003).

Beebe, S.J., et al., Nanosecond pulsed electric field (nsPEF) effects on cells and tissues: apoptosis induction and tumor growth inhibition. PPPS-2001 Pulsed Power Plasma Science 2001, 28th IEEE International Conference on Plasma Science and 13th IEEE International Pulsed Power Conference, Digest of Technical Papers (Cat. No. 01CH37251). IEEE, Part vol. 1, 2001, pp. 211-215, vol. I, Piscataway, NJ, USA.

Ben-David, et al., "Characterization of Irreversible Electroporation Ablation in Vivo Porcine Liver," Am J Roentgenol, vol. 198, pp. W62-W68, 2012.

Blad, et al., Impedance Spectra of Tumour Tissue in Comparison with Normal Tissue; a Possible Clinical Application for Electrical Impedance Tomography, Physiol. Meas. 17 (1996) A105-A115.

Bolland, F., et al., "Development and characterisation of a full-thickness acellular porcine bladder matrix for tissue engineering", Biomaterials, Elsevier Science Publishers, Barking, GB, vol. 28, No. 6, Nov. 28, 2006, pp. 1061-1070.

Boone, K., Barber, D. & Brown, B. Review—Imaging with electricity: report of the European Concerted Action on Impedance Tomography. J. Med. Eng. Technol. 21, 201-232 (1997).

Bower et al., "Irreversible electroporation of the pancreas: definitive local therapy without systemic effects." Journal of surgical oncology, 2011. 104(1): p. 22-28.

BPH Management Strategies: Improving Patient Satisfaction, Urology Times, May 2001, vol. 29, Supplement 1.

Brown, et al., Blood Flow Imaging Using Electrical Impedance Tomography, Clin. Phys. Physiol. Meas., 1992, vol. 13, Suppl. A, 175-179.

Brown, S.G., Phototherapy of tumors. World J. Surgery, 1983. 7: p. 700-9.

Cannon et al., "Safety and early efficacy of irreversible electroporation for hepatic tumors in proximity to vital structures." Journal of Surgical Oncology, 6 pages (2012).

Carpenter A.E. et al., "CellProfiler: image analysis software for identifying and quantifying cell phenotypes." Genome Biol. 2006; 7(10): R100. Published online Oct. 31, 2006, 11 pages.

Cemazar M, Parkins CS, Holder AL, Chaplin DJ, Tozer GM, et al., "Electroporation of human microvascular endothelial cells: evidence for an anti-vascular mechanism of electrochemotherapy", Br J Cancer 84: 565-570 (2001).

Chandrasekar, et al., Transurethral Needle Ablation of the Prostate (TUNA)—a Propsective Study, Six Year Follow Up, (Abstract), Presented at 2001 National Meeting, Anaheim, CA, Jun. 5, 2001.

Chang, D.C., "Cell Poration and Cell-Fusion Using an Oscillating Electric-Field". Biophysical Journal, 56(4): p. 641-652 (1989).

(56) **References Cited**

OTHER PUBLICATIONS

Charpentier, K.P., et al., "Irreversible electroporation of the pancreas in swine: a pilot study." HPB: the official journal of the International Hepato Pancreato Biliary Association, 2010. 12(5): p. 348-351.
Chen et al., "Classification of cell types using a microfluidic device for mechanical and electrical measurement on single cells." Lab on a Chip, vol. 11, pp. 3174-3181 (2011).
Chen, M.T., et al., "Two-dimensional nanosecond electric field mapping based on cell electropermeabilization", PMC Biophys, 2(1):9 (2009).
Clark et al., "The electrical properties of resting and secreting pancreas." The Journal of Physiology, vol. 189, pp. 247-260 (1967).
Coates, C.W.,et al., "The Electrical Discharge of the Electric Eel, Electrophorous Electricus," Zoologica, 1937, 22(1), pp. 1-32.
Cook, et al., ACT3: A High-Speed, High-Precision Electrical Impedance Tomograph, IEEE Transactions on Biomedical Engineering, vol. 41, No. 8, Aug. 1994.
Co-pending U.S. Appl. No. 10/571,162, filed Oct. 18, 2006 (published as 2007/0043345 on Feb. 22, 2007).
Co-Pending U.S. Appl. No. 12/432,295, filed Apr. 29, 2009.
Co-Pending U.S. Appl. No. 12/609,779, filed Oct. 30, 2009.
Co-pending U.S. Appl. No. 12/751,826, filed Mar. 31, 2010 (published as 2010/0250209 on Sep. 30, 2010).
Co-pending U.S. Appl. No. 12/751,854, filed Mar. 31, 2010 (published as 2010/0249771 on Sep. 30, 2010).
Co-Pending U.S. Appl. No. 12/757,901, filed Apr. 9, 2010.
Co-Pending U.S. Appl. No. 12/906,923, filed Oct. 18, 2010.
Co-Pending U.S. Appl. No. 14/558,631, filed Dec. 2, 2014.
Co-Pending U.S. Appl. No. 14/808,679, filed Jul. 24, 2015 and Published as U.S. Publication No. 2015/0327944 on Nov. 19, 2015.
Co-Pending Application No. PCT/US04/43477, filed Dec. 21, 2004.
Co-Pending Application No. PCT/US09/42100, filed Apr. 29, 2009.
Co-Pending Application No. PCT/US09/62806, filed Oct. 30, 2009.
Co-Pending Application No. PCT/US10/30629, filed Apr. 9, 2010.
Co-Pending Application No. PCT/US10/53077, filed Oct. 18, 2010.
Co-Pending Application No. PCT/US11/62067, filed Nov. 23, 2011.
Co-Pending Application No. PCT/US11/66239, filed Dec. 20, 2011.
Co-Pending Application No. PCT/US15/30429, filed May 12, 2015.
Co-Pending Application No. PCT/US15/30429, International Search Report and Written Opinion dated Oct. 16, 2015, 19 pages.
Co-Pending Application No. PCT/US2010/029243, filed Mar. 30, 2010, published as WO 2010/117806 on Oct. 14, 2010.
Co-Pending Application No. PCT/US2015/030429, Published on Nov. 19, 2015 as WO 2015/175570.
Co-Pending U.S. Appl. No. 12/491,151, filed Jun. 24, 2009.
Co-Pending U.S. Appl. No. 13/332,133, Final Office Action dated Dec. 26, 2014, 7 pages.
Co-Pending U.S. Appl. No. 13/332,133, Final Office Action dated Mar. 30, 2016, 15 pages.
Co-Pending U.S. Appl. No. 13/332,133, Non-Final Office Action dated Oct. 16, 2015, 9 pages.
Co-Pending U.S. Appl. No. 13/332,133, Non-Final Office Action dated Sep. 4, 2014, 13 pages.
Co-Pending U.S. Appl. No. 13/332,133, Response to Final Office Action filed Jun. 29, 2016, 9 pages.
Co-Pending U.S. Appl. No. 13/332,133, Response to Final Office Action filed Mar. 26, 2015, 11 pages.
Co-Pending U.S. Appl. No. 13/332,133, Response to Non-Final Office Action filed Dec. 4, 2014, 9 pages.
Co-Pending U.S. Appl. No. 13/332,133, Response to Non-Final Office Action filed Feb. 16, 2016, 12 pages.
Co-Pending U.S. Appl. No. 13/332,133, filed Dec. 20, 2011.
Co-Pending U.S. Appl. No. 13/550,307, filed Jul. 16, 2012.
Co-Pending U.S. Appl. No. 13/919,640, filed Jun. 17, 2013.
Co-Pending U.S. Appl. No. 13/958,152, filed Aug. 2, 2013.
Co-Pending U.S. Appl. No. 13/989,175, filed May 23, 2013.
Co-Pending U.S. Appl. No. 14/012,832, filed Aug. 28, 2013.
Co-Pending U.S. Appl. No. 14/017,210, filed Sep. 3, 2013.
Co-Pending U.S. Appl. No. 14/627,046, filed Feb. 20, 2015.
Co-Pending U.S. Appl. No. 14/686,380, filed Apr. 14, 2015.
Co-Pending U.S. Appl. No. 14/940,863, filed Nov. 13, 2015 and Published as US 2016/0066977 on Mar. 10, 2016.
Corovic et al., "Analytical and numerical quantification and comparison of the local electric field in the tissue for different electrode configurations," Biomed Eng Online, 6, 14 pages, 2007.
Cowley, Good News for Boomers, Newsweek, Dec. 30, 1996/Jan. 6, 1997.
Cox, et al., Surgical Treatment of Atrial Fibrillation: A Review, Europace (2004) 5, S20-S-29.
Crowley, Electrical Breakdown of Biomolecular Lipid Membranes as an Electromechanical Instability, Biophysical Journal, vol. 13, pp. 711-724, 1973.
Dahl et al., "Nuclear shape, mechanics, and mechanotransduction." Circulation Research vol. 102, pp. 1307-1318 (2008).
Daud, A.I., et al., "Phase I Trial of Interleukin-12 Plasmid Electroporation in Patients With Metastatic Melanoma," Journal of Clinical Oncology, 26, 5896-5903, Dec. 20, 2008.
Davalos et al., "Electrical impedance tomography for imaging tissue electroporation," IEEE Transactions on Biomedical Engineering, 51, pp. 761-767, 2004.
Davalos et al., "Theoretical analysis of the thermal effects during in vivo tissue electroporation." Bioelectrochemistry, vol. 61(1-2): pp. 99-107, 2003.
Davalos et al., "Tissue ablation with irreversible electroporation." Annals of Biomedical Engineering, vol. 33, No. 2, pp. 223-231 (Feb. 2005).
Davalos, et al., A Feasibility Study for Electrical Impedance Tomography as a Means to Monitor T issue Electroporation for Molecular Medicine, IEEE Transactions on Biomedical Engineering, vol. 49, No. 4, Apr. 2002.
Davalos, R. V. & Rubinsky, B. Temperature considerations during irreversible electroporation. International Journal of Heat and Mass Transfer 51, 5617-5622, doi:10.1016/j.ijheatmasstransfer.2008.04.046 (2008).
Davalos, Real-Time Imaging for Molecular Medicine through Electrical Impedance Tomography of Electroporation, Dissertation for Ph.D. In Engineering-Mechanical Engineering, Graduate Division of University of California, Berkeley, 2002.
De Vuyst, E., et al., "In situ bipolar Electroporation for localized cell loading with reporter dyes and investigating gap unctional coupling", Biophysical Journal, 94(2): p. 469-479 (2008).
Dean, Nonviral Gene Transfer to Skeletal, Smooth, and Cardiac Muscle in Living Animals, Am J. Physiol Cell Physiol 289: 233-245, 2005.
Demirbas, M. F., "Thermal Energy Storage and Phase Change Materials: An Overview" Energy Sources Part B 1(1), 85-95 (2006).
Dev, et al., Medical Applications of Electroporation, IEEE Transactions of Plasma Science, vol. 28, No. 1, pp. 206-223, Feb. 2000.
Dev, et al., Sustained Local Delivery of Heparin to the Rabbit Arterial Wall with an Electroporation Catheter, Catheterization and Cardiovascular Diagnosis, Nov. 1998, vol. 45, No. 3, pp. 337-343.
Duraiswami, et al., Boundary Element Techniques for Efficient 2-D and 3-D Electrical Impedance Tomography, Chemical Engineering Science, vol. 52, No. 13, pp. 2185-2196, 1997.
Duraiswami, et al., Efficient 2D and 3D Electrical Impedance Tomography Using Dual Reciprocity Boundary Element Techniques, Engineering Analysis with Boundary Elements 22, (1998) 13-31.
Duraiswami, et al., Solution of Electrical Impedance Tomography Equations Using Boundary Element Methods, Boundary Element Technology XII, 1997, pp. 226-237.
Edd et al., "Mathematical modeling of irreversible electroporation for treatment planning." Technology in Cancer Research and Treatment, vol. 6, No. 4, pp. 275-286 (2007).
Edd, J., et al., In-Vivo Results of a New Focal Tissue Ablation Technique: Irreversible Electroporaton, IEEE Trans. Biomed. Eng. 53 (2006) p. 1409-1415.
Ellis TL, Garcia PA, Rossmeisl JH, Jr., Henao-Guerrero N, Robertson J, et al., "Nonthermal irreversible electroporation for intracranial surgical applications. Laboratory investigation", J Neurosurg 114: 681-688 (2011).

(56) **References Cited**

OTHER PUBLICATIONS

Eppich et al., "Pulsed electric fields for selection of hematopoietic cells and depletion of tumor cell contaminants" Nature Biotechnology 18, pp. 882-887 (2000).

Erez, et al., Controlled Destruction and Temperature Distributions in Biological Tissues Subjected to Monoactive Electrocoagulation, Transactions of the ASME: Journal of Mechanical Design, vol. 102, Feb. 1980.

Ermolina et al., "Study of normal and malignant white blood cells by time domain dielectric spectroscopy." IEEE Transactions on Dielectrics and Electrical Insulation, 8 (2001) pp. 253-261.

Esser, A.T., et al., "Towards solid tumor treatment by irreversible electroporation: intrinsic redistribution of fields and currents in tissue". Technol Cancer Res Treat, 6(4): p. 261-74 (2007).

Esser, A.T., et al., "Towards Solid Tumor Treatment by Nanosecond Pulsed Electric Fields", Technology in Cancer Research & Treatment, 8(4): p. 289-306 (2009).

Faroja, M., et al., "Irreversible Electroporation Ablation: Is the entire Damage Nonthermal?", Radiology, 266(2), 462-470 (2013).

Fischbach et al., "Engineering tumors with 3D scaffolds." Nat Meth 4, pp. 855-860 (2007).

Flanagan et al., "Unique dielectric properties distinguish stem cells and their differentiated progeny." Stem Cells, vol. 26, pp. 656-665 (2008).

Fong et al., "Modeling Ewing sarcoma tumors in vitro with 3D scaffolds." Proceedings of the National Academy of Sciences vol. 110, pp. 6500-6505 (2013).

Foster RS, "High-intensity focused ultrasound in the treatment of prostatic disease", European Urology, 1993, vol. 23 Suppl 1, pp. 29-33.

Foster, R.S., et al., Production of Prostatic Lesions in Canines Using Transrectally Administered High-Intensity Focused Ultrasound. Eur. Urol., 1993; 23: 330-336.

Fox, et al., Sampling Conductivity Images via MCMC, Mathematics Department, Auckland University, New Zealand, May 1997.

Freeman, S.A., et al., Theory of Electroporation of Planar Bilayer-Membranes—Predictions of the Aqueous Area, Change in Capacitance, and Pore-Pore Separation. Biophysical Journal, 67(1): p. 42-56 (1994).

Garcia et al., "Irreversible electroporation (IRE) to treat brain cancer." ASME Summer Bioengineering Conference, Marco Island, FL, Jun. 25-29, 2008, 2 pages.

Garcia et al., "Non-thermal irreversible electroporation (N-TIRE) and adjuvant fractionated radiotherapeutic multimodal therapy for intracranial malignant glioma in a canine patient," Technol Cancer Res Treat, 10, pp. 73-83, 2011.

Garcia et al., "Towards a Predictive Model of Electroporation-Based Therapies using Pre-Pulse Electrical Measurements" Abstract presented in the IEEE Engineering in Medicine and Biology Conference in Aug. 28, 2012 in San Diego, California, 4 pages.

Garcia P.A., et al., "7.0-T Magnetic Resonance Imaging Characterization of Acute Blood-Brain-Barrier Disruption Achieved with Intracranial Irreversible Electroporation", PLOS ONE, Nov. 2012, 7:11, e50482.

Garcia P.A., et al., "Pilot study of irreversible electroporation for intracranial surgery", Conf Proc IEEE Eng Med Biol Soc, 2009:6513-6516, 2009.

Garcia, et al., "A Parametric Study Delineating Irreversible Electroporation from Thermal Damage Based on a Minimally Invasive Intracranial Procedure," Biomed Eng Online, vol. 10:34, 22 pages, 2011.

Garcia, P. et al. Intracranial nonthermal irreversible electroporation: in vivo analysis. J Membr Biol 236, 127-136 (2010).

Gascoyne et al., "Membrane changes accompanying the induced differentiation of Friend murine erythroleukemia cells studied by dielectrophoresis." Biochimica et Biophysica Acta (BBA)-Biomembranes, vol. 1149, pp. 119-126 (1993).

Gauger, et al., A Study of Dielectric Membrane Breakdown in the Fucus Egg, J. Membrane Biol., vol. 48, No. 3, pp. 249-264, 1979.

Gehl, et al., In Vivo Electroporation of Skeletal Muscle: Threshold, Efficacy and Relation to Electric Field Distribution, Biochimica et Biphysica Acta 1428, 1999, pp. 233-240.

Gençer, et al., Electrical Impedance Tomography: Induced-Current Imaging Achieved with a Multiple Coil System, IEEE Transactions on Biomedical Engineering, vol. 43, No. 2, Feb. 1996.

Gilbert, et al., Novel Electrode Designs for Electrochemotherapy, Biochimica et Biophysica Acta 1334, 1997, pp. 9-14.

Gilbert, et al., The Use of Ultrasound Imaging for Monitoring Cryosurgery, Proceedings 6th Annual Conference, IEEE Engineering in Medicine and Biology, 107-111, 1984.

Gilbert, T. W., et al., "Decellularization of tissues and organs", Biomaterials, Elsevier Science Publishers, Barking, GB, vol. 27, No. 19, Jul. 1, 2006, pp. 3675-3683.

Gimsa et al., "Dielectric spectroscopy of single human erythrocytes at physiological ionic strength: dispersion of the cytoplasm." Biophysical Journal, vol. 71, pp. 495-506 (1996).

Glidewell, et al., The Use of Magnetic Resonance Imaging Data and the Inclusion of Anisotropic Regions in Electrical Impedance Tomography, Biomed, Sci. Instrum. 1993; 29: 251-7.

Golberg, A. and Rubinsky, B., "A statistical model for multidimensional irreversible electroporation cell death in tissue." Biomed Eng Online, 9, 13 pages, 2010.

Gothelf, et al., Electrochemotherapy: Results of Cancer Treatment Using Enhanced Delivery of Bleomycin by Electroporation, Cancer Treatment Reviews 2003: 29: 371-387.

Gowrishankar T.R., et al., "Microdosimetry for conventional and supra-electroporation in cells with organelles". Biochem Biophys Res Commun, 341(4): p. 1266-76 (2006).

Griffiths, et al., A Dual-Frequency Electrical Impedance Tomography System, Phys. Met Biol., 1989, vol. 34, No. 10, pp. 1465-1476.

Griffiths, The Importance of Phase Measurement in Electrical Impedance Tomography, Phys. Med. Biol., 1987, vol. 32, No. 11, pp. 1435-1444.

Griffiths, Tissue Spectroscopy with Electrical Impedance Tomography: Computer Simulations, IEEE Transactions on Biomedical Engineering, vol. 42, No. 9, Sep. 1995.

Gumerov, et al., The Dipole Approximation Method and Its Coupling with the Regular Boundary Element Method for Efficient Electrical Impedance Tomography, Boundary Element Technology XIII, 1999.

Hapala, Breaking the Barrier: Methods for Reversible Permeabilization of Cellular Membranes, Critical Reviews in Biotechnology, 17(2): 105-122, 1997.

Helczynska et al., "Hypoxia promotes a dedifferentiated phenotype in ductal breast carcinoma in situ." Cancer Research, vol. 63, pp. 1441-1444 (2003).

Heller, et al., Clinical Applications of Electrochemotherapy, Advanced Drug Delivery Reviews, vol. 35, pp. 119-129, 1999.

Hjouj, M., et al., "Electroporation-Induced BBB Disruption and Tissue Damage Depicted by MRI", Neuro-Oncology 13: Issue suppl 3, abstract ET-32 (2011).

Hjouj, M., et al., "MRI Study on Reversible and Irreversible Electroporation Induced Blood Brain Barrier Disruption", PLOS ONE, Aug. 2012, 7:8, e42817.

Hjouj, Mohammad et al., "Electroporation-Induced BBB Disruption and Tissue Damage Depicted by MRI," Abstracts from 16th Annual Scientific Meeting of the Society for Neuro-Oncology in Conjunction with the AANS/CNS Section on Tumors, Nov. 17-20, 2011, Orange County California, Neuro-Oncology Supplement, vol. 13, Supplement 3, p. iii114.

Ho, et al., Electroporation of Cell Membranes: A Review, Critical Reviews in Biotechnology, 16(4): 349-362, 1996.

Holder, et al., Assessment and Calibration of a Low-Frequency System for Electrical Impedance Tomography (EIT), Optimized for Use in Imaging Brain Function in Ambulant Human Subjects, Annals of the New York Academy of Science, vol. 873, Issue 1, Electrical BI, pp. 512-519, 1999.

Huang, et al., Micro-Electroporation: Improving the Efficiency and Understanding of Electrical Permeabilization of Cells, Biomedical Microdevices, vol. 2, pp. 145-150, 1999.

(56) **References Cited**

OTHER PUBLICATIONS

Hughes, et al., An Analysis of Studies Comparing Electrical Impedance Tomography with X-Ray Videofluoroscopy in the Assessment of Swallowing, Physiol. Meas. 15, 1994, pp. A199-A209.

Ibey et al., "Selective cytotoxicity of intense nanosecond-duration electric pulses in mammalian cells." Biochimica Et Biophysica Acta-General Subjects, vol. 1800, pp. 1210-1219 (2010).

Mir, L.M., et al., Electric Pulse-Mediated Gene Delivery to Various Animal Tissues, in Advances in Genetics, Academic Press, 2005, p. 83-114.

Mir, Therapeutic Perspectives of In Vivo Cell Electropermeabilization, Bioelectrochemistry, vol. 53, pp. 1-10, 2000.

Mulhall et al., "Cancer, pre-cancer and normal oral cells distinguished by dielectrophoresis." Analytical and Bioanalytical Chemistry, vol. 401, pp. 2455-2463 (2011).

Narayan, et al., Establishment and Characterization of a Human Primary Prostatic Adenocarcinoma Cell Line (ND-1), The Journal of Urology, vol. 148, 1600-1604, Nov. 1992.

Naslund, Cost-Effectiveness of Minimally Invasive Treatments and Transurethral Resection (TURP) in Benign Prostatic Hyperplasia (BPH), (Abstract), Presented at 2001 AUA National Meeting,, Anaheim, CA, Jun. 5, 2001.

Naslund, Michael J., Transurethral Needle Ablation of the Prostate, Urology, vol. 50, No. 2, Aug. 1997.

Neal II et al., "A Case Report on the Successful Treatment of a Large Soft-Tissue Sarcoma with Irreversible Electroporation," Journal of Clinical Oncology, 29, pp. 1-6, 2011.

Neal II et al., "Experimental Characterization and Numerical Modeling of Tissue Electrical Conductivity during Pulsed Electric Fields for Irreversible Electroporation Treatment Planning," Biomedical Engineering, IEEE Transactions on Biomedical Engineering, vol. 59, pp. 1076-1085, 2012.

Neal II, R. E., et al., "Successful Treatment of a Large Soft Tissue Sarcoma with Irreversible Electroporation", Journal of Clinical Oncology, 29:13, e372-e377 (2011).

Neal II, R.E., et al., "Treatment of breast cancer through the application of irreversible electroporation using a novel minimally invasive single needle electrode." Breast Cancer Research and Treatment, 2010. 123(1): p. 295-301.

Neal II, Robert E. and R.V. Davalos, The Feasibility of Irreversible Electroporation for the Treatment of Breast Cancer and Other Heterogeneous Systems, Ann Biomed Eng, 2009, 37(12): p. 2615-2625.

Nesin et al., "Manipulation of cell volume and membrane pore comparison following single cell permeabilization with 60- and 600-ns electric pulses." Biochimica et Biophysica Acta (BBA)—Biomembranes, vol. 1808, pp. 792-801 (2011).

Neumann, et al., Gene Transfer into Mouse Lyoma Cells by Electroporation in High Electric Fields, J. Embo., vol. 1, No. 7, pp. 841-845, 1982.

Neumann, et al., Permeability Changes Induced by Electric Impulses in Vesicular Membranes, J. Membrane Biol., vol. 10, pp. 279-290, 1972.

Nikolova, B., et al., "Treatment of Melanoma by Electroporation of Bacillus Calmette-Guerin". Biotechnology & Biotechnological Equipment, 25(3): p. 2522-2524 (2011).

Nuccitelli, R., et al., "A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence", Int J Cancer, 125(2): p. 438-45 (2009).

O'Brien et al., "Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity." European Journal of Biochemistry, vol. 267, pp. 5421-5426 (2000).

Okino, et al., Effects of High-Voltage Electrical Impulse and an Anticancer Drug on In Vivo Growing Tumors, Japanese Journal of Cancer Research, vol. 78, pp. 1319-1321, 1987.

Onik, et al., Sonographic Monitoring of Hepatic Cryosurgery in an Experimental Animal Model, AJR American J. of Roentgenology, vol. 144, pp. 1043-1047, May 1985.

Onik, et al., Ultrasonic Characteristics of Frozen Liver, Cryobiology, vol. 21, pp. 321-328, 1984.

Onik, G. and B. Rubinsky, eds. "Irreversible Electroporation: First Patient Experience Focal Therapy of Prostate Cancer. Irreversible Electroporation", ed. B. Rubinsky 2010, Springer Berlin Heidelberg, pp. 235-247.

Onik, G., P. Mikus, and B. Rubinsky, "Irreversible electroporation: implications for prostate ablation." Technol Cancer Res Treat, 2007. 6(4): p. 295-300.

Organ, L.W., Electrophysiological principles of radiofrequency lesion making, Apply. Neurophysiol., 1976. 39: p. 69-76.

Ott, H. C., et al., "Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart", Nature Medicine, Nature Publishing Group, New York, NY, US, vol. 14, No. 2, Feb. 1, 2008, pp. 213-221.

Paszek et al., "Tensional homeostasis and the malignant phenotype." Cancer Cell, vol. 8, pp. 241-254 (2005).

Payselj, et al., "The course of tissue permeabilization studied on a mathematical model of a subcutaneous tumor in small animals," IEEE Trans Biomed Eng, vol. 52, pp. 1373-1381, 2005.

PCT International Preliminary Report on Patentability of Corresponding International Application No. PCT/2011/062067, dated May 28, 2013.

PCT International Preliminary Report on Patentability of Corresponding International Application No. PCT/2011/066239, dated Jun. 25, 2013.

PCT International Search Report (dated Aug. 2, 2011), Written Opinion (dated Aug. 2, 2011), and International Preliminary Report on Patentability (dated Apr. 17, 2012) of PCT/US10/53077.

PCT International Search Report (dated Aug. 22, 2012), and Written Opinion (dated Aug. 22, 2012) of PCT/US11/66239.

PCT International Search Report (dated Aug. 26, 2005), Written Opinion (dated Aug. 26, 2005), and International Preliminary Report on Patentability (dated Jun. 26, 2006) of PCT/US2004/043477.

PCT International Search Report (dated Jan. 19, 2010), Written Opinion (dated Jan. 19, 2010), and International Preliminary Report on Patentability (dated Jan. 4, 2010) of PCT/US09/62806, 15 pgs.

PCT International Search Report (dated Jul. 15, 2010), Written Opinion (dated Jul. 15, 2010), and International Preliminary Report on Patentability (dated Oct. 11, 2011) from PCT/US2010/030629.

PCT International Search Report (dated Jul. 9, 2009), Written Opinion (dated Jul. 9, 2009), and International Preliminary Report on Patentability (dated Nov. 2, 2010) of PCT/US2009/042100.

PCT International Search Report and Written Opinion (dated Jul. 25, 2012) of PCT/US2011/062067.

PCT International Search Report, 4 pgs, (dated Jul. 30, 2010), Written Opinion, 7 pgs, (dated Jul. 30, 2010), and International Preliminary Report on Patentability, 8 pgs, (dated Oct. 4, 2011) from PCT/US2010/029243.

Phillips, M., Maor E. & Rubinsky, B. Non-Thermal Irreversible Electroporation for Tissue Decellularization. J. Biomech. Eng, doi: 10.1115/1.4001882 (2010).

Piñero, et al., Apoptotic and Necrotic Cell Death Are Both Induced by Electroporation in HL60 Human Promyeloid Leukaemia Cells, Apoptosis, vol. 2, No. 3, 330-336, Aug. 1997.

Polak et al., "On the Electroporation Thresholds of Lipid Bilayers: Molecular Dynamics Simulation Investigations." The Journal of Membrane Biology, vol. 246, pp. 843-850 (2013).

Precision Office TUNA System, When Patient Satisfaction is Your Goal, VidaMed 2001.

Pucihar et al., "Numerical determination of transmembrane voltage induced on irregularly shaped cells." Annals of Biomedical Engineering, vol. 34, pp. 642-652 (2006).

Rajagopal, V. and S.G. Rockson, Coronary restenosis: a review of mechanisms and management, The American Journal of Medicine, 2003, 115(7): p. 547-553.

Reberšek, M. and D. Miklavčič, "Advantages and Disadvantages of Different Concepts of Electroporation Pulse Generation," Automatika 52(2011) 1, 12-19.

Rols, M.P., et al., Highly Efficient Transfection of Mammalian Cells by Electric Field Pulses: Application to Large Volumes of Cell Culture by Using a Flow System, Eur. J. Biochem. 1992, 206, pp. 115-121.

(56) **References Cited**

OTHER PUBLICATIONS

Ron et al., "Cell-based screening for membranal and cytoplasmatic markers using dielectric spectroscopy." Biophysica chemistry, 135 (2008) pp. 59-68.

Rossmeisl et al., "Pathology of non-thermal irreversible electroporation (N-TIRE)—induced ablation of the canine brain." Journal of Veterinary Science vol. 14, pp. 433-440 (2013).

Rossmeisl, "New Treatment Modalities for Brain Tumors in Dogs and Cats." Veterinary Clinics of North America: Small Animal Practice 44, pp. 1013-1038 (2014).

Rubinsky et al., "Optimal Parameters for the Destruction of Prostate Cancer Using Irreversible Electroporation." The Journal of Urology, 180 (2008) pp. 2668-2674.

Rubinsky, B., "Irreversible Electroporation in Medicine", Technology in Cancer Research and Treatment, vol. 6, No. 4, Aug. 1, 2007, pp. 255-259.

Rubinsky, B., ed, Cryosurgery. Annu Rev. Biomed. Eng. vol. 2 2000. 157-187.

Rubinsky, B., et al., "Irreversible Electroporation: A New Ablation Modality—Clinical Implications" Technol. Cancer Res. Treatment 6(1), 37-48 (2007).

Sabuncu et al., "Dielectrophoretic separation of mouse melanoma clones." Biomicrofluidics, vol. 4, 7 pages (2010).

Salford, L.G., et al., "A new brain tumour therapy combining bleomycin with in vivo electropermeabilization", Biochem. Biophys. Res. Commun., 194(2): 938-943 (1993).

Salmanzadeh et al., "Investigating dielectric properties of different stages of syngeneic murine ovarian cancer cells" Biomicrofluidics 7, 011809 (2013), 12 pages.

Salmanzadeh et al., "Dielectrophoretic differentiation of mouse ovarian surface epithelial cells, macrophages, and fibroblasts using contactless dielectrophoresis." Biomicrofluidics, vol. 6, 13 Pages (2012).

Salmanzadeh et al., "Sphingolipid Metabolites Modulate Dielectric Characteristics of Cells in a Mouse Ovarian Cancer Progression Model." Integr. Biol., 5(6), pp. 843-852 (2013).

Sano et al., "Contactless Dielectrophoretic Spectroscopy: Examination of the Dielectric Properties of Cells Found in Blood." Electrophoresis, 32, pp. 3164-3171, 2011.

Sano et al., "In-vitro bipolar nano- and microsecond electro-pulse bursts for irreversible electroporation therapies." Bioelectrochemistry vol. 100, pp. 69-79 (2014).

Sano et al., "Modeling and Development of a Low Frequency Contactless Dielectrophoresis (cDEP) Platform to Sort Cancer Cells from Dilute Whole Blood Samples." Biosensors & Bioelectronics, 8 pages (2011).

Sano, M. B., et al., "Towards the creation of decellularized organ constructs using irreversible electroporation and active mechanical perfusion", Biomedical Engineering Online, Biomed Central LTD, London, GB, vol. 9, No. 1, Dec. 10, 2010, p. 83.

Saur et al., "CXCR4 expression increases liver and lung metastasis in a mouse model of pancreatic cancer." Gastroenterology, vol. 129, pp. 1237-1250 (2005).

Schmukler, Impedance Spectroscopy of Biological Cells, Engineering in Medicine and Biology Society, Engineering Advances: New Opportunities for Biomedical Engineers, Proceedings of the 16th Annual Internal Conference of the IEEE, vol. 1, p. A74, downloaded from IEEE Xplore website, 1994.

Schoenbach et al., "Intracellular effect of ultrashort electrical pulses." Bioelectromagnetics, 22 (2001) pp. 440-448.

Seibert et al., "Clonal variation of MCF-7 breast cancer cells in vitro and in athymic nude mice." Cancer Research, vol. 43, pp. 2223-2239 (1983).

Seidler et al., "A Cre-loxP-based mouse model for conditional somatic gene expression and knockdown in vivo by using avian retroviral vectors." Proceedings of the National Academy of Sciences, vol. 105, pp. 10137-10142 (2008).

Sel, D., Lebar, A. M. & Miklavcic, D. Feasibility of employing model-based optimization of pulse amplitude and electrode distance for effective tumor electropermeabilization. IEEE Trans Biomed Eng 54, 773-781 (2007).

Sel, et al., "Sequential finite element model of tissue electropermeabilization," IEEE Trans Biomed Eng, vol. 52, pp. 816-827, 2005.

Sersa, et al., Reduced Blood Flow and Oxygenation in SA-1 Tumours after Electrochemotherapy with Cisplatin, British Journal of Cancer, 87, 1047-1054, 2002.

Sersa, et al., Tumour Blood Flow Modifying Effects of Electrochemotherapy: a Potential Vascular Targeted Mechanism, Radiol. Oncol., 37(1): 43-8, 2003.

Sharma, A. , et al., "Review on Thermal Energy Storage with Phase Change Materials and Applications", Renewable Sustainable Energy Rev. 13(2), 318-345 (2009).

Sharma, et al., Poloxamer 188 Decreases Susceptibility of Artificial Lipid Membranes to Electroporation, Biophysical Journal, vol. 71, No. 6, pp. 3229-3241, Dec. 1996.

Shiina, S., et al, Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. AJR, 1993, 160: p. 1023-8.

Szot et al., "3D in vitro bioengineered tumors based on collagen I hydrogels." Biomaterials vol. 32, pp. 7905-7912 (2011).

Talele, S., et al., "Modelling single cell electroporation with bipolar pulse parameters and dynamic pore radii". Journal of Electrostatics, 68(3): p. 261-274 (2010).

Tekle, Ephrem, R. Dean Astumian, and P. Boon Chock, Electroporation by using bipolar oscillating electric field: An improved method for DNA transfection of NIH 3T3 cells, Proc. Natl. Acad. Sci., vol. 88, pp. 4230-4234, May 1991, Biochemistry.

Thompson, et al., To determine whether the temperature of 2% lignocaine gel affects the initial discomfort which may be associated with its instillation into the male urethra, BJU International (1999), 84, 1035-1037.

Thomson et al., "Investigation of the safety of irreversible electroporation in humans," J Vasc Interv Radiol, 22, pp. 611-621, 2011.

TUNA—Suggested Local Anesthesia Guidelines, no date available.

Verbridge et al., "Oxygen-Controlled Three-Dimensional Cultures to Analyze Tumor Angiogenesis." Tissue Engineering, Part A vol. 16, pp. 2133-2141 (2010).

Vernier, P.T., et al., "Nanoelectropulse-driven membrane perturbation and small molecule permeabilization", Bmc Cell Biology, 7 (2006).

Vidamed, Inc., Transurethral Needle Ablation (TUNA): Highlights from Worldwide Clinical Studies, Vidamed's Office TUNA System, 2001.

Weaver et al., "A brief overview of electroporation pulse strength-duration space: A region where additional Intracellular effects are expected." Bioelectrochemistry vol. 87, pp. 236-243 (2012).

Weaver, Electroporation: A General Phenomenon for Manipulating Cells and Tissues, Journal of Cellular Biochemistry, 51: 426-435, 1993.

Weaver, et al., Theory of Electroporation: A Review, Bioelectrochemistry and Bioenergetics, vol. 41, pp. 136-160, 1996.

Weaver, J. C., Electroporation of biological membranes from multicellular to nano scales, IEEE Tms. Dielectr. Electr. Insul. 10, 754-768 (2003).

Weaver, J.C., "Electroporation of cells and tissues", IEEE Transactions on Plasma Science, 28(1): p. 24-33 (2000).

Weisstein: Cassini Ovals. From MathWorld—A. Wolfram Web Resource; Apr. 30, 2010; http://mathworld.wolfram.com/ (updated May 18, 2011).

Yang et al., "Dielectric properties of human leukocyte subpopulations determined by electrorotation as a cell separation criterion." Biophysical Journal, vol. 76, pp. 3307-3314 (1999).

Yao et al., "Study of transmembrane potentials of inner and outer membranes induced by pulsed-electric-field model and simulation." IEEE Trans Plasma Sci, 2007. 35(5): p. 1541-1549.

Zhang Y. et al., MR imaging to assess immediate response to irreversible electroporation for targeted ablation of liver tissues: preclinical feasibility studies in a rodent model. Radiology, 2010. 256(2): p. 424-32.

Zimmermann, et al., Dielectric Breakdown of Cell Membranes, Biophysical Journal, vol. 14, No. 11, pp. 881-899, 1974.

(56) **References Cited**

OTHER PUBLICATIONS

Zlotta, et al., Long-Term Evaluation of Transurethral Needle Ablation of the Prostate (TUNA) for Treatment of Benign Prostatic Hyperplasia (BPH): Clinical Outcome After 5 Years. (Abstract) Presented at 2001 AUA National Meeting, Anaheim, CA—Jun. 5, 2001.

Zlotta, et al., Possible Mechanisms of Action of Transurethral Needle Ablation of the Prostate on Benign Prostatic Hyperplasia Symptoms: a Neurohistochemical Study, Reprinted from Journal of Urology, vol. 157, No. 3, Mar. 1997, pp. 894-899.

Co-Pending U.S. Appl. No. 13/332,133, Advisory Action dated Apr. 6, 2016, 3 pages.

Co-Pending U.S. Appl. No. 13/332,133, Interview Summary dated Jan. 22, 2016, 4 pages.

Issa, et al., The TUNA Procedure for BPH: Review of the Technology: The TUNA Procedure for BPH: Basic Procedure and Clinical Results, Reprinted from Infections in Urology, Jul./Aug. 1998 and Sep./Oct. 1998.

Ivanuša, et al., MRI Macromolecular Contrast Agents as Indicators of Changed Tumor Blood Flow, Radiol. Oncol. 2001; 35(2): 139-47.

Ivorra et al., "In vivo electric impedance measurements during and after electroporation of rat live." Bioelectrochemistry, vol. 70, pp. 287-295 (2007).

Ivorra et al., "In vivo electrical conductivity measurements during and after tumor electroporation: conductivity changes reflect the treatment outcome." Physics in Medicine and Biology, vol. 54, pp. 5949-5963 (2009).

Ivorra, "Bioimpedance monitoring for physicians: an overview." Biomedical Applications Group, 35 pages (2002).

J.F. Edd and R.V. Davalos, "Mathematical modeling of irreversible electroporation for treatment planning," Technology in Cancer Research and Treatment, 6, pp. 275-286, 2007.

Jarm et al., "Antivascular effects of electrochemotherapy: implications in treatment of bleeding metastases." Expert Rev Anticancer Ther. vol. 10, pp. 729-746 (2010).

Jaroszeski, et al., In Vivo Gene Delivery by Electroporation, Advanced Drug Delivery Review, vol. 35, pp. 131-137, 1999.

Jensen et al., "Tumor volume in subcutaneous mouse xenografts measured by microCT is more accurate and reproducible than determined by 18FFDG-microPET or external caliper." BMC medical Imaging vol. 8:16, 9 Pages (2008).

Jossinet et al., Electrical Impedance Endo-Tomography: Imaging Tissue From Inside, IEEE Transactions on Medical Imaging, vol. 21, No. 6, Jun. 2002, pp. 560-565.

Kingham et al., "Ablation of perivascular hepatic malignant tumors with irreversible electroporation." Journal of the American College of Surgeons, 2012. 215(3), p. 379-387.

Kinosita and Tsong, "Formation and resealing of pores of controlled sizes in human erythrocyte membrane." Nature, vol. 268 (1977) pp. 438-441.

Kinosita and Tsong, "Voltage-induced pore formation and hemolysis of human erythrocytes." Biochimica et Biophysica Acta (BBA)—Biomembranes, 471 (1977) pp. 227-242.

Kinosita et al., "Electroporation of cell membrane visualized under a pulsed-laser fluorescence microscope." Biophysical Journal, vol. 53, pp. 1015-1019 (1988).

Kinosita, et al., Flemolysis of Human Erythrocytes by a Transient Electric Field, Proc. Natl. Acad. Sci. USA, vol. 74, No. 5, pp. 1923-1927, 1977.

Kirson et al., "Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors." Proceedings of the National Academy of Sciences vol. 104, pp. 10152-10157 (2007).

Kotnik and Miklavcic, "Theoretical evaluation of voltage inducement on internal membranes of biological cells exposed to electric fields." Biophysical Journal, vol. 90(2), pp. 480-491 (2006).

Kotnik, T. and D. Miklavcic, "Theoretical evaluation of the distributed power dissipation in biological cells exposed to electric fields", Bioelectromagnetics, 21(5): p. 385-394 (2000).

Kotnik, T., et al., "Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses. Part II. Reduced electrolytic contamination", Bioelectrochemistry, 54(1): p. 91-5 (2001).

Kotnik, T., et al., "Role of pulse shape in cell membrane electropermeabilization", Biochimica Et Biophysica Acta-Biomembranes, 1614(2): p. 193-200 (2003).

Labeed et al., "Differences in the biophysical properties of membrane and cytoplasm of apoptotic cells revealed using dielectrophoresis." Biochimica et Biophysica Acta (BBA)—General Subjects, vol. 1760, pp. 922-929 (2006).

Lackovic, I., et al., "Three-dimensional Finite-element Analysis of Joule Heating in Electrochemotherapy and in vivo Gene Electrotransfer", Ieee Transactions on Dielectrics and Electrical Insulation, 16(5): p. 1338-1347 (2009).

Laufer et al., "Electrical impedance characterization of normal and cancerous human hepatic tissue." Physiological Measurement, vol. 31, pp. 995-1009 (2010).

Lebar et al., "Inter-pulse interval between rectangular voltage pulses affects electroporation threshold of artificial lipid bilayers." IEEE Transactions on NanoBioscience, vol. 1 (2002) pp. 116-120.

Lee, E. W. et al. Advanced Hepatic Ablation Technique for Creating Complete Cell Death : Irreversible Electroporation Radiology 255, 426-433, doi:10.1148/radiol.10090337 (2010).

Lee, E.W., et al., "Imaging guided percutaneous irreversible electroporation: ultrasound and immunohistological correlation", Technol Cancer Res Treat 6: 287-294 (2007).

Li, W., et al., "The Effects of Irreversible Electroporation (IRE) on Nerves" PloS One, Apr. 2011, 6(4), e18831.

Liu, et al., Measurement of Pharyngeal Transit Time by Electrical Impedance Tomography, Clin. Phys. Physiol. Meas., 1992, vol. 13, Suppl. A, pp. 197-200.

Long, G., et al., "Targeted Tissue Ablation With Nanosecond Pulses". IEEE Transactions on Biomedical Engineering, 58(8) (2011).

Lundqvist, et al., Altering the Biochemical State of Individual Cultured Cells and Organelles with Ultramicroelectrodes, Proc. Natl. Acad. Sci. USA, vol. 95, pp. 10356-10360, Sep. 1998.

Lurquin, Gene Transfer by Electroporation, Molecular Biotechnology, vol. 7, 1997.

Lynn, et al., A New Method for the Generation and Use of Focused Ultrasound in Experimental Biology, The Journal of General Physiology, vol. 26, 179-193, 1942.

M. Marty et al., "Electrochemotherapy—An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study," European Journal of Cancer Supplements, 4, pp. 3-13, 2006.

Maček Lebar and Miklavčič, "Cell electropermeabilization to small molecules in vitro: control by pulse parameters." Radiology and Oncology, vol. 35(3), pp. 193-202 (2001).

Mahmood, F., et al., "Diffusion-Weighted MRI for Verification of Electroporation-Based Treatments", Journal of Membrane Biology 240: 131-138 (2011).

Mahnic-Kalamiza, et al., "Educational application for visualization and analysis of electric field strength in multiple electrode electroporation," BMC Med Educ, vol. 12:102, 13 pages, 2012.

Malpica et al., "Grading ovarian serous carcinoma using a two-tier system." The American Journal of Surgical Pathology, vol. 28, pp. 496-504 (2004).

Maor et al., The Effect of Irreversible Electroporation on Blood Vessels, Tech. in Cancer Res. and Treatment, vol. 6, No. 4, Aug. 2007, pp. 307-312.

Maor, E., A. Ivorra, and B. Rubinsky, Non Thermal Irreversible Electroporation: Novel Technology for Vascular Smooth Muscle Cells Ablation, PLoS ONE, 2009, 4(3): p. e4757.

Maor, E., A. Ivorra, J. Leor, and B. Rubinsky, Irreversible electroporation attenuates neointimal formation after angioplasty, IEEE Trans Biomed Eng, Sep. 2008, 55(9): p. 2268-74.

Marszalek et al., "Schwan equation and transmembrane potential induced by alternating electric field." Biophysical Journal, vol. 58, pp. 1053-1058 (1990).

Martin, n.R.C.G., et al., "Irreversible electroporation therapy in the management of locally advanced pancreatic adenocarcinoma." Journal of the American College of Surgeons, 2012. 215(3): p. 361-369.

(56) **References Cited**

OTHER PUBLICATIONS

Miklavcic et al., "A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy," Biochimica et Biophysica Acta, 1523, pp. 73-83, 2000.
Miklavčič, et al., The Importance of Electric Field Distribution for Effective in Vivo Electroporation of Tissues, Biophysical Journal, vol. 74, May 1998, pp. 2152-2158.
Miller, L., et al., Cancer cells ablation with irreversible electroporation, Technology in Cancer Research and Treatment 4 (2005) 699-706.
Mir et al., "Mechanisms of Electrochemotherapy" Advanced Drug Delivery Reviews 35:107-118 (1999).
Mir, et al., Effective Treatment of Cutaneous and Subcutaneous Malignant Tumours by Electrochemotherapy, British Journal of Cancer, vol. 77, No. 12, pp. 2336-2342, 1998.
Mir, et al., Electrochemotherapy Potentiation of Antitumour Effect of Bleomycin by Local Electric Pulses, European Journal of Cancer, vol. 27, No. 1, pp. 68-72, 1991.
Mir, et al., Electrochemotherapy, a Novel Antitumor Treatment: First Clinical Trial, C.R. Acad. Sci. Paris, Ser. III, vol. 313, pp. 613-618, 1991.
Mir, L.M. and Orlowski, S., The basis of electrochemotherapy, in Electrochemotherapy, electrogenetherapy, and transdermal drug delivery: electrically mediated delivery of molecules to cells, M.J. Jaroszeski, R. Heller, R. Gilbert, Editors, 2000, Humana Press, p. 99-118.
Co-pending U.S. Appl. No. 13/332,133, Non-Final Office Action dated Nov. 22, 2016, 8 pages.
PCT IPRP for PCT/US15/30429 (WO2015175570), dated Nov. 15, 2016.
Co-pending U.S. Appl. No. 15/423,986, filed Feb. 3, 2017.
Co-pending U.S. Appl. No. 15/424,335, filed Feb. 3, 2017.
Co-Pending U.S. Appl. No. 14/808,679, Restriction Requirement dated Mar. 19, 2018, 7 pages.
Co-Pending U.S. Appl. No. 14/808,679, Final Office Action dated Jan. 11, 2019, 12 pages.
Co-Pending U.S. Appl. No. 14/808,679, Preliminary Amendment, filed Jul. 27, 2015, 9 pages.
Co-Pending U.S. Appl. No. 14/808,679, Response to Mar. 19, 2018 Restriction Requirement dated May 21, 2018, 2 pages.
Co-Pending U.S. Appl. No. 14/808,679, Response to Sep. 10, 2018 Non-Final Office Action dated Dec. 10, 2018, 9 pages.
Co-Pending U.S. Appl. No. 14/017,210, Final Office Action dated Aug. 30, 2016, 11 pages.
Co-Pending U.S. Appl. No. 14/017,210, Non-Final Office Action dated Dec. 15, 2016, 8 pages.
Co-Pending U.S. Appl. No. 14/017,210, Non-Final Office Action dated Sep. 8, 2015, 8 pages.
Co-Pending U.S. Appl. No. 14/017,210, Notice of Allowance (after Dec. 12, 2018 RCE) dated Jan. 9, 2019, 5 pages.
Co-Pending U.S. Appl. No. 14/017,210, Petition dated Dec. 11, 2015, 5 pages.
Co-Pending U.S. Appl. No. 14/017,210, Petition Decision dated Aug. 12, 2016, 9 pages.
Co-Pending U.S. Appl. No. 14/017,210, Petition Decision dated Aug. 2, 2016, 5 pages.
Co-Pending U.S. Appl. No. 14/017,210, RCE dated Nov. 30, 2016, 13 pages.
Co-Pending U.S. Appl. No. 14/017,210, Response to Non-Final Office Action dated Mar. 8, 2016, 16 pages.
Co-pending U.S. Appl. No. 15/843,888, filed Dec. 15, 2017.
Co-pending U.S. Appl. No. 15/881,414, filed Jan. 26, 2018.
Co-Pending Application U.S. Appl. No. 13/332,133, Appeal Brief and Appendices filed Aug. 15, 2017, 26 pages.
Co-Pending U.S. Appl. No. 13/332,133, Examiner's Answer to Appeal Brief dated Oct. 5, 2017, 16 pages.
Co-Pending U.S. Appl. No. 13/332,133, Final Office Action dated Mar. 15, 2017, 17 pages.
Co-Pending U.S. Appl. No. 13/332,133, Response to Nov. 22, 2016 Non-Final Office Action filed Feb. 21, 2017, 14 pages.
Garcia, Paulo A., Robert E. Neal II and Rafael V. Davalos, Chapter 3, Non-Thermal Irreversible Electroporation for Tissue Ablation, In: Electroporation in Laboratory and Clinical Investigations ISBN 978-1-61668-327-6 Editors: Enrico R Spugnini and Alfonso Baldi, 2010, 22 pages.
Kotnik et al., "Sensitivity of transmembrane voltage induced by applied electric fields—A theoretical analysis", Bioelectrochemistry and Bioenergetics,vol. 43, Issue 2, 1997, pp. 285-291.
Neal RE II, et al. (2013) Improved Local and Systemic Anti-Tumor Efficacy for Irreversible Electroporation in Immunocompetent versus Immunodeficient Mice. PLoS ONE 8(5): e64559. https://doi.org/10.1371/journal.pone.0064559.
Wimmer, Thomas, et al., "Planning Irreversible Electroporation (IRE) in the Porcine Kidney: Are Numerical Simulations Reliable for Predicting Empiric Ablation Outcomes?", Cardiovasc Intervent Radiol. Feb. 2015; 38(1): 182-190. doi:10.1007/s00270-014-0905-2.
Co-Pending U.S. Appl. No. 12/757,901, File History 2018.
Co-Pending U.S. Appl. No. 14/808,679, Non-Final Office Action dated Sep. 10, 2018, 12 pages.
Co-pending U.S. Appl. No. 15/881,414 Amendment and Petition for Priority Claim dated Jul. 26, 2018, 26 pages.
Co-pending U.S. Appl. No. 15/881,414, filed Apr. 26, 2018 Non-Final Office Action, 8 pages.
Co-pending U.S. Appl. No. 15/881,414 Notice of Allowance dated Oct. 24, 2018, 7 pages.
Co-pending U.S. Appl. No. 16/177,745, filed Nov. 1, 2018.
Co-Pending U.S. Appl. No. 14/017,210, Acceptance of 312 Amendment dated Sep. 12, 2018, 1 page.
Co-Pending U.S. Appl. No. 14/017,210, AFCP dated Aug. 13, 2018, 9 pages.
Co-Pending U.S. Appl. No. 14/017,210, Final Office Action dated Apr. 11, 2018, 10 pages.
Co-Pending U.S. Appl. No. 14/017,210, Final Office Action dated May 1, 2017, 11 pages.
Co-Pending U.S. Appl. No. 14/017,210, Non-Final Office Action dated Oct. 25, 2017, 9 pages.
Co-Pending U.S. Appl. No. 14/017,210, Notice of Allowance dated Sep. 12, 2018, 7 pages.
Co-Pending U.S. Appl. No. 14/017,210, RCE dated Aug. 1, 2017, 13 pages.
Co-Pending U.S. Appl. No. 14/017,210, Response to Dec. 15, 2016 Non-Final Office Action dated Mar. 20, 2017, 9 pages.
Co-Pending U.S. Appl. No. 14/017,210, Response to Oct. 25, 2017 Non-Final Office Action dated Jan. 25, 2018, 11 pages.
Co-Pending U.S. Appl. No. 14/627,046, Amendment dated Jun. 29, 2017, 8 pages.
Co-Pending U.S. 14/627,046, Final Office Action dated Sep. 14, 2017, 11 pages.
Co-Pending U.S. Appl. No. 14/627,046, Interview Summary dated dated Apr. 27, 2018, 3 pages.
Co-Pending U.S. Appl. No. 14/627,046, Non-Final Office Action dated Feb. 15, 2018, 12 pages.
Co-Pending U.S. Appl. No. 14/627,046, Non-Final Office Action dated Mar. 29, 2017, 9 pages.
Co-Pending U.S. 14/627,046, Notice of Allowance dated Sep. 19, 2018, 7 pages.
Co-Pending U.S. Appl. No. 14/627,046, Response to Sep. 14, 2017 Final Office Action dated Dec. 14, 2017, 7 pages.
Co-Pending U.S. Appl. No. 14/627,046, Rule 132 Affidavit and Response to Feb. 15, 2018 Non-Final Office Action, dated Jun. 15, 2018, 13 pages.
Co-pending U.S. Appl. No. 16/152,743, filed Oct. 5, 2018.
Co-Pending U.S. Appl. No. 15/310,114, NFOA dated Mar. 6, 2019, 13 pages.

* cited by examiner

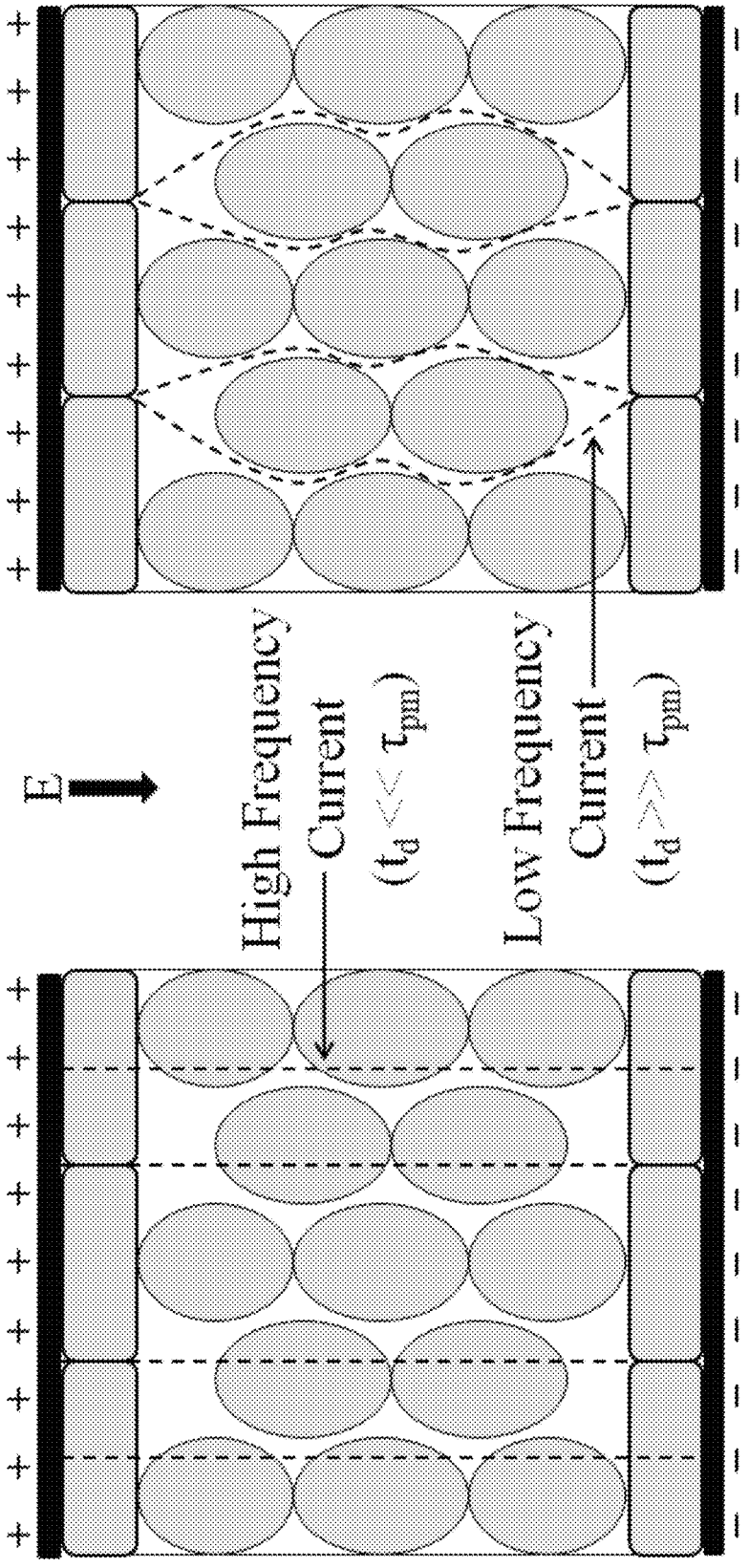

E
High Frequency
Current
($t_d \ll \tau_{pm}$)
Low Frequency
Current
($t_d \gg \tau_{pm}$)

FIG. 1A

FIG. 1B

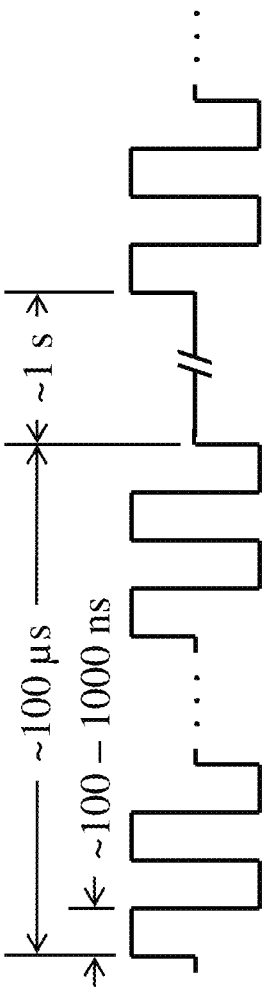


FIG. 2

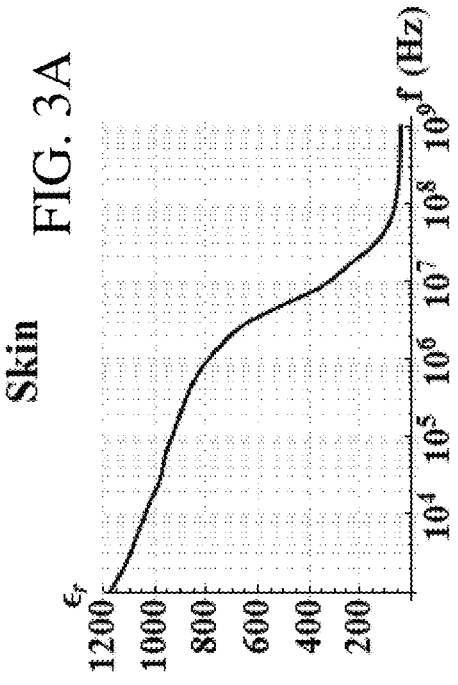


FIG. 3A

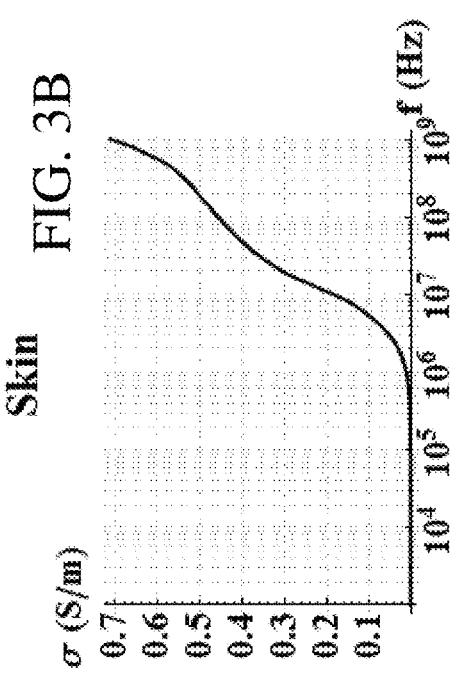


FIG. 3B

FIG. 3C

FIG. 3D

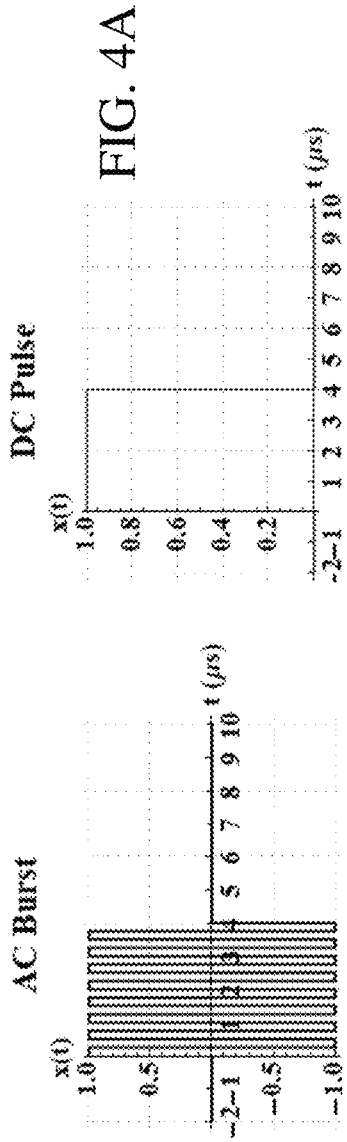

DC Pulse
x(t)
1.0
0.8
0.6
0.4
0.2
-2-1 1 2 3 4 5 6 7 8 9 10 t (μs)
AC Burst
x(t)
1.0
0.5
-0.5
-1.0
-2-1 1 2 3 4 5 6 7 8 9 10 t (μs)

FIG. 4A

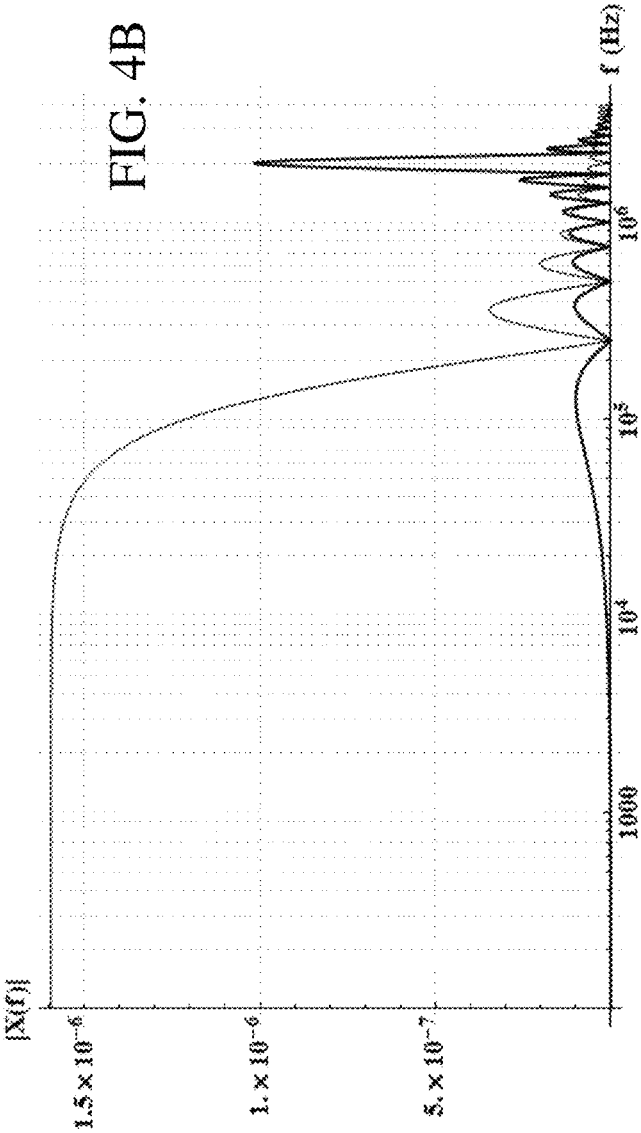
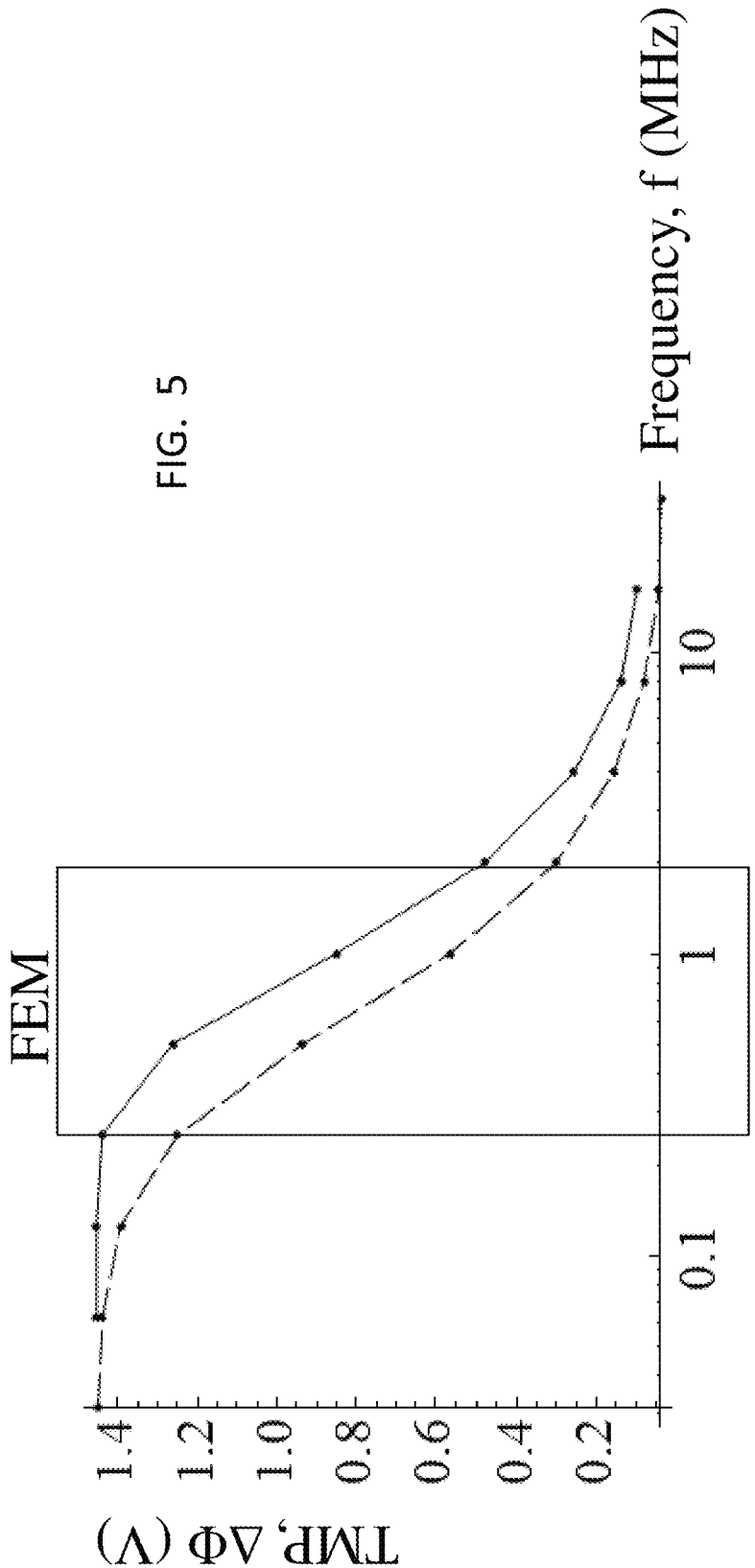

Magnitude Spectrum
|X(f)|
1.5×10⁻⁶
1.×10⁻⁶
5.×10⁻⁷
1000
10⁴
10⁵
10⁶
f (Hz)

FIG. 4B

FIG. 5



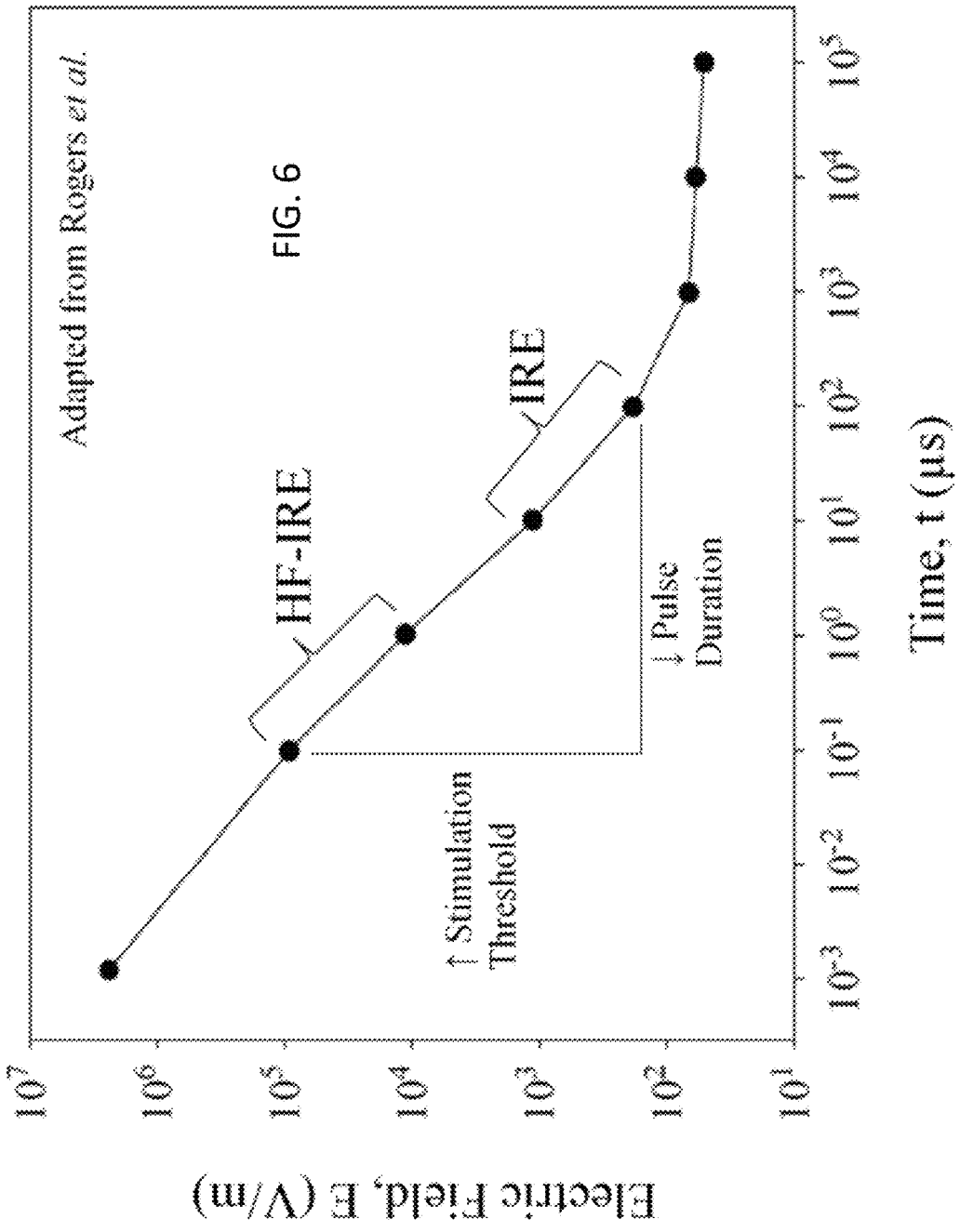

Adapted from Rogers et al.
HF-IRE
IRE
↑ Stimulation Threshold
↓ Pulse Duration
Electric Field, E (V/m)
Time, t (μs)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
10^2
10^3
10^4
10^5

FIG. 6

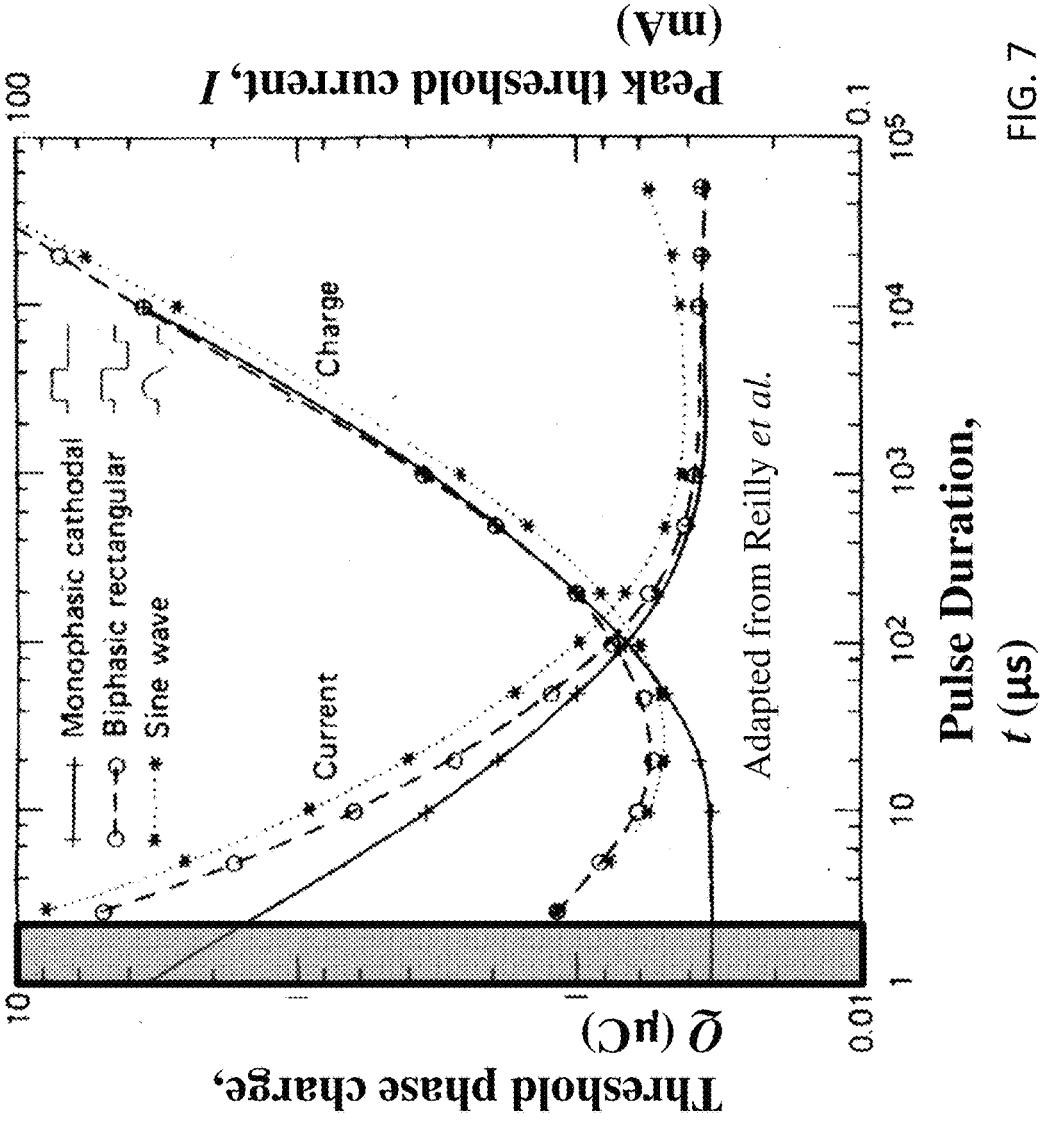

Peak threshold current, I
(mA)
100
10
1
0.1
0.01
Threshold phase charge,
Q (μC)
Monophasic cathodal
Biphasic rectangular
Sine wave
Charge
Current
Adapted from Reilly et al.
1
10
10²
10³
10⁴
10⁵
Pulse Duration,
t (μs)

FIG. 7

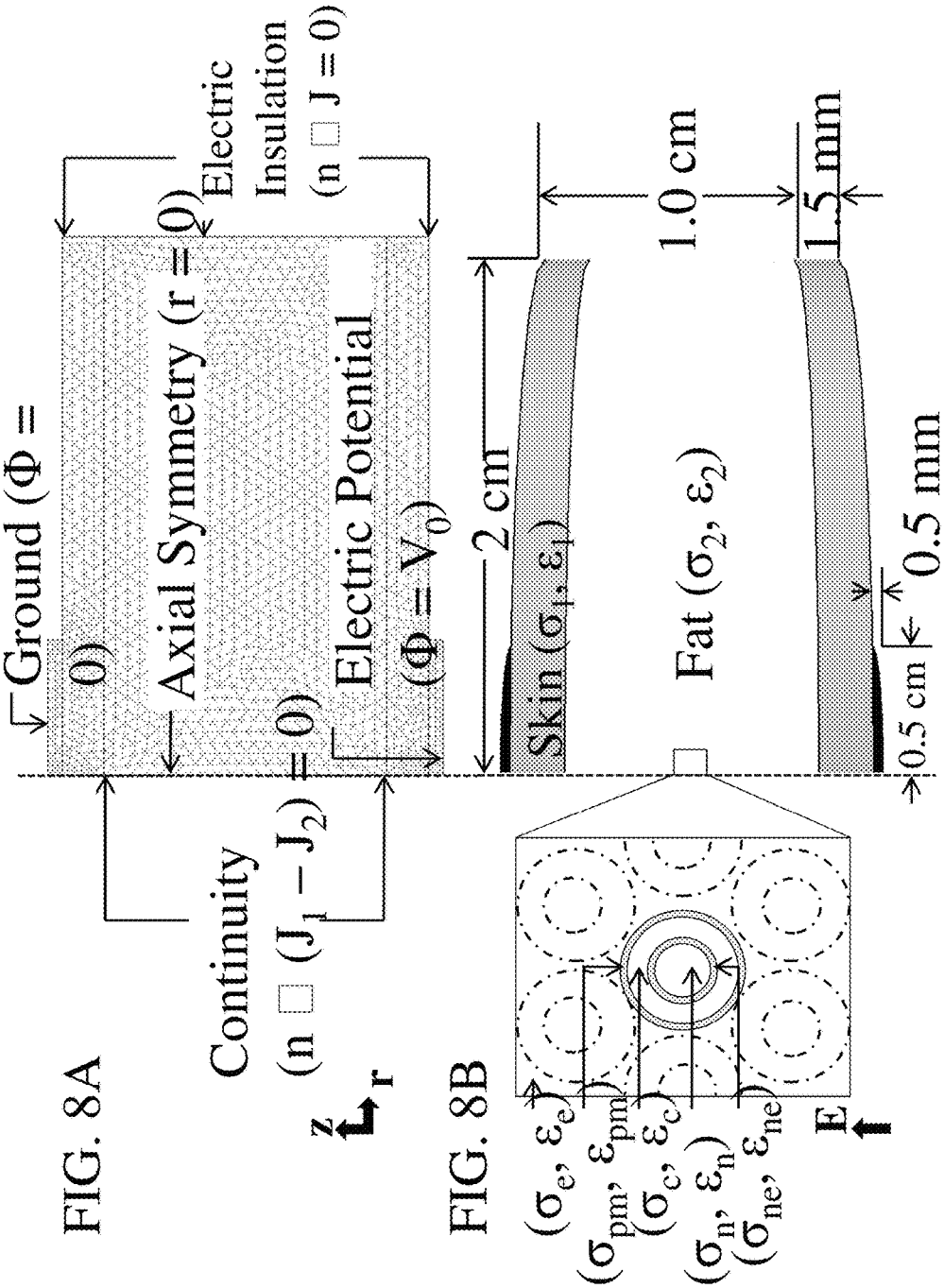

Ground (Φ = 0)
Axial Symmetry (r = 0)
Electric Insulation (n □ J = 0)
Electric Potential (Φ = V0)
Continuity (n □ (J1 – J2) = 0)
z
r
2 cm
1.0 cm
1.5 mm
Skin (σ1, ε1)
Fat (σ2, ε2)
0.5 mm
0.5 cm
(σe, εe)
(σpm, εpm)
(σc, εc)
(σn, εn)
(σne, εne)
E

FIG. 8A

FIG. 8B

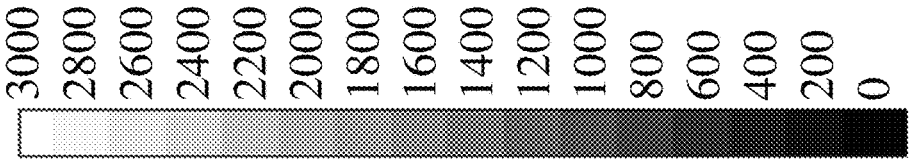


FIG. 9B

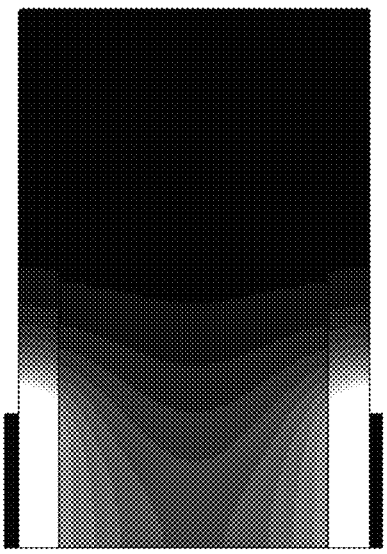


FIG. 9D

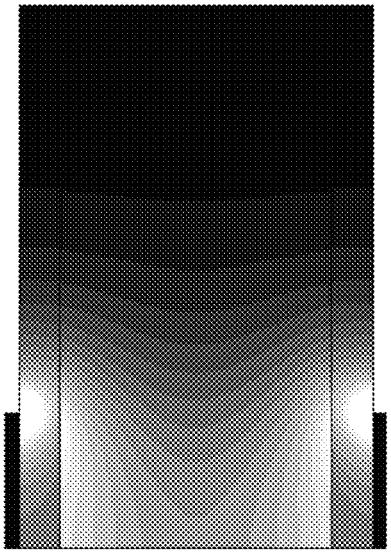


FIG. 9A

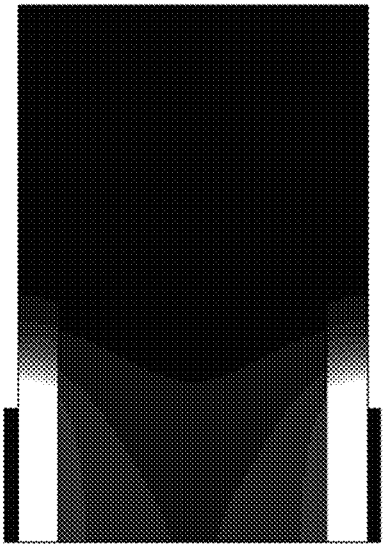
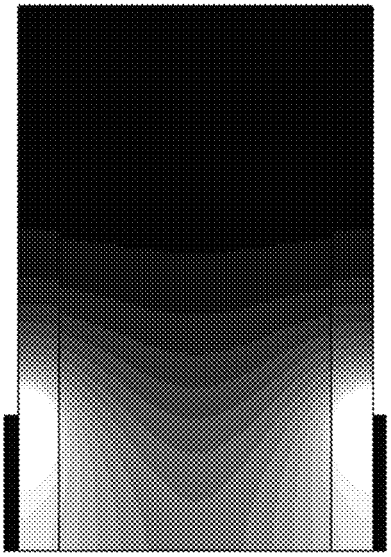


FIG. 9C



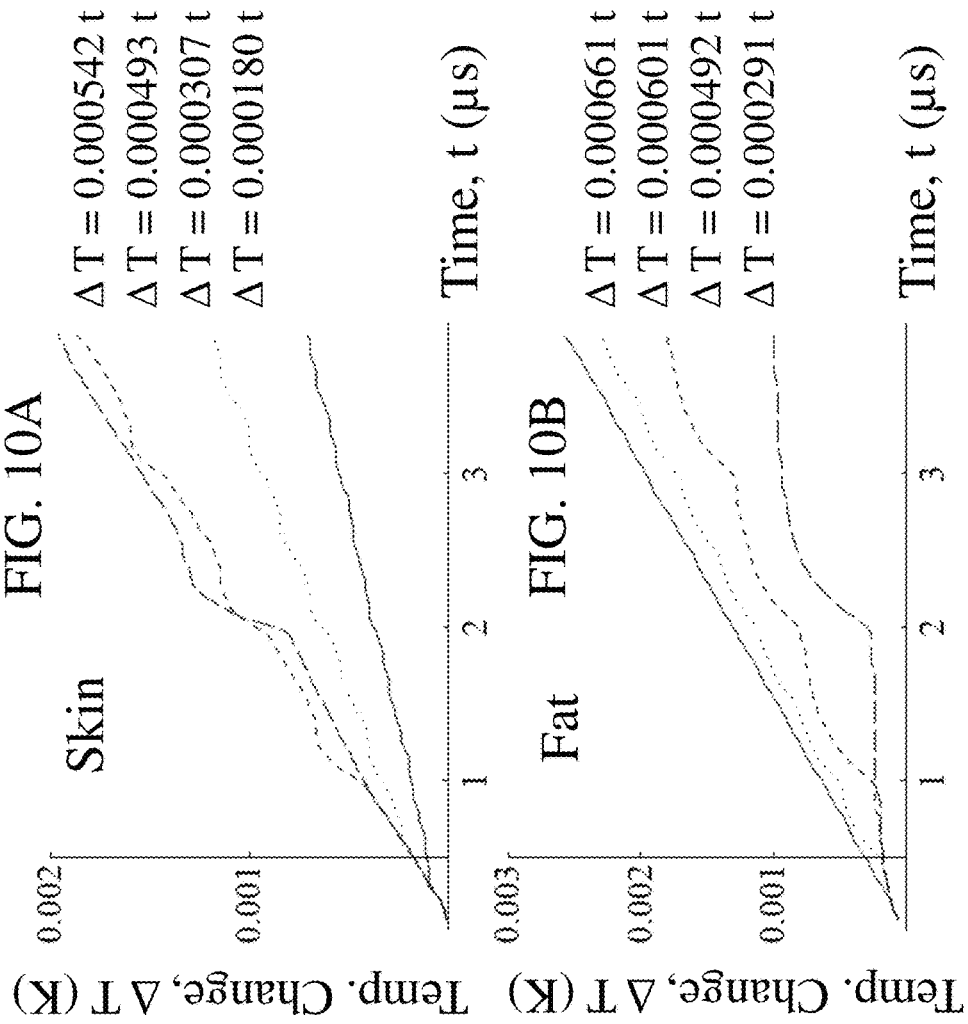


FIG. 10A
Skin
Temp. Change, Δ T (K)
0.002
0.001
1
2
3
Time, t (μs)
Δ T = 0.000542 t
Δ T = 0.000493 t
Δ T = 0.000307 t
Δ T = 0.000180 t
FIG. 10B
Fat
Temp. Change, Δ T (K)
0.003
0.002
0.001
1
2
3
Time, t (μs)
Δ T = 0.000661 t
Δ T = 0.000601 t
Δ T = 0.000492 t
Δ T = 0.000291 t

FIG. 11A

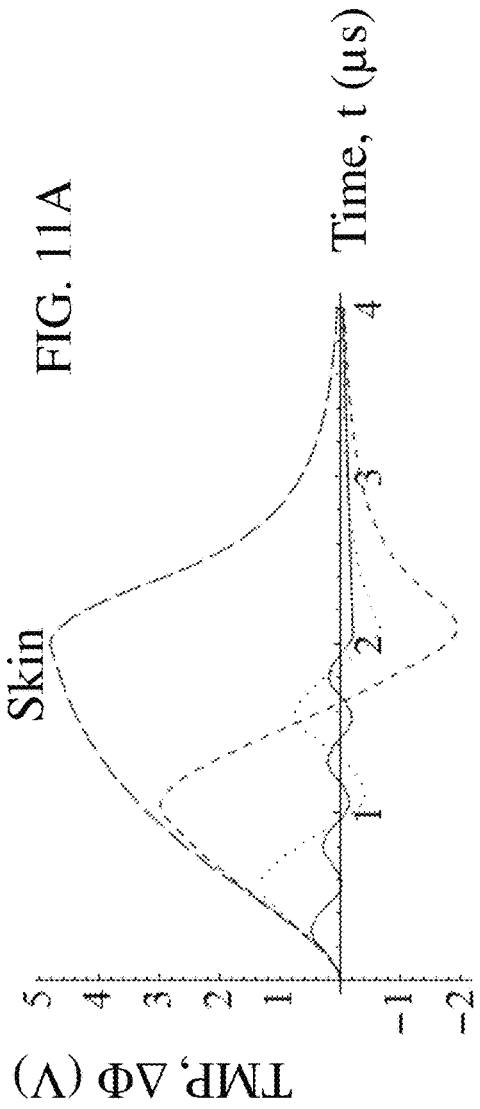
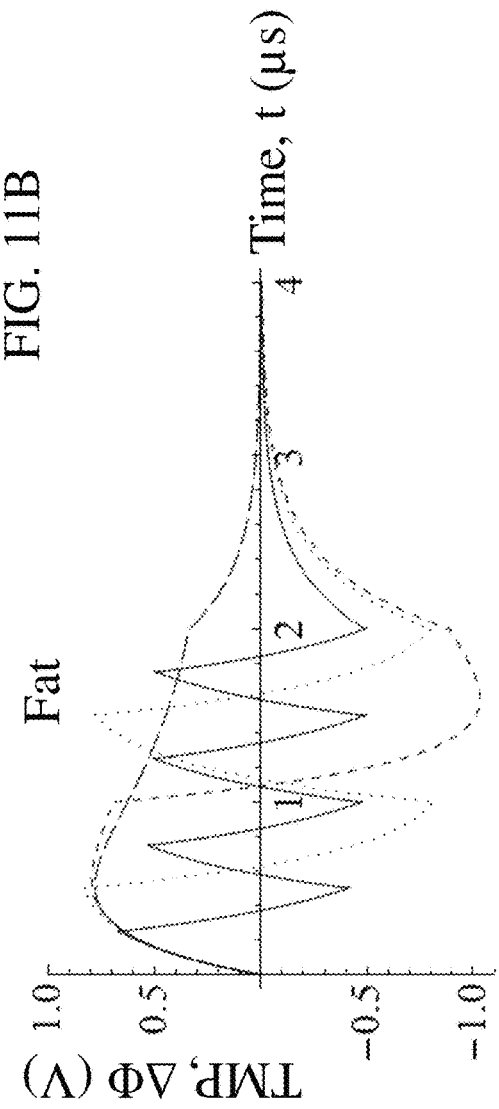


FIG. 11B



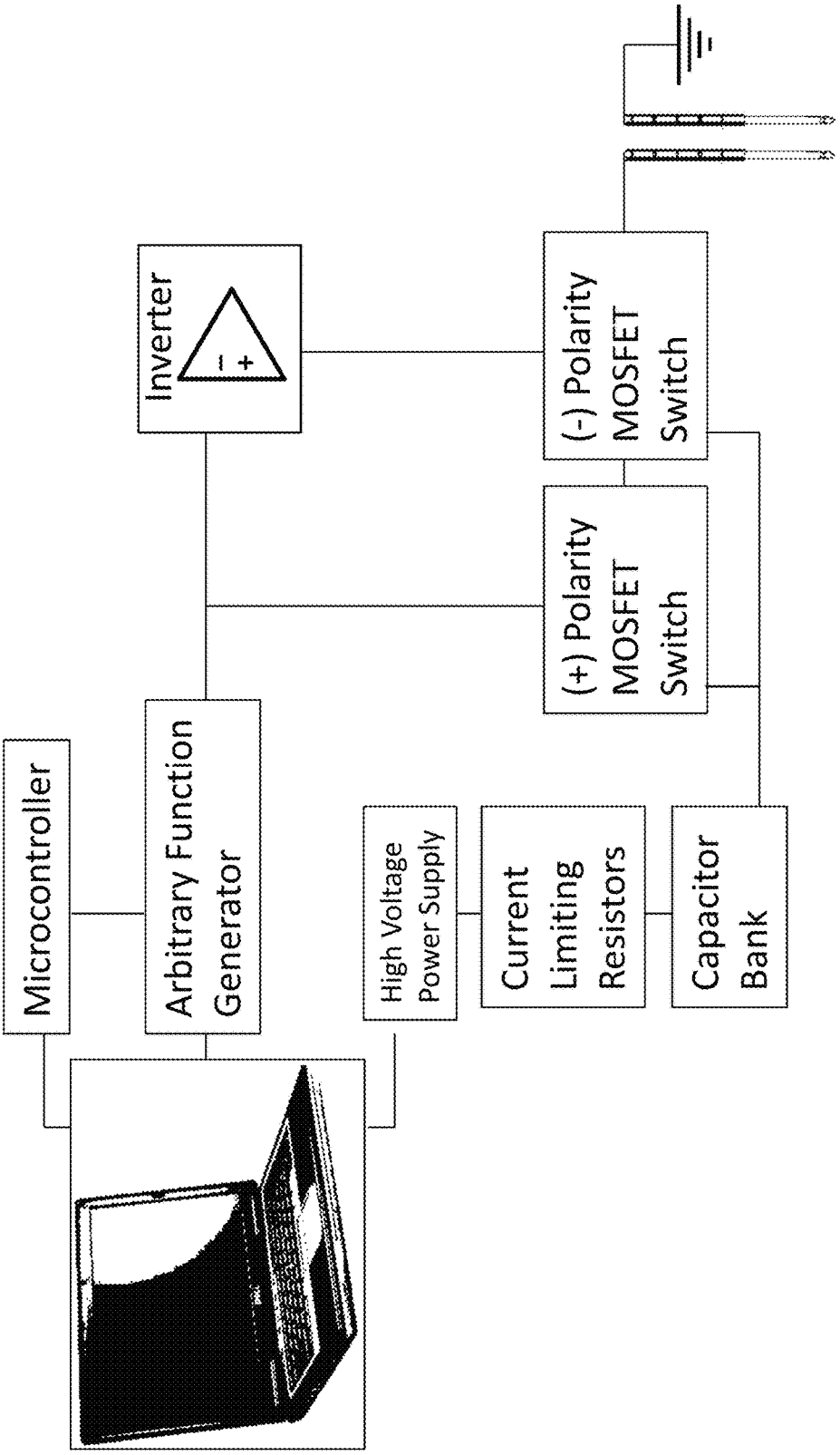

Inverter
(-) Polarity MOSFET Switch
(+) Polarity MOSFET Switch
Microcontroller
Arbitrary Function Generator
High Voltage Power Supply
Current Limiting Resistors
Capacitor Bank

FIG. 12

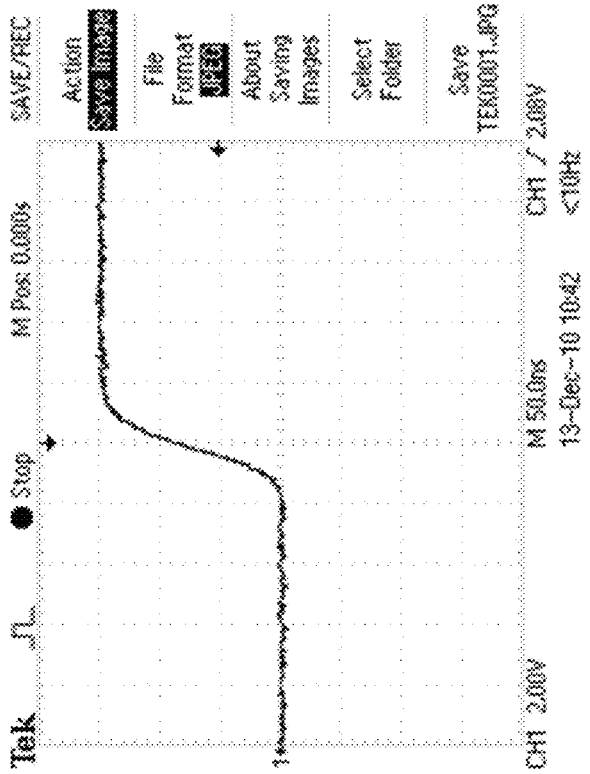


FIG. 13B

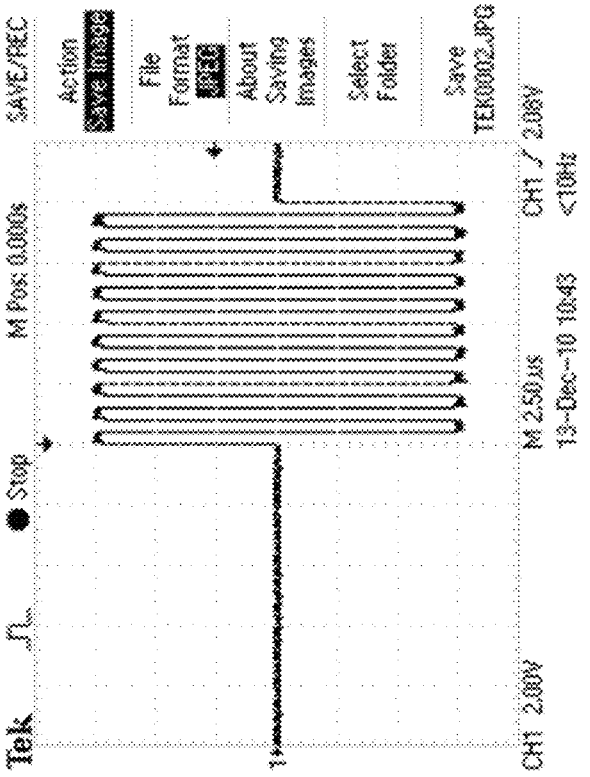


FIG. 13A

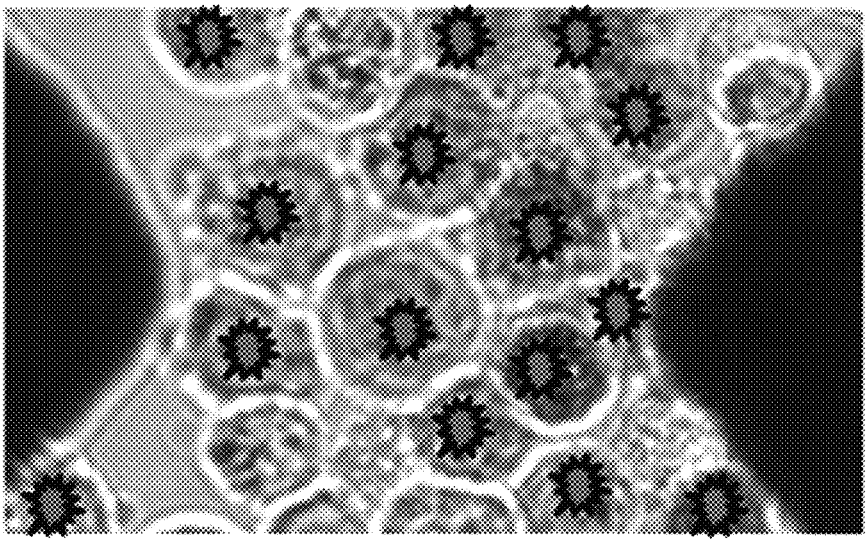


FIG. 14B

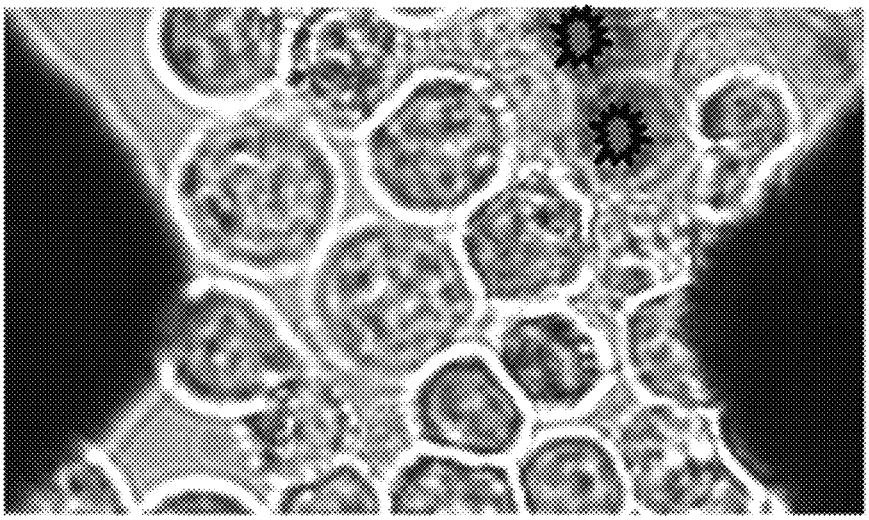


FIG. 14A

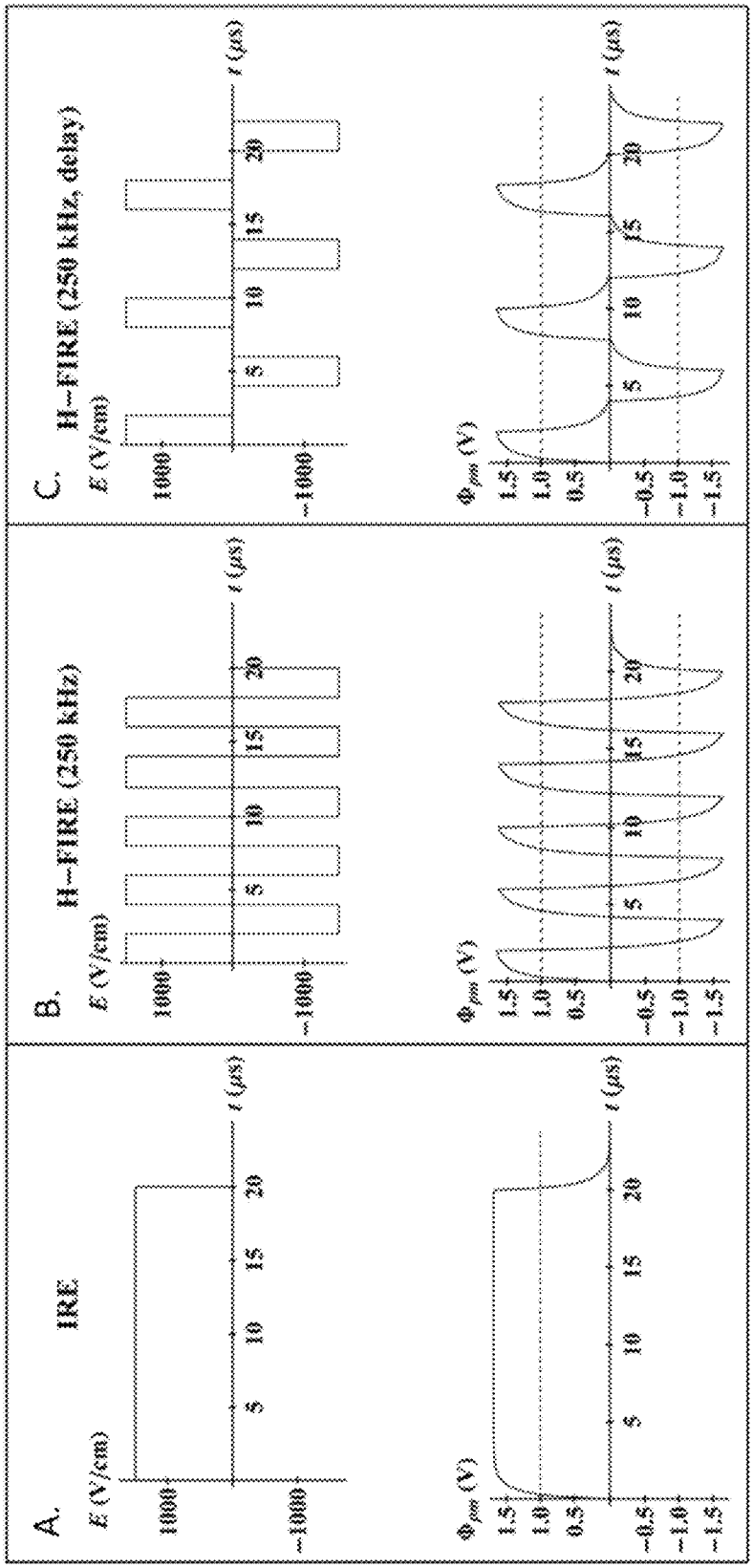


FIG. 15A

FIG. 15B

FIG. 15C

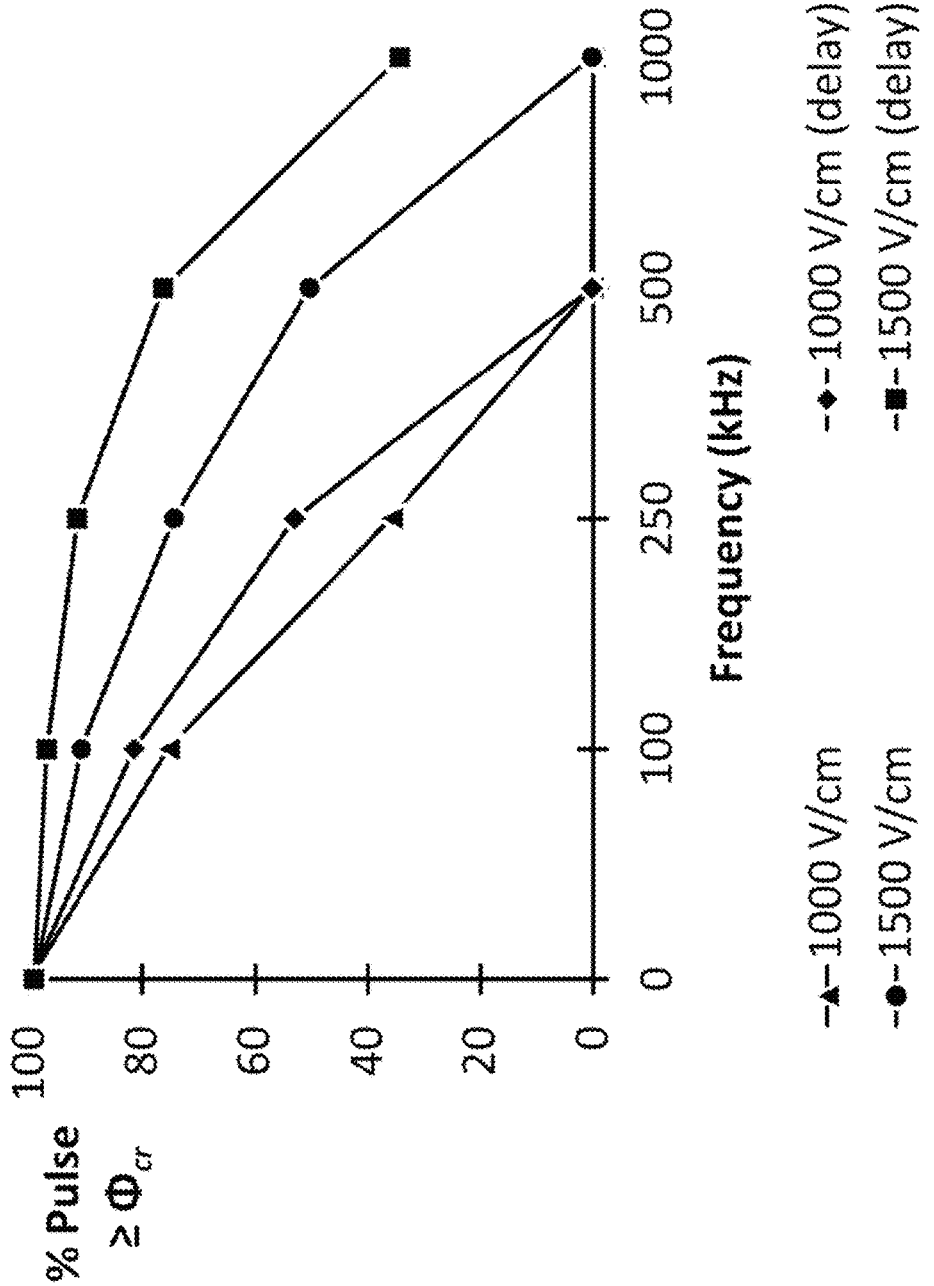

% Pulse
≥ Φcr
100
80
60
40
20
0
0
100
250
500
1000
Frequency (kHz)
1000 V/cm
1500 V/cm
1000 V/cm (delay)
1500 V/cm (delay)

FIG. 16

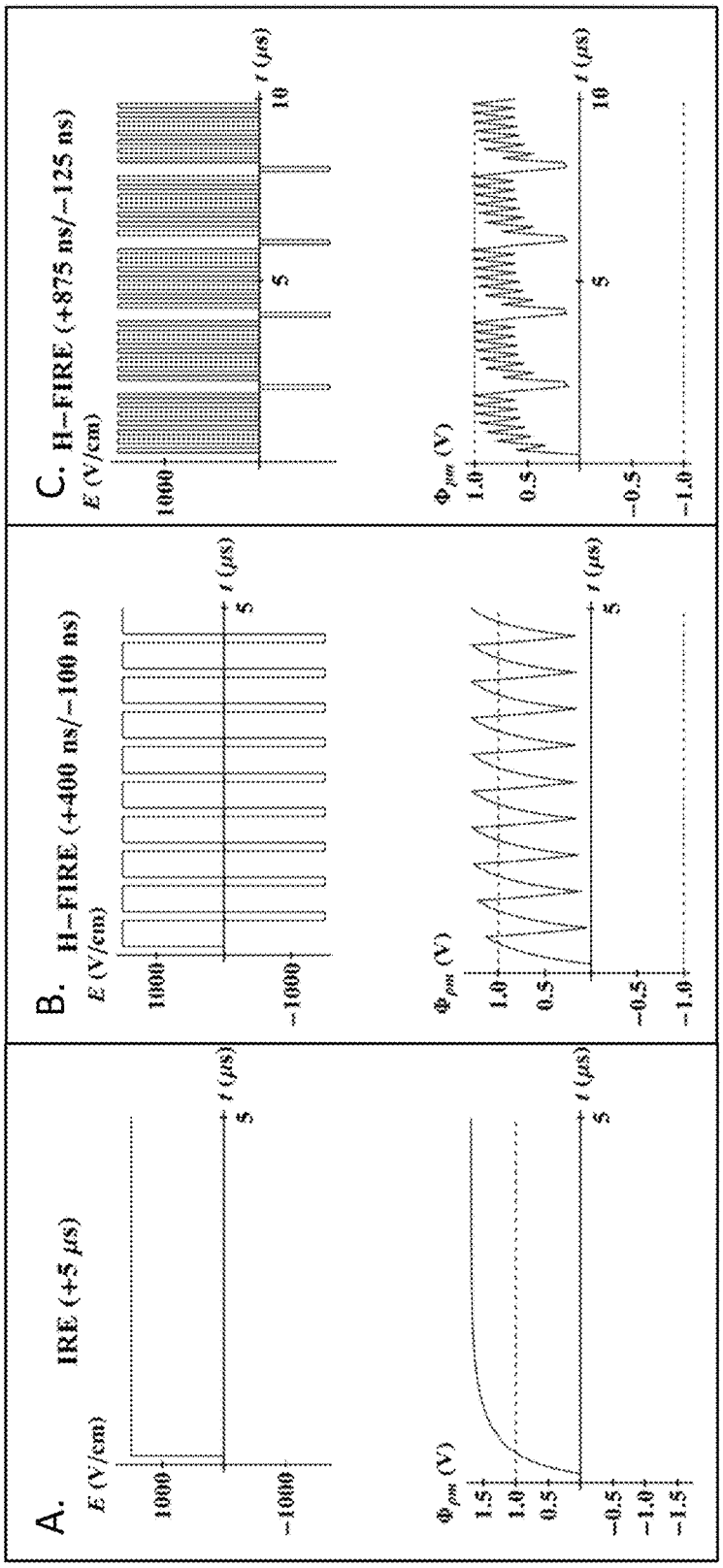


FIG. 17A

FIG. 17B

FIG. 17C

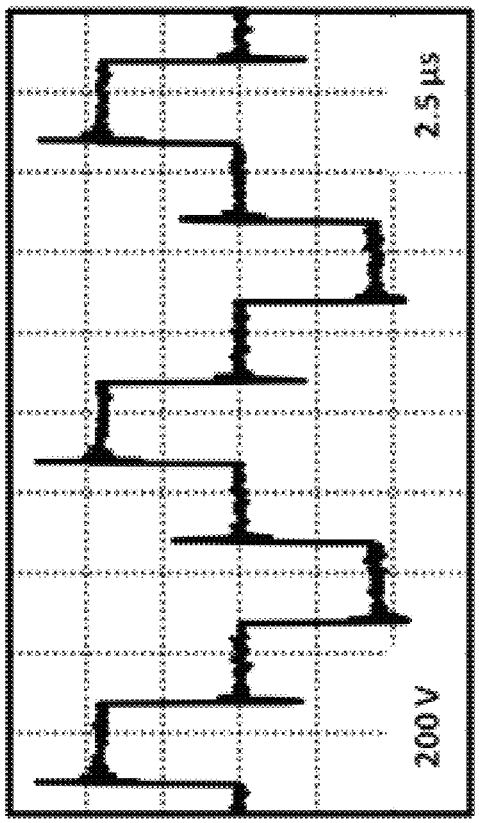


FIG. 18

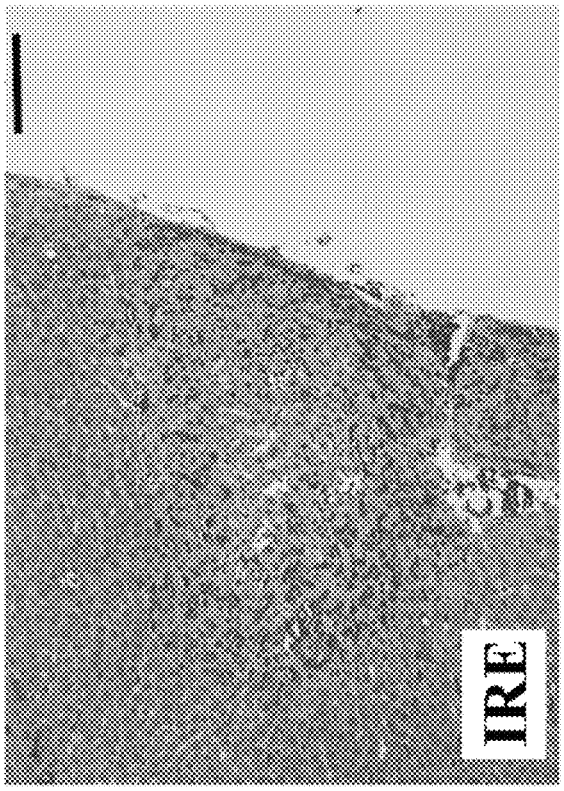


FIG. 19B

FIG. 19A

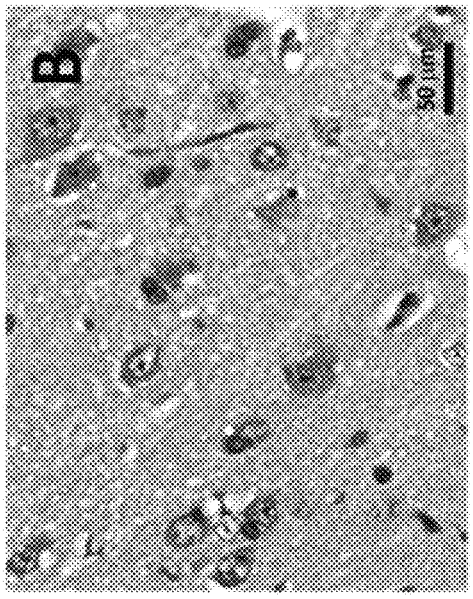


FIG. 20B

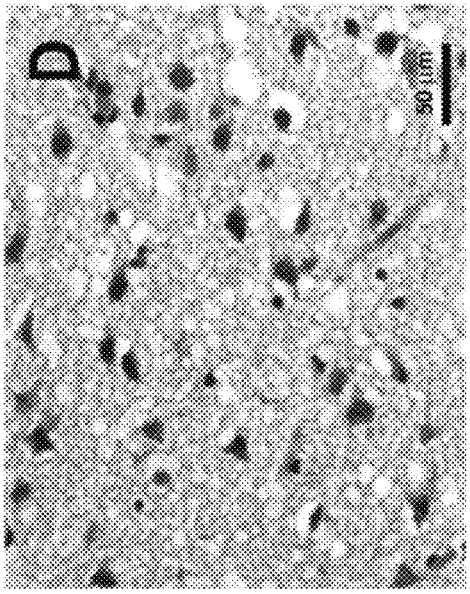


FIG. 20D

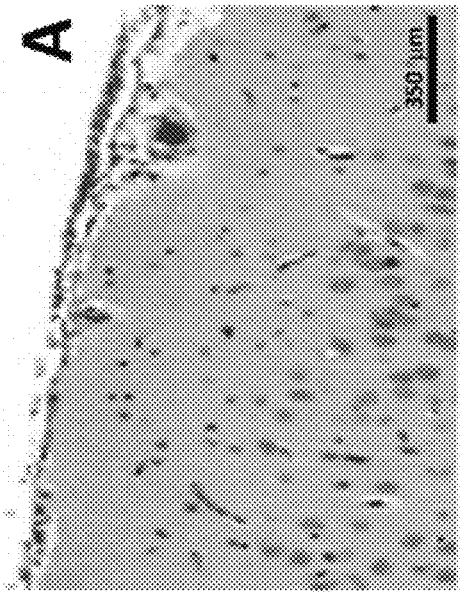


FIG. 20A

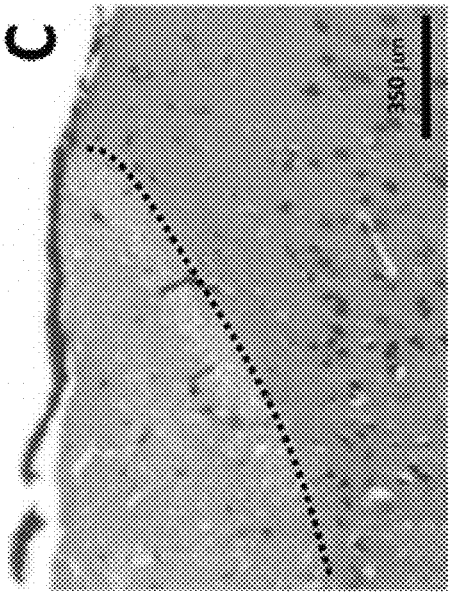


FIG. 20C

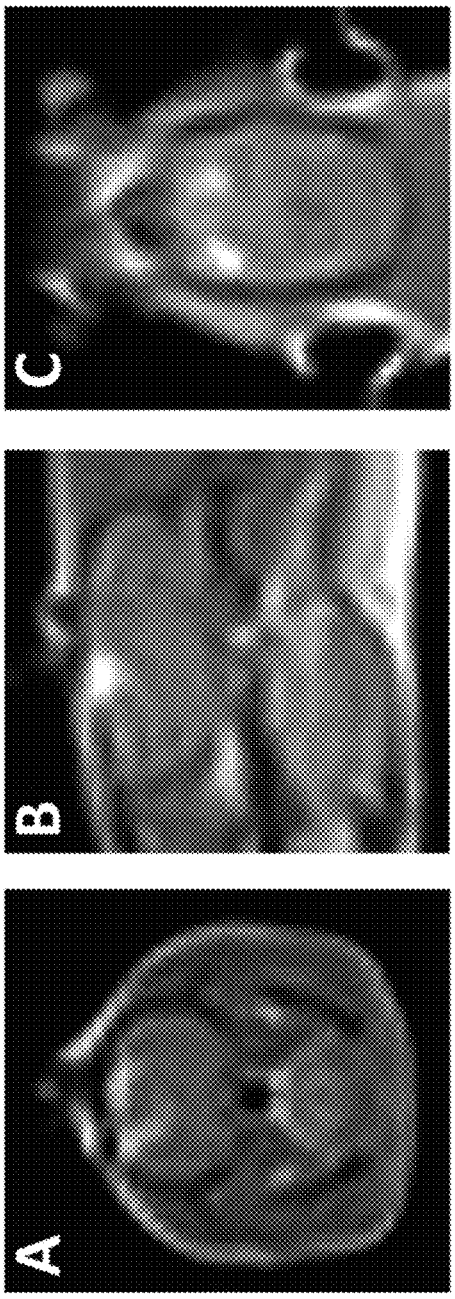


FIG. 21A

FIG. 21B

FIG. 21C

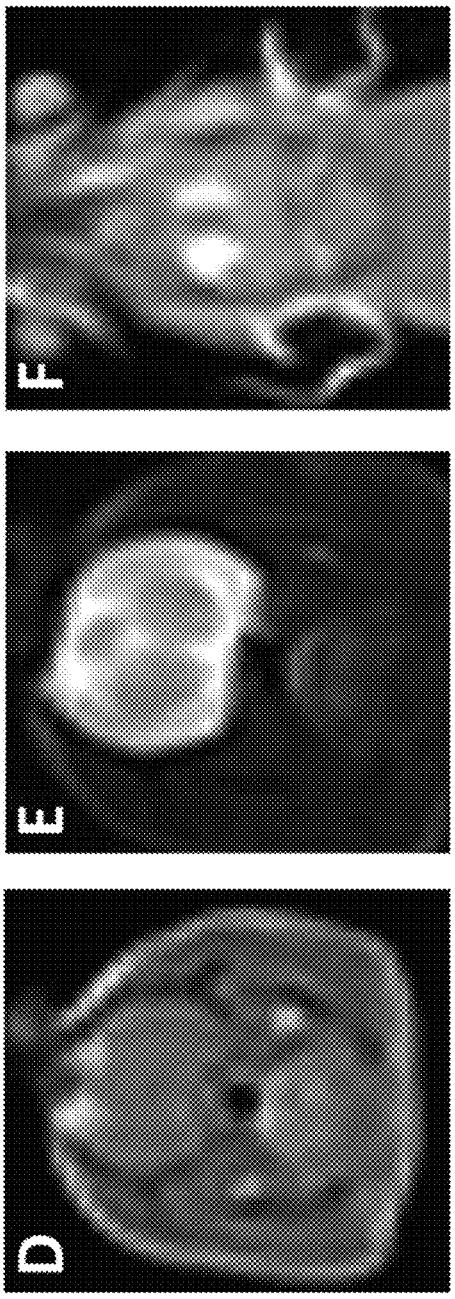


FIG. 21D

FIG. 21E

FIG. 21F

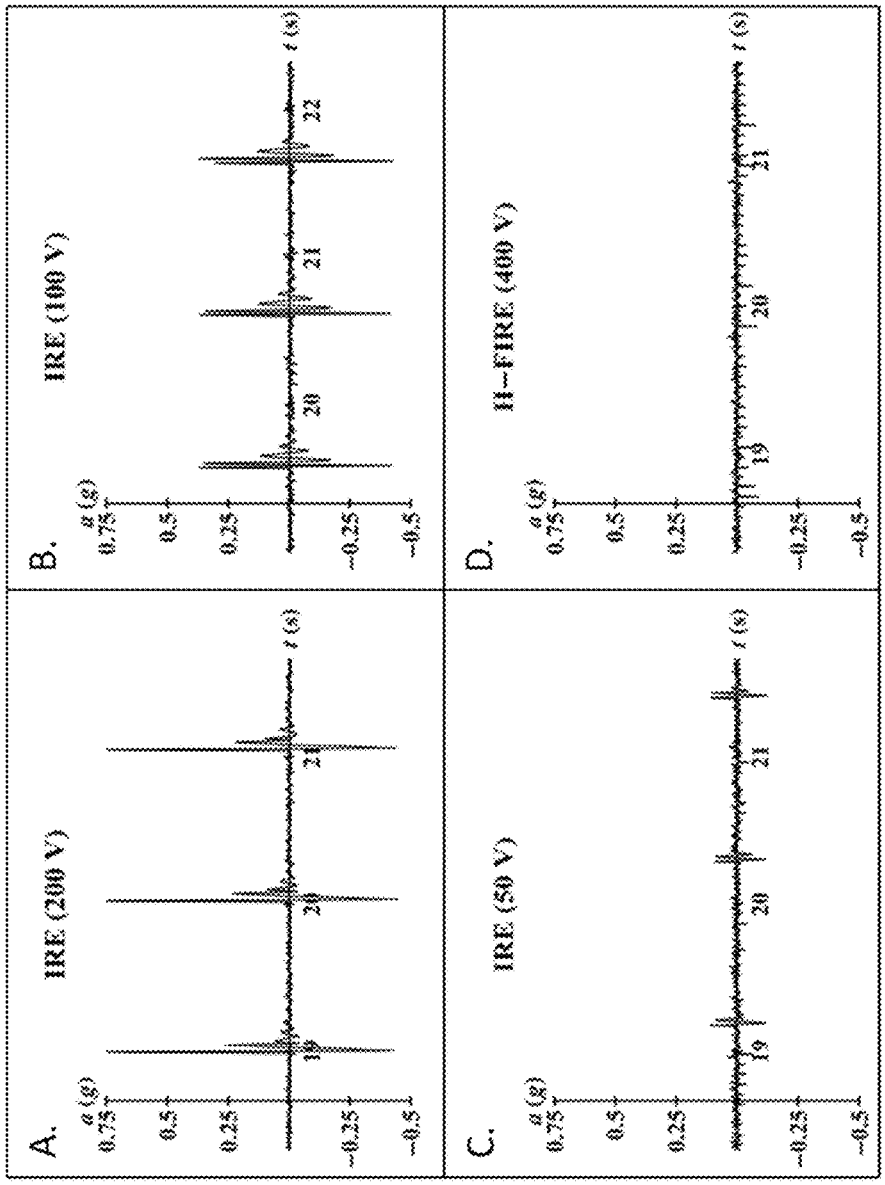


FIG. 22A

FIG. 22B

FIG. 22C

FIG. 22D

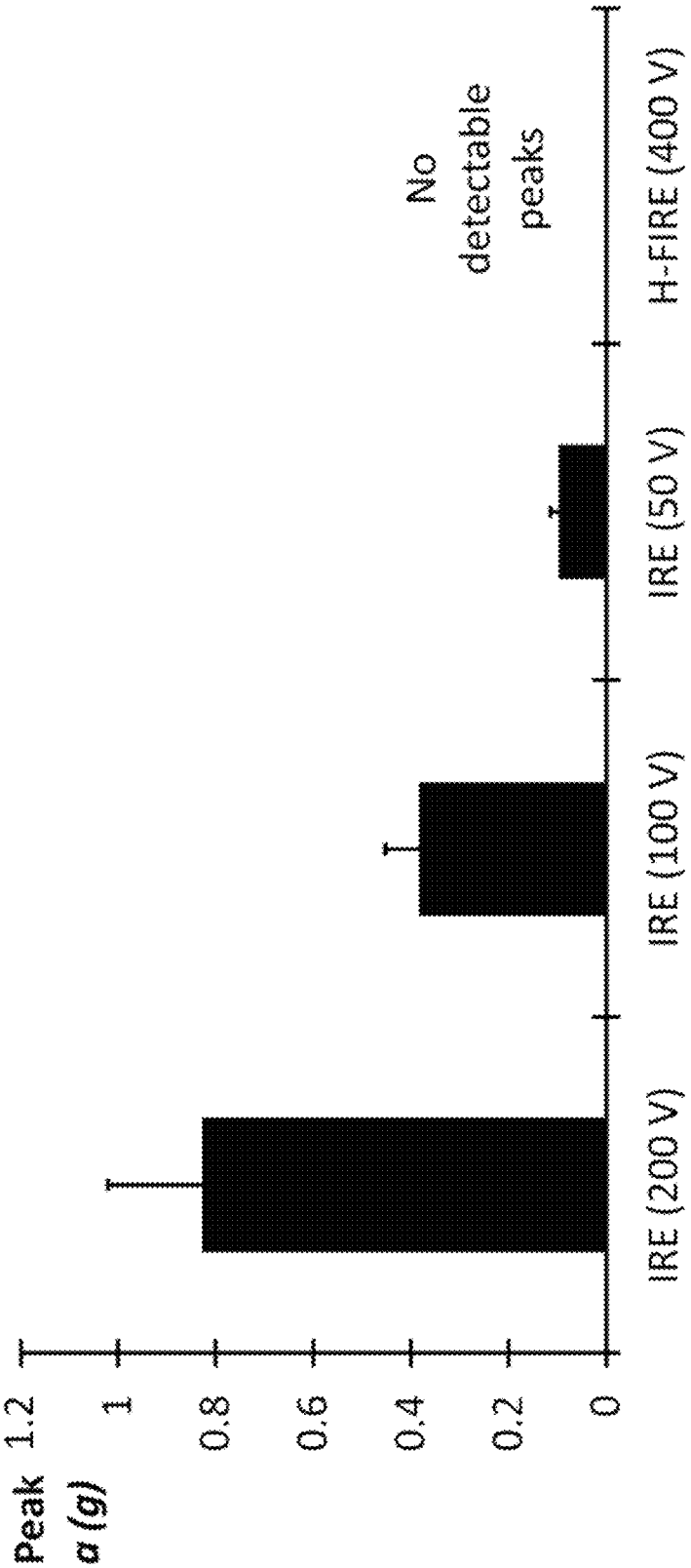

Peak
a (g)
1.2
1
0.8
0.6
0.4
0.2
0
IRE (200 V)
IRE (100 V)
IRE (50 V)
H-FIRE (400 V)
No
detectable
peaks

FIG. 23

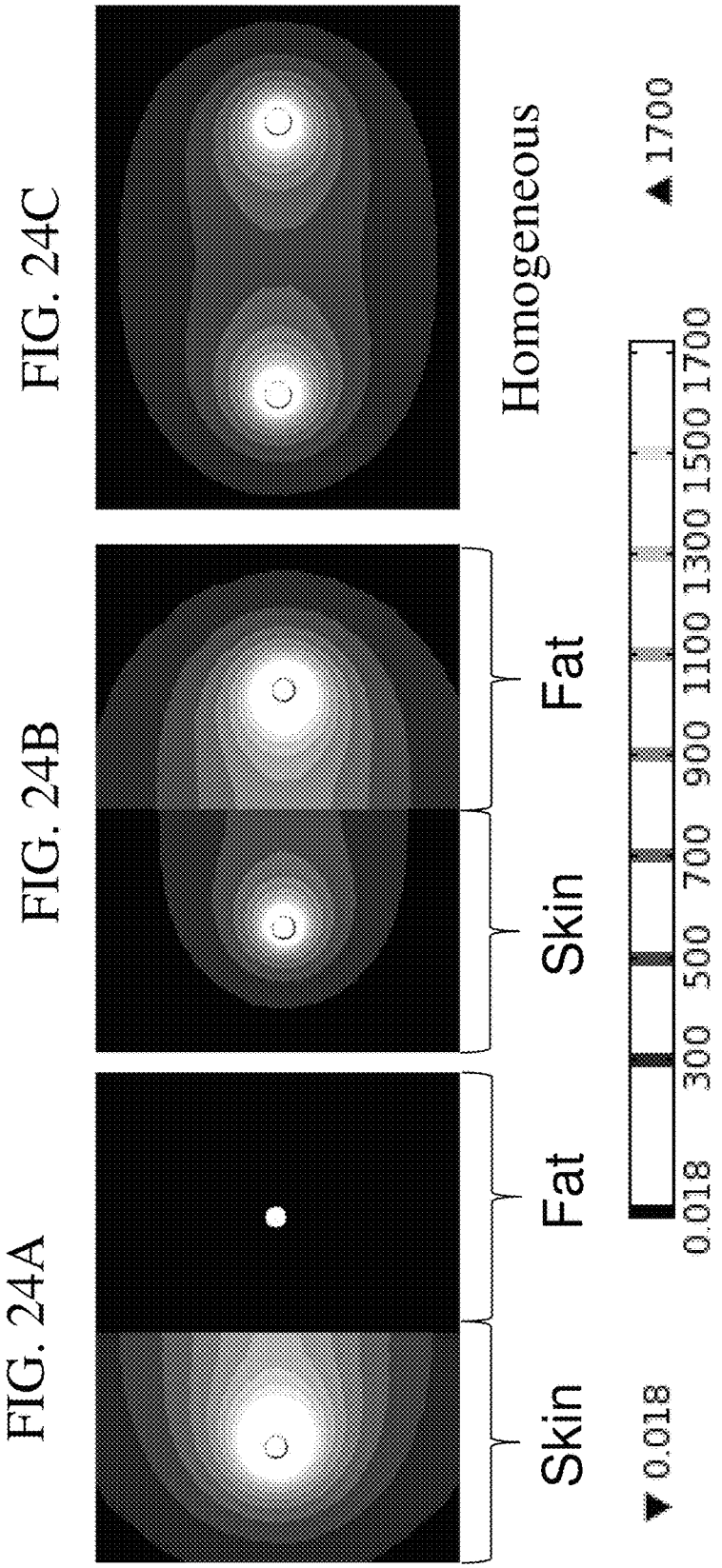


FIG. 24A
FIG. 24B
FIG. 24C
Skin
Fat
Skin
Fat
Homogeneous
▼ 0.018
0.018 300 500 700 900 1100 1300 1500 1700
▲ 1700

HIGH FREQUENCY ELECTROPORATION FOR CANCER THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to and is a Divisional of parent application U.S. patent application Ser. No. 13/332,133 filed Dec. 20, 2011, which published as U.S. Patent Application Publication No. 20120109122 on May 3, 2012. The '133 application relies on and claims priority to and the benefit of the filing date of U.S. Provisional Patent Application No. 61/424,872 filed Dec. 20, 2010. The '133 application is a Continuation-In-Part (CIP) application of U.S. patent application Ser. No. 12/757,901, filed Apr. 9, 2010 (patented as U.S. Pat. No. 8,926,606 on Jan. 6, 2015), which relies on and claims priority to and the benefit of the filing date of U.S. Provisional Patent Application No. 61/167,997, filed Apr. 9, 2009, and 61/285,618 filed Dec. 11, 2009. The present application is also related to International Patent Application No. PCT/US 11/66239, filed Dec. 20, 2011, which published as WO 2012/088149 on Jun. 28, 2012. The entire disclosures of all of these patent applications are hereby incorporated herein by reference.

STATEMENT OF GOVERNMENT INTEREST

This invention was made with government support under Contract No. CBET-0933335 awarded by National Science Foundation. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the field of biomedical engineering and medical treatment of diseases and disorders. More specifically, embodiments of the invention relate to a device and method for destroying aberrant cells, including tumor tissues, using high-frequency, bipolar electrical pulses having a burst width on the order of microseconds and duration of single polarity on the microsecond to nanosecond scale.

Description of Related Art

Electroporation based therapies typically involve delivering multiple, unipolar pulses with a short duration (~100 μs) through electrodes inserted directly into, or adjacent to, the target tissue. See Nuccitelli, R., X. Chen, A. G. Pakhomov, W. H. Baldwin, S. Sheikh, J. L. Pomicter, W. Ren, C. Osgood, R. J. Swanson, J. F. Kolb, S. J. Beebe, and K. H. Schoenbach, *A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence*. Int J Cancer, 2009. 125(2): p. 438-45; Davalos, R. V., L. M. Mir, and B. Rubinsky, Tissue ablation with irreversible electroporation. Ann Biomed Eng, 2005. 33(2): p. 223-31 ("Davalos 2005"); Payselj, N., V. Preat, and D. Miklavcic, A numerical model of skin electroporation as a method to enhance gene transfection in skin. 11th Mediterranean Conference on Medical and Biological Engineering and Computing 2007, Vols 1 and 2, 2007. 16(1-2): p. 597-601 ("Payselj 2007"); and Payselj, N., Z. Bregar, D. Cukjati, D. Batiuskaite, L. M. Mir, and D. Miklavcic, The course of tissue permeabilization studied on a mathematical model of a subcutaneous tumor in small animals. Ieee Transactions on Biomedical Engineering, 2005. 52(8): p. 1373-1381.

The extent of electroporation is attributed to the induced buildup of charge across the plasma membrane, or transmembrane potential (TMP). See Abidor, I. G., V. B. Arakelyan, L. V. Chernomordik, Y. A. Chizmadzhev, V. F. Pastushenko, and M. R. Tarasevich, *Electric Breakdown of Bilayer Lipid-Membranes* 0.1. *Main Experimental Facts and Their Qualitative Discussion . Bioelectrochemistry and Bioenergetics,* 1979. 6(1): p. 37-52; Benz, R., F. Beckers, and U. Zimmermann, *Reversible electrical breakdown of lipid bilayer membranes: a charge-pulse relaxation study*. J Membr Biol, 1979. 48(2): p. 181-204; Neumann, E. and K. Rosenheck, *Permeability changes induced by electric impulses in vesicular membranes*. J Membr Biol, 1972. 10(3): p. 279-90; Teissie, J. and T. Y. Tsong, *Electric-Field Induced Transient Pores in Phospholipid-Bilayer Vesicles . Biochemistry,* 1981. 20(6): p. 1548-1554; Zimmermann, U., G. Pilwat, and F. Riemann, Dielectric breakdown of cell membranes . Biophys J, 1974. 14(11): p. 881-99; and Kinosita, K. and T. Y. Tsong, Formation and Resealing of Pores of Controlled Sizes in Human *Erythrocyte-Membrane . Nature,* 1977. 268(5619): p. 438-441.

Once the TMP reaches a critical voltage, it is thought that permeabilizing defects, or pores, form in the plasma membrane in attempt to limit further TMP rise. Pore formation can either be reversible to allow for the introduction of foreign particles into viable cells, or irreversible to promote cell death through a loss of homeostasis. Known devices and methods of performing electroporation clinically involve several drawbacks, including painful muscle contractions, unpredictable treatment outcomes, and a high potential for thermal damage in low passive conductivity tissues.

IRE performed with unipolar pulses causes intense muscle contractions. Therefore, clinical applications of IRE require the administration of general anesthesia and neuroparalytic agents in order to eliminate the discomfort caused by muscle contractions seen during each pulse. See Talele, S. and P. Gaynor, *Non-linear time domain model of electropermeabilization: Response of a single cell to an arbitrary applied electric field*. Journal of Electrostatics, 2007. 65(12): p. 775-784. Receiving paralytic agents is undesirable for patients, and may deter them from seeking an electroporation based therapy. Further, in some cases, even with an adequate neuromuscular blockade, muscle contractions are still visible (see Payselj 2007), and questions remain as to what constitutes an appropriate dosage. Muscle contractions may affect the location of implanted needle electrodes, which can invalidate treatment planning algorithms Additionally, in treatments near vital structures, displacement of the implanted electrodes may cause unavoidable collateral damage.

The time course of the pulsed electric field and dielectric properties of the tissue control the TMP development and the extent to which the transient defects form and reseal within the membrane. Knowledge of these two components can be used to predict treatment outcomes. However, predictions are complicated in heterogeneous tissues, or organs with multiple types of parenchymal tissue. There is often an intricate and unknown geometrical arrangement between tissues of low and high electrical conductivity, and the conductivity can change in real-time due to the phenomenon of electroporation, the extent of which is highly unpredictable without prior knowledge.

Low conductivity tissues, such as epithelial layers, often contain a dense packing of cells with reduced extracellular current pathways. As such, the resistance of the extracellular space is increased. Additionally, when pulses much longer than the charging time of the plasma membrane (~1 μs) are

applied (see T. R., A. T. Esser, Z. Vasilkoski, K. C. Smith, and J. C. Weaver, *Microdosimetry for conventional and supra-electroporation in cells with organelles*. Biochem Biophys Res Commun, 2006. 341(4): p. 1266-76, "Gowrishankar 2006"), the current is confined to the extracellular space prior to the onset of electroporation, as shown in FIGS. **1**A-B. As shown, when the pulse duration (t_d) is much less than the plasma membrane time constant (τ_{pm}), current flows through both intracellular and extracellular spaces (FIG. **1**A). In the case that t_d is much greater than τ_{pm}, current flow is restricted to the narrower extracellular spaces (FIG. **1**B). Consequently, there is a large voltage drop across tissues with low conductivity, which increases the potential for deleterious Joule heating effects, such as thermal damage.

SUMMARY OF THE INVENTION

The present invention provides advancements over conventional tissue electroporation by utilizing high-frequency, bipolar pulses. Pulsing protocols according to embodiments of the invention involve bursts of bipolar pulses with a burst width on the order of microseconds and duration of single polarity on the microsecond to nanosecond scale, as shown in FIG. **2**. The total burst width of the high-frequency pulses (~100-1000 ns duration of single polarity) is on the order of hundreds of microseconds, the time delay in between bursts is on the order of seconds, and the total number of bursts can be adjusted.

It is possible for the electric field to penetrate tissue heterogeneities when high-frequency electric fields are employed, because capacitive coupling is enhanced allowing current to flow through both extracellular and intracellular spaces. See Gowrishankar, T. R. and J. C. Weaver, An approach to electrical modeling of single and multiple cells. Proceedings of the National Academy of Sciences of the United States of America, 2003. 100(6): p. 3203-3208; and Ivorra, A., ed. Tissue Electroporation as a Bioelectric Phenomenon: Basic Concepts . Irreversible Electroporation, ed. B. Rubinsky. 2010, Springer Berlin Heidelberg. 23-61. In this case, all cells present in the organ, regardless of their packing and morphology, experience a macroscopically homogeneous electric field distribution. See Esser, A. T., K. C. Smith, T. R. Gowrishankar, and J. C. Weaver, Towards Solid Tumor Treatment by Nanosecond Pulsed Electric Fields. Technology in Cancer Research & Treatment, 2009. 8(4): p. 289-306. This results in more predictable and uniform treatment outcomes without the electric energy being preferentially deposited into regions of tissue with a lower passive conductivity. As a result, Joule heating is also more uniformly distributed throughout the tissue, which mitigates the potential for thermal damage in regions with a low passive conductivity.

Enhanced capacitive coupling also limits the change in tissue electrical conductivity due to electroporation. Therefore, prior knowledge of how the conductivity of a tissue is modulated in response to electroporation is not required to accurately predict the electric field distribution. As a result, simplified algorithms can be implemented for treatment planning.

High-frequency, bipolar waveforms are also included in embodiments of the invention for mitigating or completely eliminating muscle contractions during electroporation based therapies. It is well known in the field of functional electrical stimulation that the threshold for nerve stimulation increases as the center frequency of bipolar waveforms increases. Further, muscle twitch forces are reduced as frequency increases. The present invention demonstrates that a range of frequencies exist where non-thermal tissue ablation can be achieved without causing nerve excitation or muscle contraction. In the context of this specification, it is noted that the term ablation is used to indicate destruction of cells, but not necessarily destruction of the supportive stroma.

Clinically, this translates to performing IRE without the requirement of paralytic agents (or a reduction in the amount of paralytic agents administered) in all procedures, and without the further requirement of general anesthesia in minimally invasive procedures. Additionally, other complications caused by IRE with unipolar electric pulses are alleviated, including electrode displacement and pain associated with intense muscle contractions.

Examples of heterogeneous systems include, but are not limited to, tumors surrounded by or containing any type of epithelial layer, such as a skin fold geometry, or systems comprised of multiple tissue types including, brain, bone, breast, pancreatic, kidney, or lung. In this specification, an epithelial layer is defined as a dense packing of cells that restrict the flow of materials (e.g., electrical current) resulting in a low passive electrical conductivity.

The present invention applies to all electroporation based therapies. Recently, electroporation has been utilized in vivo as a means to destroy cancer cells within tissues in both reversible and irreversible modalities. Reversible electroporation is being studied to facilitate the delivery of anticancer drugs and DNA into cancer cells through the plasma membrane in the form of electrochemotherapy (ECT) and electrogenetherapy (EGT), respectively. Irreversible electroporation (IRE) promotes cell death resulting in the development of a tissue lesion. It is an independent means to ablate substantial volumes of targeted tissue without the use of harmful adjuvant chemicals if used prior to the onset of thermal injury. See Davalos 2005. By not relying on thermal processes, IRE has been shown to spare the extracellular matrix and architecture of nerves and blood vessels.

More specifically, the present invention provides new devices and methods for the treatment of diseases and disorders, such as hemic and solid neoplasias, which improves conventional clinical practice associated with electroporating target tissues.

Included in embodiments of the invention is a method of treating a subject suffering from a neoplasia comprising: implanting at least one device for emitting electric pulses into or adjacent a neoplastic site within the body of a subject; and delivering one or more electric pulse to the neoplastic site, such that amplitude and duration of the pulse are in the range of about 1500 V/cm to 2500 V/cm for 10 μs or less which is capable of inducing irreversible electroporation. Methods of the invention also include non-invasive methods of treating a subject comprising non-invasively placing at least one device for emitting electric pulses around a region of the body containing a neoplastic site within; and delivering one or more electric pulse, such that amplitude and duration of the pulse are in the range of about 1500 V/cm to 2500 V/cm for 10 μs or less which is capable of inducing irreversible electroporation.

According to embodiments of the invention, such methods can employ multiple pulses administered in a pulse burst having a duration of less than 10 ms.

Such methods can employ one or more pulses or a plurality of pulses in a pulsing protocol, wherein the amplitude of the pulse is in the range of about 500 V/cm to 1500 V/cm. Amplitude in the context of this specification refers to

the voltage-distance ratio of a pulse, such as for 1500 V/cm the voltage is 750V over a distance of 0.5 cm.

Such methods can have a pulse duration in the range of about 2 MHz (250 ns) to about 500 kHz (1 μs). For example, the pulse duration can be about 1 MHz (500 ns). In preferred embodiments, the duration of each pulse is in the range of about 100 to 10,000 ns.

Any number of probes or electrodes can be used invasively, semi-invasively, or non-invasively according to embodiment of the invention. In preferred embodiments, two or more electrically conductive regions are used within a single device for emitting the electrical pulses. Similarly, in any of the methods according to the invention, two or more devices can be used to deliver multiple electric pulses at different positions within, on, or near a body.

Custom treatment area shapes can be created through varying electrode activation patterns in combination with any of the embodiments of the invention.

The methods can also employ delivery of a bipolar burst of pulses. In embodiments, a bipolar burst of pulses can be delivered with multiple pulses in a single phase before a polarity switch. Even further, total burst width of any pulse protocol according to the invention can be between 1 μs and 10,000 μs. In preferred embodiments, the methods can have a duration of single polarity within a bipolar burst of between about 100 ns and 100,000 ns.

The shape of the electric pulses delivered using methods of the invention can be square, ramp, sinusoidal, exponential, or trapezoidal.

In preferred embodiments, two or more electric pulse bursts can be administered with a delay between bursts. In preferred embodiments, a delay between bursts can be on the order of seconds. For example, in bipolar protocols a selected positive voltage (+V) can be applied for a selected period of time (e.g., 50 μs), then a zero voltage applied for a selected period of time (e.g., 75 μs), then a negative voltage (−V) can be applied (e.g., 50 μs). The voltage can be applied in any number of individual pulses, as a pulse or pulse burst.

Also included in embodiments of the invention is a method of delivering electric pulses such that amplitude and duration of single polarity are selected to be capable of administering electroporation to electrically excitable tissue without stimulation of the tissue.

Further included is a method of delivering electric pulses such that amplitude and duration of single polarity are selected to be capable of administering electroporation to electrically excitable tissue with reduced stimulation of the tissue as compared with higher amplitude and longer duration pulse protocols. Preferably tissue stimulation that is avoided or prevented refers to a muscle contraction.

In embodiments, the neoplastic site, region of the body, or electrically excitable tissue can be nerve tissue, muscle, or an organ containing nerves and/or muscle tissue.

Any embodiment of the invention can employ applying electric pulses having an amplitude and duration in the range of about 1500 V/cm to 2500 V/cm for 10 ms or less which is capable of inducing irreversible electroporation.

Method embodiments of the invention can be used to build up the transmembrane potential of a tissue to a critical value (~1V) by delivering trains of less than 1 μs bipolar pulses. For example, multiple monopolar pulses can be delivered at a pulse duration of about 5 MHz prior to a polarity switch, then delivered at a pulse duration of about 5 MHz after polarity switch.

Methods of the invention may or may not employ administering of a drug designed to induce a neural blockade. The methods can include administration of general, local, or no anesthesia for treatment of tissues with electroporation based therapies. In preferred embodiments, no neural blockade is required for treatment of tissues with electroporation based therapies, or lower dosages of a neural blockade can be used in embodiments of the invention to achieve the same results as using higher doses with lower frequency pulsing protocols.

The pulses of any method of the invention can be delivered on a short enough timescale to flow through epithelial cells but are long enough to induce electroporation in underlying cells. In specific embodiments, a frequency of 500 kHz or 1 MHz or 250 kHz is used to treat underlying fat cells in a layer of fat disposed under the epidermis.

Methods according to the invention can be modified to provide for administering non-thermal IRE, IRE, and/or reversible electroporation.

Treatment planning according to embodiments of the invention can result in more predictable outcomes in homogeneous and heterogeneous tissues than compared with lower frequency pulsing protocols.

Any one or more of the methods, devices, or systems, or parts thereof, can be combined with other methods, devices, systems, or parts thereof mentioned in this specification to obtain additional embodiments within the scope of this invention.

Devices and systems for implementing any one or more of the above mentioned methods are also within the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings illustrate certain aspects of some of the embodiments of the present invention, and should not be used to limit the invention. Together with the written description the drawings serve to explain certain principles of the invention.

FIGS. **1**A-B are schematic illustrations showing electrical current pathways through epithelial layers and bulk tissue prior to the onset of electroporation.

FIG. **2** is a schematic diagram of a representative pulsing protocol for electroporation based therapy according to embodiments of the present invention.

FIGS. **3**A-D are graphs showing dielectric properties (τ and $\in_r$) as a function of frequency for skin and fat.

FIGS. **4**A-B are graphs showing respectively 2 MHz AC burst with a width of 4 μs, and a DC pulse with a duration of 4 μs of equal amplitude (FIG. **4**A); and magnitude spectrum of the AC burst (thick) and DC pulse (thin) (FIG. **4**B).

FIG. **5** is a graph showing frequency (f) response of the TMP at the cell pole (θ=0) for rectangular bipolar pulses (-) and sinusoidal waveforms (- -). The box illustrates the frequency window implemented in the FEM.

FIG. **6** is a graph of the strength-duration curve for unipolar electric pulses expressed as electric field strength in tissue. Adapted from Rogers, W. R., J. H. Merritt, J. A. Comeaux, C. T. Kuhnel, D. F. Moreland, D. G. Teltschik, J. H. Lucas, and M. R. Murphy, *Strength-duration curve for an electrically excitable tissue extended down to near 1nanosecond*. Ieee Transactions on Plasma Science, 2004. 32(4): p. 1587-1599 ("Reilly 2004").

FIG. **7** is a strength-duration graph comparing unipolar to bipolar rectangular and sine waveforms expressed as phase charge and current. Adapted from Reilly 2004.

FIGS. **8**A-B are schematic diagrams showing meshed geometry of the FEM with boundary settings (FIG. **8**A) and the geometry with dimensions (FIG. **8**B).

FIGS. **9**A-D are schematic diagrams showing the electric field, norm (V/cm) contours predicted by the FEM at the end of a 2 μs burst with an amplitude of 2600 V and a frequency of 250 kHz (FIG. **9**A), 500 kHz (FIG. **9**B), 1 MHz (FIG. **9**C), and 2 MHz (FIG. **9**D).

FIGS. **10**A-B are graphs showing temperature changes predicted by the FEM at the center of the skin (FIG. **10**A) and fat (FIG. **10**B) for various frequencies of 250 kHz (- -), 500 kHz (- - -), 1 MHz (. . .), and 2 MHz (-).

FIGS. **11**A-B are graphs of TMP predicted by the FEM at the center of the skin (FIG. **11**A) and fat (FIG. **11**B) for frequencies of 250 kHz (- -), 500 kHz (- - -), 1 MHz (. . .), and 2 MHz (-).

FIG. **12** is a diagram of a system for implementing high-frequency, bipolar pulses for tissue electroporation.

FIGS. **13**A-B are graphs showing output of the arbitrary function generator prior to signal amplification by the high voltage MOSFET positive and negative polarity switches.

FIGS. **14**A-B are micrographs showing in vitro experimental results on electroporation with high-frequency bipolar, pulses using a trypan blue dye exclusion assay.

FIG. **15**A-C are waveforms of IRE with unipolar pulses and high-frequency IRE with the corresponding TMP development across the plasma membrane (Φ_{pm}) for a 1500 V/cm unipolar pulse (FIG. **15**A) and a 1500 V/cm bipolar burst without a delay (FIG. **15**B) and with a delay (FIG. **15**C).

FIG. **16** is a graph comparing time above the critical threshold (Φ_{pm}) for IRE at various center frequencies.

FIGS. **17**A-C are waveforms of IRE with unipolar pulses and high-frequency IRE with the corresponding TMP development across the plasma membrane (Φ_{pm}) for a 1500 V/cm unipolar pulse (FIG. **17**A), a 1500 V/cm bipolar burst without a delay and with a shortened negative phase (FIG. **17**B), and a 1500 V/cm bipolar burst with a delay and with a shortened, lower amplitude negative phase (FIG. **17**C).

FIG. **18** is a chart showing an exemplary output from an in vivo treatment of the brain with high-frequency, bipolar pulses, where the snapshot is taken within a single burst.

FIGS. **19**A-B are photographs showing histological sections of liver tissue treated with high-frequency IRE (FIG. **19**A) and conventional IRE with unipolar (FIG. **19**B), with cross sections of tissue taken between the electrodes (scale bar=250 μm).

FIGS. **20**A-D are micrographs showing the histopathology of rat brain tissue for untreated rats (FIGS. **20**A-B) and treated with high-frequency, bipolar pulses at 200 V/250 kHz (FIG. **20**C-D, Rat #2, right hemisphere), with the delineation between treated and untreated tissue shown in FIG. **20**C (black, dotted line).

FIGS. **21**A-F are MRIs of lesions in rat brain appearing as focal hyper-intense regions (white) compared to adjacent untreated cerebrocortical tissue (gray). FIGS. **21**A-C were obtained from Rat #3, in which both the left and right cerebral hemispheres were treated with high-frequency waveforms at 300 V/250 kHz and 400 V/250 kHz, respectively. FIGS. D-F were obtained from Rat #4, which underwent high-frequency, bipolar pulses in the right cerebrum at 400 V/500 kHz, and conventional IRE with unipolar pulses at 50 V in the left cerebrum.

FIGS. **22**A-D are data recordings of acceleration (a) versus time during treatments with unipolar IRE pulses and high-frequency IRE pulses.

FIG. **23** is a chart showing peak acceleration (a) during pulsing protocols averaged over the first 90 pulses.

FIGS. **24**A-C are schematic diagrams showing electric field, norm (V/cm) contours predicted by the FEM during a 1000 V amplitude burst with a center frequency of 1 kHz (FIG. **24**A) and 1 MHz (FIG. **24**B). In FIG. **24**C, the homogeneous solution is shown for a constant pulse.

DETAILED DESCRIPTION OF VARIOUS EMBODIMENTS OF THE INVENTION

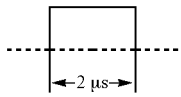
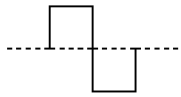
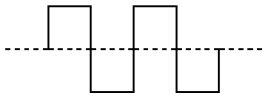
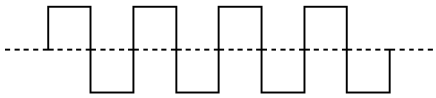
Reference will now be made in detail to various exemplary embodiments of the invention. It is to be understood that the following discussion of exemplary embodiments is not intended as a limitation on the invention. Rather, the following discussion is provided to give the reader a more detailed understanding of certain aspects and features of the invention.

Despite being a well-known technique, there is significant controversy about the mechanisms governing electroporation. Weaver, J. C., *Electroporation of cells and tissues*. IEEE Transactions on Plasma Science, 2000. 28(1): p. 24-33. Even though the biophysical phenomenon at the molecular level is not known, the hypothesis is that in the presence of an externally applied electric field, the lipid bilayer in cellular membranes rearranges to create water-filled structures. These structures (or pores) provide a pathway for ions and molecules through the membranes that normally are impermeable. The dynamics of membrane poration is considered a four-step process: pore induction, expansion, stabilization and resealing. Weaver, J. C. and Y. A. Chizmadzhev, *Theory of electroporation: a review*. Bioelectrochem. Bioenerg., 1996. 41: p. 135-60. Initial thermal fluctuations are responsible for the presence of hydrophobic pores. There exists a critical radius where it is more energetically favorable for a hydrophobic pore to transition to a hydrophilic pore. In addition, increasing the TMP reduces this critical radius and increases the stability of a hydrophilic pore. Kinosita, K., Jr., S. Kawato, and A. Ikegami, *A theory of fluorescence polarization decay in membranes*. Biophys J, 1977. 20(3): p. 289-305. When the pore reaches this metastable state, it becomes permeable to small molecules. The presence of the induced transmembrane potential lowers the energy required for the pore's existence. Freeman, S. A., M. A. Wang, and J. C. Weaver, *Theory of Electroporation of Planar Bilayer-Membranes—Predictions of the Aqueous Area, Change in Capacitance, and Pore-Pore Separation*. Biophysical Journal, 1994. 67(1): p. 42-56. When the electric field has been turned off, the membrane starts to return to its normal membrane potential and resealing of the pores takes place.

The dielectric permittivity and conductivity of a given tissue are typically functions of frequency. A comparison of the dielectric properties between skin and fat is presented in Table 1. This data was obtained by interpolating the results from Gabriel et al. (FIGS. **3**A-D). Gabriel, S., R. W. Lau, and C. Gabriel, The dielectric properties of biological tissues 0.2. Measurements in the frequency range 10Hz to 20GHz . Physics in Medicine and Biology, 1996.41(11): p. 2251-2269. At varying frequencies, different mechanisms of charge transfer contribute differently to the permittivity and conductivity. Stoy, R. D., K. R. Foster, and H. P. Schwan, Dielectric properties of mammalian tissues from 0.1to 100MHz: a summary of recent data . Phys Med Biol, 1982. 27(4): p. 501-13.

TABLE 1

Conductivity of skin and fat as a function of frequency.

Frequency	Waveform	Property	Tissue: Skin	Tissue: Fat
250 kHz		σ [S/m]	0.00216	0.0263
		ϵ_r	888	47
500 kHz		σ [S/m]	0.00485	0.0265
		ϵ_r	851	33
1 MHz		σ [S/m]	0.0119	0.0267
		ϵ_r	792	25
2 MHz		σ [S/m]	0.0290	0.0270
		ϵ_r	700	20

In general, as the frequency increases, so does the conductivity of the skin and fat. According to Table 1, the difference in conductivity between skin (s) and fat (f) is reduced as the frequency increases from 250 kHz to 2 MHz ($\sigma_s/\sigma_f \sim 1$).

Therefore, if electroporation is used to treat a tumor within a heterogeneous skin fold geometry, the electric field distribution in the surrounding skin and fat would be more homogenous if high-frequency waveforms are utilized. Oftentimes tissue impedance has patient-to-patient variability and the impedance distribution and any impedance changes may be difficult to determine for a particular patient. This point is emphasized further in EXAMPLE 1. Because rectangular waveforms are comprised of components with various frequencies and amplitudes, tissue properties at frequencies associated with the center frequency, defined as the inverse of twice the duration of single polarity, are chosen when studying AC bursts. This is illustrated in FIGS. **4**A-B. By taking the absolute value of the Fourier Transform of an AC burst and a DC pulse, the magnitude spectrum can be obtained. While the DC pulse transmits a majority of its power at low frequencies (0 Hz), the AC burst has a characteristic peak at the center frequency (2 MHz in this case).

The benefits of bipolar pulses have been studied for electroporation applications at the single-cell level. Theoretically, Talele et al. have shown that asymmetrical electroporation due to the resting TMP (~0.1 V) (see Gowrishankar 2006) of cells seen when unipolar pulses are delivered (see Chang, D. C., *Cell Poration and Cell-Fusion Using an Oscillating Electric-Field*. Biophysical Journal, 1989. 56(4): p. 641-652, "Chang 1989"; and Tekle, E., R. D. Astumian, and P. B. Chock, Electroporation by Using Bipolar Oscillating Electric - Field—an Improved Method for DNA Transfection of Nih 3t3Cells . Proceedings of the National Academy of Sciences of the United States of America, 1991. 88(10): p. 4230-4234, "Tekle 1991") can be alleviated by switching to bipolar pulses. Talele, S. and P. Gaynor, *Non-linear time domain model of electropermeabilization: Response of a single cell to an arbitrary applied electric field*. Journal of Electrostatics, 2007. 65(12): p. 775-784. Experimentally, this leads to increased efficiency of macromolecule uptake through the membrane. Chang 1989; and Tekle 1991. Depending on the extracellular conductivity, bipolar pulses with a frequency of 1 MHz (i.e. 500 ns duration of single polarity) can also lessen the dependence of electroporation on cell size, allowing more cells to be electroporated. Talele, S. and P. Gaynor, *Non-linear time domain model of electropermeabilization: Effect of extracellular conductivity and applied electric field parameters*. Journal of Electrostatics, 2008. 66(5-6): p. 328-334; and Talele, S., P. Gaynor, M. J. Cree, and J. van Ekeran, *Modelling single cell electroporation with bipolar pulse parameters and dynamic pore radii*. Journal of Electrostatics, 2010. 68(3): p. 261-274. In general, pore formation increases as long as the TMP is sustained above a critical threshold (~1 V). Gowrishankar 2006. Bipolar pulses require higher field strengths to induce a given TMP as compared to a unipolar pulse of equivalent duration. This is accentuated when the frequency of the bipolar pulses is increased, because the time interval above the critical TMP is reduced. Talele, S., P. Gaynor, M. J. Cree, and J. van Ekeran, *Modelling single cell electroporation with bipolar pulse parameters and dynamic pore radii*. Journal of Electrostatics, 2010.68(3): p. 261-274. Kotnik et al. have explored the benefits of bipolar pulse trains at significantly lower frequencies, up to 1 kHz (i.e. 500 μs duration of single polarity). At lower frequencies, theoretical results show that the pore formation symmetry can also be normalized with bipolar pulses. Kotnik, T., L. M. Mir, K. Flisar, M. Puc, and D. Miklavcic, *Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses. Part I. Increased efficiency of permeabilization*. Bioelectrochemistry, 2001. 54(1): p. 83-90, "Kotnik I 2001." Experimentally, bipolar pulses reduce electrolytic contamination (see Kotnik, T., D. Miklavcic, and L. M. Mir, *Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses. Part II. Reduced electrolytic contamination*. Bioelectrochemistry, 2001. 54(1): p. 91-5) and the required field strength for reversible electroporation, while the field strength required for IRE remains unchanged. Kotnik I 2001. The authors attribute this to the fact that when the duration of single

polarity is much longer than the plasma membrane charging time, permeabilized area differences on the membrane between unipolar and bipolar pulses decreases as pulse amplitude increases.

Bipolar pulse delivery has been studied in vivo for reversible applications of electroporation using center frequencies that are two orders of magnitude lower than that used in embodiments of the present invention. Daskalov et al. have demonstrated that pulses delivered at 1 kHz are associated with less patient pain in during electrochemotherapy. Daskalov, I., N. Mudrov, and E. Peycheva, *Exploring new instrumentation parameters for electrochemotherapy—Attacking tumors with bursts of biphasic pulses instead of single pulses*. IEEE Eng Med Biol Mag, 1999. 18(1): p. 62-66. Similarly, Nikolova et al. has recently demonstrated the same findings during electrochemotherapy with a Bacillus Calmette-Guerin vaccine. Nikolova, B., I. Tsoneva, and E. Peycheva, *Treatment of Melanoma by Electroporation of Bacillus Calmette-Guerin*. Biotechnology & Biotechnological Equipment, 2011.25(3): p. 2522-2524. Both authors attribute the reduction in patient pain due to the associated reduction in muscle contractions seen with bipolar pulses.

There is a balance between employing pulses that are delivered at a high enough frequency to reduce the conductivity mismatch between different tissues but have a duration of single polarity long enough to induce electroporation of cells comprising the tissues. As mentioned, electrical current associated with pulses longer than ~1 μs is confined to extracellular spaces prior to the onset of electroporation. Ivorra, A., ed. *Tissue Electroporation as a Bioelectric Phenomenon: Basic Concepts*. Irreversible Electroporation, ed. B. Rubinsky. 2010, Springer Berlin Heidelberg. 23-61; and Esser, A. T., K. C. Smith, T. R. Gowrishankar, and J. C. Weaver, *Towards solid tumor treatment by irreversible electroporation: intrinsic redistribution of fields and currents in tissue*. Technol Cancer Res Treat, 2007. 6(4): p. 261-74. This can be attributed to the migration of charges towards biological membranes following the application of an external electric field. The time required for a membrane to become charged to 63% of its steady state value is defined as the charging time constant of the membrane. Displacement currents across the plasma membrane allow organelles to be exposed to fields during the time that it takes the plasma membrane to reach steady state. Esser, A. T., K. C. Smith, T. R. Gowrishankar, and J. C. Weaver, *Towards Solid Tumor Treatment by Nanosecond Pulsed Electric Fields*. Technology in Cancer Research & Treatment, 2009. 8(4): p. 289-306. Once steady state is achieved, the counter-field developed along the plasma membrane due to the accumulation of charges is significant enough to shield the field from entering the cell, and current is directed through extracellular spaces. Only after permeabilization of the membrane does ionic conduction allow the field to re-enter the cell. Kolb, J. F., S. Kono, and K. H. Schoenbach, *Nanosecond pulsed electric field generators for the study of subcellular effects*. Bioelectromagnetics, 2006. 27(3): p. 172-187. If extracellular current pathways between cells are reduced, as in layers of epithelial cells connected by tight or gap junctions (see Jones, D. M., R. H. Smallwood, D. R. Hose, B. H. Brown, and D. C. Walker, Modelling of epithelial tissue impedance measured using three different designs of probe . Physiological Measurement, 2003. 24(2): p. 605-623), the field is highly concentrated across the layer, and the extent of electroporation in underlying cells is reduced. This problem is alleviated when the duration of single polarity approaches the membrane time constant.

By treating cells as a series of spherical, dielectric shells containing and surrounded by a conductive medium, the analytical solution for induced TMP across the plasma membrane ($\Delta\Phi$) can be obtained according to the law of total current (see Yao, C. G., D. B. Mo, C. X. Li, C. X. Sun, and Y. Mi, *Study of transmembrane potentials of inner and outer membranes induced by pulsed-electric-field model and simulation*. IEEE Trans Plasma Sci, 2007. 35(5): p. 1541-1549, "Yao 2007"):

$$\nabla\cdot\left(\varepsilon_0\varepsilon_r\frac{\partial E}{\partial t}+\sigma E\right)=\Lambda_k\nabla\cdot E=0 \tag{1}$$

$$\Lambda_k=\sigma+\varepsilon_0\varepsilon_r\frac{\partial}{\partial t} \tag{2}$$

where Λ is the admittivity operator and the subscript k denotes cellular regions including the nucleoplasm (n), nuclear envelop (ne), cytoplasm (c), plasma membrane (pm), and extracellular space (e). Transforming (2), (5), and (6) into the frequency domain (see Yao 2007):

$$E=-\nabla\Phi(s) \tag{3}$$

$$\Lambda_k\nabla\cdot E(s)=0 \tag{4}$$

$$\Lambda_k(S)=\sigma+\in_0\in_r s \tag{5}$$

where $s=j\omega=j2\pi f$, and applying the appropriate boundary conditions of potential continuity and normal vector continuity of current density at the interface between the different regions yields the solution for TMP (see Yao 2007):

$$\Delta\Phi(s)=F(\Lambda_n,\Lambda_{ne},\Lambda_c,\Lambda_{pm},\Lambda_e)E(s)\cos\theta \tag{6}$$

where θ represents the polar angle at the cell center between the electric field and the point of interest along the membrane. TMP is defined as the potential directly outside the membrane minus the inside. The natural, resting component of the plasma membrane TMP was ignored in all simulations, because it is typically an order of magnitude less than the induced TMP. See Gowrishankar 2006. Further, the TMP across the nuclear envelope never reached a permeabilizing threshold with the chosen pulsing protocols, and reference to TMP from this point forward refers only to the plasma membrane. As shown in Table 2, the term $F(\Lambda_k)$ represents a transfer function of the TMP that reflects the geometric and dielectric properties of the cellular regions as a function of the admittivity. See Hu, Q., S. Viswanadham, R. P. Joshi, K. H. Schoenbach, S. J. Beebe, and P. F. Blackmore, *Simulations of transient membrane behavior in cells subjected to a high-intensity ultrashort electric pulse*. Physical Review E, 2005. 71(3). Dielectric properties at the cellular level are assumed to be frequency independent, which is valid for predicting TMP up to around 100 MHz. Kotnik, T. and D. Miklavcic, *Theoretical evaluation of the distributed power dissipation in biological cells exposed to electric fields*. Bioelectromagnetics, 2000. 21(5): p. 385-394.

TABLE 2

Dielectric properties of various cellular regions.

Geometry	σ [S/m]	ε_r	Dimensions [m]
Extracellular Space	0.6	80.0	—
Plasma Membrane	5.3×10^{-6}	7.0	7.0×10^{-9} (thickness)
Cytoplasm	0.13	60.0	5.0×10^{-6} (radius)

TABLE 2-continued

Dielectric properties of various cellular regions.

Geometry	σ [S/m]	ε_r	Dimensions [m]
Nuclear Envelope	4.3×10^{-3}	22.8	40.0×10^{-9} (thickness)
Nucleoplasm	0.18	120.0	2.5×10^{-6} (radius)

The exact formulation for $F(\Lambda_k)$ is lengthy and can be found in (see Kotnik, T. and D. Miklavcic, *Theoretical evaluation of voltage inducement on internal membranes of biological cells exposed to electric fields*. Biophysical Journal, 2006. 90(2): p. 480-491), but is not included here for brevity. The term E(s) represents the Laplace transform of the pulsed electric field as a function of time.

Using the analytical model, the frequency dependence of the induced TMP can be investigated for both rectangular and sinusoidal electric fields with identical maximum amplitude. By substituting the transient electric fields into (6) the results of a parametric study on TMP for frequencies spanning from 62.5 kHz to 16 MHz are shown in FIG. **5**. The maximum amplitude of the sinusoidal and bipolar rectangular electric fields was 2000 V/cm (peak). For this applied field and the given geometric and dielectric properties of the modeled cell, the TMP never exceeds 1.46 V. Additionally, the time constant of the plasma membrane is 345 ns. All measurement were taken at the cell pole (θ=0) to depict the maximum achieved TMP after the system reached a steady oscillatory state. From the curve, as the frequency increases, the magnitude of the TMP is reduced. For the sinusoidal waveform, the reduction is evident at lower frequencies compared to the rectangular waveform. This has to do with the fact that the rectangular waveform maintains its maximum amplitude for a longer period of time than the sinusoidal waveform. It is not until the frequency of the rectangular waveform surpasses 250 kHz that a dramatic decrease in TMP occurs. For this reason, only rectangular pulses in a frequency window of 250 kHz to 2 MHz are best suited for electroporation with high-frequency, bipolar pulses.

Based on the analytical model for TMP presented above, the time constant of the plasma membrane for a constant field (2000 V/cm) is 345 ns. The time constant of 345 ns falls between the 2 MHz (250 ns pulse duration) and 1 MHz (500 ns pulse duration) bursts. Further, the 500 kHz burst (1 μs pulse duration) is close to the time it takes the TMP to reach steady state. As frequency is increased, the dielectric properties different tissues become more macroscopically homogeneous, but above 2 MHz, the pulse duration is not adequate for the cell to charge and induce electroporation. According to in vitro experiments that utilize bipolar rectangular pulses, the typical burst width required to induce either reversible electroporation or IRE increases with the frequency of the applied field. For EGT, a 60 kHz bipolar square with a burst width of 400 μs and an amplitude of 1600 V/cm has a six times greater transfection efficiency than a 1 MHz bipolar square wave with equal amplitude and width. Tekle, E., R. D. Astumian, and P. B. Chock, *Electroporation by Using Bipolar Oscillating Electric-Field—an Improved Method for DNA Transfection of Nih* 3t3 Cells. Proceedings of the National Academy of Sciences of the United States of America, 1991. 88(10): p. 4230-4234 (Telke 1991). In terms of IRE, a 60 kHz bipolar square with a burst width of 400 μs and an amplitude of 4000 V/cm results in 19% cell viability. Telke 1991. These results were obtained when a single burst was delivered to the sample. The inventors, however, appear to be the first in providing data on high-frequency electroporation with rectangular pulses that implemented multiple bursts. Similar to how multiple unipolar pulses are typically delivered in ECT, EGT, or IRE protocols to enhance the desired outcome (see Belehradek, J., S. Orlowski, L H Ramirez, G. Pron, B. Poddevin, and L. M. Mir, Electropermeabilization of Cells in Tissues Assessed by the Qualitative and Quantitative Electroloading of Bleomycin . Biochimica Et Biophysica Acta-Biomembranes, 1994. 1190(1): p. 155-163; and Garcia, P. A., J. H. Rossmeisl, R. E. Neal, T. L. Ellis, J. D. Olson, N. Henao-Guerrero, J. Robertson, and R. V. Davalos, *Intracranial Nonthermal Irreversible Electroporation: In Vivo Analysis*. Journal of Membrane Biology, 2010. 236(1): p. 127-136) multiple bipolar bursts would likely produce similar trends. Data is also available for burst sinusoidal waveforms in the frequency range of 2 kHz to 50 MHz (see Jordan, D. W., R. M. Gilgenbach, M. D. Uhler, L. H. Gates, and Y. Y. Lau, *Effect of pulsed, high-power radiofrequency radiation on electroporation of mammalian cells*. Ieee Transactions on Plasma Science, 2004. 32(4): p. 1573-1578; and Katsuki, S., N. Nomura, H. Koga, H. Akiyama, I. Uchida, and S. I. Abe, *Biological effects of narrow band pulsed electric fields*. Ieee Transactions on Dielectrics and Electrical Insulation, 2007. 14(3): p. 663-668), but the results are inconclusive, and sinusoidal waveforms are less efficient than rectangular bipolar pulses for inducing electroporation. Kotnik, T., G. Pucihar, M. Rebersek, D. Miklavcic, and L. M. Mir, *Role of pulse shape in cell membrane electropermeabilization*. Biochimica Et Biophysica Acta-Biomembranes, 2003. 1614(2): p. 193-200.

There is a narrow window of pulse parameters where ECT and EGT have proven to be effective without reducing cell viability by IRE. For ECT, the field for inducing optimal reversible electroporation conditions is between 300 and 500 V/cm in tumors, when eight square-wave pulses 100 μs in duration are delivered at a frequency of 1 Hz. Mir, L. M., *Therapeutic perspectives of in vivo cell elect ropermeabilization*. Bioelectrochemistry, 2001. 53: p. 1-10 (Mir 2001). For EGT, permeabilization conditions are optimal when eight square-wave pulses 20 ms in duration are delivered at a frequency of 1 Hz, which constitutes a field of around 90 V/cm. Mir 2001. To maintain its non-thermal benefits, the pulse parameters for IRE procedures are restricted to those that minimize any associated Joule heating. Davalos, R. V. and B. Rubinsky, *Temperature considerations during irreversible electroporation*. International Journal of Heat and Mass Transfer, 2008. 51(23-24): p. 5617-5622. However, a similar field strength and duration to those required for ECT can induce IRE when the number of pulses is raised above the traditional 8 pulses to 90 pulses, and the temperature of the tissue remains below 50° C. Rubinsky, J., G. Onik, P. Mikus, and B. Rubinsky, *Optimal Parameters for the Destruction of Prostate Cancer Using Irreversible Electroporation*. Journal of Urology, 2008. 180(6): p. 2668-2674.

In addition to being bipolar, the pulses used according to methods of the invention can have a duration of single polarity (~1 μs) that is two orders of magnitude less than the duration of a conventional electroporation pulse (~100 μs) and an amplitude that is one order of magnitude less than supraporation protocols with nanosecond pulsed electric field (nsPEF). Supraporation involves pulses with a duration ranging from 1-100 ns and an amplitude ranging from 10-100 kV/cm. These electric fields are capable of causing electroporation within the membranes of intracellular organelles. Vernier, P. T., Y. H. Sun, and M. A. Gundersen, Nanoelectropulse-driven membrane perturbation and small

molecule permeabilization. Bmc Cell Biology, 2006. 7. When the pulse length is shorter than the charging time of the plasma membrane, the field can penetrate the plasma membrane to reach the cell interior. Beebe, S. J., P. M. Fox, L. J. Rec, L. K. Willis, and K. H. Schoenbach, *Nanosecond, high-intensity pulsed electric fields induce apoptosis in human cells*. FASEB J, 2003. 17(9): p. 1493-5. Because organelles are smaller in diameter than cells, the amplitude required to raise the TMP on organelles up to ~1 V is greater than that in ECT and IRE procedures. However, due to the ultra-short nature of the pulses, the accompanying Joule heating is still negligible. Schoenbach, K. H., S. J. Beebe, and E. S. Buescher, *Intracellular effect of ultrashort electrical pulses*. Bioelectromagnetics, 2001. 22(6): p. 440-8. While immediate necrosis is suspected as the primary mechanism of cell death following IRE, apoptosis triggered by DNA fragmentation and the release of calcium from intracellular stores occurs in cells exposed to sufficiently high nsPEFs. Beebe, S. J., J. White, P. F. Blackmore, Y. P. Deng, K. Somers, and K. H. Schoenbach, *Diverse effects of nanosecond pulsed electric fields on cells and tissues*. DNA and Cell Biology, 2003. 22(12): p. 785-796.

In vivo experiments on supraporation have shown that the ultra-short, unipolar pulses do not cause stimulation of excitable tissue, such as muscle and nerves. Long, G., P. K. Shires, D. Plescia, S. J. Beebe, J. F. Kolb, and K. H. Schoenbach, *Targeted Tissue Ablation With Nanosecond Pulses*. Ieee Transactions on Biomedical Engineering, 2011. 58(8). This is a consequence of the pulses being below the strength-duration threshold determined by Rogers et al. Rogers, W. R., J. H. Merritt, J. A. Comeaux, C. T. Kuhnel, D. F. Moreland, D. G. Teltschik, J. H. Lucas, and M. R. Murphy, *Strength-duration curve for an electrically excitable tissue extended down to near 1nanosecond*. Ieee Transactions on Plasma Science, 2004. 32(4): p. 1587-1599. As seen in FIG. **6**, for IRE pulses, the electric field threshold for stimulation is between 1-10 V/cm. The present invention describes pulses where the duration of single polarity is as low as 100 ns. At this duration, the electric field threshold for stimulation increases to 1000 V/cm, which is above the amplitude required for reversible electroporation and on the order of the amplitude for IRE.

In addition to the duration of single polarity being reduced, the fact that the inventive waveforms are inherently bipolar offers an additional benefit in terms of the stimulation of excitable tissue. As shown in FIG. **7**, biphasic waveforms have a higher threshold current for inducing nerve stimulation. Reilly, J. P., V. T. Freeman, and W. D. Larkin, *Sensory Effects of Transient Electrical-Stimulation—Evaluation with a Neuroelectric Model*. IEEE Trans Biomed Eng, 1985. 32(12): p. 1001-1011. Further, the threshold increases exponentially as the duration of single polarity is decreased. While the mechanism of this phenomenon is unknown, it is thought that the reversal in polarity prevents an action potential from being generated by limiting the flow of sodium ions down their concentration gradient. This has been shown to translate to a reduced muscle twitch force during bipolar functional electrical stimulation as opposed to monopolar. Vandenhonert, C. and J. T. Mortimer, *Response of the Myelinated Nerve-Fiber to Short Duration Biphasic Stimulating Currents*. Annals of Biomedical Engineering, 1979.7(2): p. 117-125.

The inventors have shown that bipolar waveforms can induce IRE at center frequencies high enough to eliminate muscle contraction completely. This procedure is termed high-frequency IRE (H-FIRE). Overall, the results indicate that H-FIRE can produce more predictable treatment outcomes, reduce the potential for thermal damage, and obviate the need for (or reduce the necessity of) neuroparalytic agents delivered prior to or during treatment.

The following examples show that bursts of bipolar, nanosecond pulses can maintain a critical TMP beneath epithelial layers, while minimizing Joule heating. This has to do with the ability of high-frequency waveforms to achieve a macroscopically homogeneous field distribution in a heterogeneous system. At high-frequencies, tissues with a low passive DC conductivity become more conductive. Additionally, it is proven that high-frequency IRE (H-FIRE) can be applied to non-thermally ablate tissue while eliminating muscle contractions seen in conventional IRE protocols with longer duration unipolar pulses. These results have implications not only for skin, brain, and liver as presented here, but for other tissues, such as bone, breast, pancreas, kidney, and lung. These examples should not be considered as limiting the invention in any way.

As a general background to the examples, it is noted that the inventors and their colleagues have successfully demonstrated that finite element models (FEMs) can accurately predict treatment outcomes of pulsed electric field therapies for cancer treatment. See Edd, J. F. and R. V. Davalos, Mathematical modeling of irreversible electroporation for treatment planning. Technol Cancer Res Treat, 2007. 6: p. 275-286; and Edd, J. F., L. Horowitz, R. V. Davalos, L. M. Mir, and B. Rubinsky, In vivo results of a new focal tissue ablation technique: irreversible electroporation . IEEE Trans Biomed Eng, 2006. 53(7): p. 1409-15.

EXAMPLE 1

High-Frequency Electroporation Results in More Uniform and Predictable Treatment Outcomes in Heterogeneous Tissues

A 2D axisymmetric FEM representative of a cylindrical section of non-infiltrated fat encapsulated by dry skin was simulated using COMSOL 3.5a (Burlington, Mass.). The electric potential distribution within the tissue was obtained by transiently solving:

$$-\nabla\cdot(\sigma\nabla\Phi)-\varepsilon_0\varepsilon_r\nabla\cdot\left(\frac{\partial\nabla\Phi}{\partial t}\right)=0 \tag{7}$$

where Φ is the electric potential and σ and $\in_r$ are the conductivity and relative permittivity of each tissue layer, respectively, which depends on frequency (Table 1). Equation (7) is obtained from Maxwell's equations assuming no external current density ($J=\sigma E$), no remnant displacement ($D=\in_0\in_r E$), and the quasi-static approximation. This approximation implies a negligible coupling between the electric and magnetic fields ($\nabla\times E=0$), which allows for the expression of electric field only in terms of electric potential:

$$E=-\nabla\Phi \tag{8}$$

Dielectric properties of the bulk tissue were chosen from data generated by Gabriel et al. (see Gabriel, S., R. W. Lau, and C. Gabriel, *The dielectric properties of biological tissues* 0.2. Measurements in the frequency range 10 Hz to 20 GHz. Physics in Medicine and Biology, 1996. 41(11): p. 2251-2269) available at (http://niremf.ifac.cnr.it/docs/dielectric/home.html). The data was interpolated in Mathematica 7 (Wolfram Research, Inc.) in order to estimate the dielectric properties at the desired frequencies. Dielectric properties of the electrode were chosen to be stainless steel,

as incorporated in the Comsol material library. All electrical boundary conditions are shown in FIGS. **8**A-B, which provides in FIG. **8**A, a meshed geometry of the FEM with boundary settings. The mesh consists of 3028 elements and was refined until there was <0.1% change in the magnitude of the electric field at the center of the tissue. FIG. **8**B provides a schematic diagram of the geometry with dimensions. The box represents an expanded view of the tissue that describes the link between the macroscopic electric field (E) and the microscopic analysis of TMP. Adjacent cells are drawn with dashed lines, indicating their role was ignored in calculating TMP.

Because rectangular waveforms are comprised of components with various frequencies and amplitudes, tissue properties at frequencies associated with the center frequency, defined as the inverse of twice the duration of single polarity, are chosen. Intuitively, the duration of single polarity defines the frequency at which the current changes direction in the tissue. The pulses were constructed by multiplying the applied voltage by a function consisting of two smoothed Heaviside functions with a continuous second derivative and a tolerance of 5 ns (rise and fall times). The quasi-static assumption is confirmed based on the fact that the primary frequency of the pulses is lower than 200 MHz (rise and fall times), which corresponds to a wavelength that is greater than the longest dimension in the geometry. Chen, M. T., C. Jiang, P. T. Vernier, Y. H. Wu, and M. A. Gundersen, *Two-dimensional nanosecond electric field mapping based on cell electropermeabilization*. PMC Biophys, 2009. 2(1): p. 9. The inclusion of a permittivity term in (1) differs from previous, simplified models (see Edd, J. F. and R. V. Davalos, *Mathematical Modeling of irreversible Electroporation for treatment planning*. Technology in Cancer Research & Treatment, 2007. 6(4): p. 275-286; and Neal, R. E. and R. V. Davalos, The *Feasibility of Irreversible Electroporation for the Treatment of Breast Cancer and Other Heterogeneous Systems*. Annals of Biomedical Engineering, 2009. 37(12): p. 2615-2625), and accounts for reactive component of tissue to time dependent pulsing, which is required for obtaining accurate potential distributions in heterogeneous models. Yousif, N., R. Bayford, and X. Liu, *The Influence of Reactivity of the Electrode-Brain Interface on the Crossing Electric Current in Therapeutic Deep Brain Stimulation*. Neuroscience, 2008. 156(3): p. 597-606.

FIGS. **9**A-D show the electric field distribution at the end of a 2 μs burst with various frequencies given in Table 1. In each case, the maximum applied voltage was set to 2600 V (peak) in order to set up a voltage to distance ratio of 2000 V/cm between the electrodes (1.3 cm spacing). From the surface contour map, as frequency is increased, the electric field in the fat rises while the field in the skin drops. This trend extends to the point that at 2 MHz the field in the skin is lower than the fat, which is a direct result of the tissue dielectric properties at that frequency (greater conductivity and permittivity of skin as compared to fat). Therefore, high-frequency fields, or pulses with shorter duration, are better suited to penetrate epithelial layers, such as the skin, and reach underlying tissue.

EXAMPLE 2

High-Frequency Electroporation Results in Homogeneous Energy Deposition and Reduces the Potential for Thermal Damage in Low Passive Conductivity Tissue

The temperature distribution in the model described in EXAMPLE 1 was obtained by transiently solving a modified version of the Pennes bioheat equation (see Pennes, H. H., *Analysis of tissue and arterial blood temperatures in the resting human forearm*. J Appl Physiol, 1948. 1(2): p. 93-122) with the inclusion of a Joule heating term:

$$\rho C\frac{\partial T}{\partial t} = \nabla\cdot(k\nabla T) + \rho_b\omega_b C_b(T_b - T) + Q_m + |J\cdot E| \tag{9}$$

where T is the tissue temperature, T_b is the blood temperature, k is the thermal conductivity of the tissue, C and C_b are the tissue and blood specific heat, respectively, ρ and ρ_b are the tissue and blood density, respectively, Q_m is the metabolic heat source term, ω_b is the blood perfusion coefficient, and |J·E| is the Joule heating term. All thermal tissue properties are given in Table 3. Fiala, D., K. J. Lomas, and M. Stohrer, *A computer model of human thermoregulation for a wide range of environmental conditions: the passive system*. Journal of Applied Physiology, 1999. 87(5): p. 1957-1972.

TABLE 3

Thermal tissue properties of various tissues.

	Tissue		
Property	Blood	Skin	Fat
ρ [kg/m^3]	1069	1085	850
C [J/(Kg□K)]	3650	3680	2300
k [W/(m□K)]	—	0.47	0.16
ω [1/s]	—	1.1	0.0036
Q_m [kg/m^3]	—	368	58

Due to the presence of different tissue layers and the high frequencies under consideration (250 kHz-2 MHz), displacement currents are considered along with conduction currents in the formulation of Joule heating:

$$J = J_D + J_C = \varepsilon_0\varepsilon_r\frac{\partial E}{\partial t} + \sigma E \tag{10}$$

where J is the total current density, J_D is the displacement current density, and J_C is the conduction current density. In order to ensure that negative current components due to polarity changes add to the total current in the tissue, the absolute value of the resistive heating term was taken prior to temperature calculations. It was assumed that all subdomains were initially at physiologic temperature (T_0=310.15 K). The boundaries between the electrode-skin interface and the skin-fat interface were treated as continuous ($n\cdot(k_1\nabla T_1 - k_2\nabla T_2)=0$), the centerline was defined as axial symmetry (r=0), and the remaining boundaries were thermally insulated ($n\cdot(k\nabla T)=0$) for conservative temperature estimates. Temperature profiles were investigated along the centerline (r=0 mm) in the middle of the fat (z=0 mm) and skin (z=5.75 mm) layers. Data was imported into Mathematica, and a moving average with a period of 100 ns was taken to smooth the plots. Additionally, the data was fit with a linear trendline in order to extrapolate to longer burst widths and predict the onset of thermal damage.

Temperature changes predicted by the FEM at the center of the skin and fat are shown in FIGS. **10**A-B, which provides temperature changes predicted by the FEM at the center of the skin (FIG. **10**A) and fat (FIG. **10**B) for

frequencies of 250 kHz (- -), 500 kHz (- - -), 1 MHz (. . .), and 2 MHz (-). Equations represent a linear fit to the data. In this case, a burst width of 4 μs was simulated in order to capture the trends in temperature development. Polarity of the 2 μs pulse (250 kHz) was switched between pulses to maintain consistency with the other waveforms that are inherently bipolar. With respect to the skin, as the frequency of the applied field increases, the temperature rises at a slower rate. This is a consequence of the fact that the electric field within the skin also decreases with increasing frequency. In the case of the fat, the temperature rises at a faster rate when the frequency of the applied field is increased. At first glance, this seems to be detrimental, however, it is merely an indication that energy is preferentially being deposited more uniformly into the fat at higher frequencies. Again, this can be correlated to the electric field profile. In both tissues, the sharp rises in temperature are due to the spikes in displacement current that occur at the onset and offset of each pulse (data not shown). The total temperature increase in all cases is less than 0.003 K for a burst width of 4 μs. As explained in the discussion, even for bursts of longer widths, the temperature increase is not enough to promote thermal damage.

The onset of protein denaturation and loss of cell structure occurs above 318.15 K (see Bilchik, A. J., T. F. Wood, and D. P. Allegra, *Radiofrequency ablation of unresectable hepatic malignancies: Lessons learned*. Oncologist, 2001. 6(1): p. 24-33), which correlates to an increase in temperature of 8 K above physiological temperature. Using this information, the maximum energy delivery period (number of pulses multiplied by pulse duration) can be calculated for an amplitude of 2000 V/cm at each of the frequencies investigated using the trendlines generated by the FEM data (FIGS. **9**A-D). In the skin layer, heating is reduced by increasing the frequency of the applied field. This shows that the potential for thermal damage in the skin is reduced when the frequency of the applied field is increased. At higher frequencies, the energy is preferentially deposited in the fat layer. For 2 MHz, the total energy delivery period required to cause an 8 K increase in temperature is 12 ms. An example treatment plan can include 12, 1 ms pulses separated by a delay of 1 s. If the frequency is reduced to 500 kHz, which shows the greatest electroporation efficiency (Table 4, see EXAMPLE 3), the allowable energy delivery period increases to 16 ms, which would permit the delivery of an additional 4, 1 ms pulses before the onset of thermal damage.

TABLE 4

Various exemplary treatment protocols.

Frequency (pulse duration)	Time (μs), |TMP| > 0.5 V	% of Pulse, |TMP| > 0.5 V
250 kHz (2 μs)	1.2	60
500 kHz (1 μs (×2))	1.9	95
1 MHz (500 ns (×4))	1.3	65
2 MHz (250 ns (×8))	0.1	5

The restrictions could be increased if less conservative estimates are obtained that account for heat dissipation between pulses and heat convection at the tissue surface. Lackovic, I., R. Magjarevic, and D. Miklavcic, *Three-dimensional Finite-element Analysis of Joule Heating in Electrochemotherapy and in vivo Gene Electrotransfer*. Ieee Transactions on Dielectrics and Electrical Insulation, 2009. 16(5): p. 1338-1347. These projected protocols represent a maximum, and it is likely that the desired effects will be induced at a significantly lower energy. See Belehradek, J., S. Orlowski, L. H. Ramirez, G. Pron, B. Poddevin, and L. M. Mir, *Electropermeabilization of Cells in Tissues Assessed by the Qualitative and Quantitative Electroloading of Bleomycin*. Biochimica Et Biophysica Acta-Biomembranes, 1994. 1190(1): p. 155-163; and Garcia, P. A., J. H. Rossmeisl, R. E. Neal, T. L. Ellis, J. D. Olson, N. Henao-Guerrero, J. Robertson, and R. V. Davalos, *Intracranial Nonthermal Irreversible Electroporation: In Vivo Analysis*. Journal of Membrane Biology, 2010. 236(1): p. 127-136.

EXAMPLE 3

High-Frequency Electroporation can Overcome Shielding Effects of Low Passive Conductivity Tissues and Induce Electroporation in Underlying Layers

The analytical model for TMP described in this specification was utilized to investigate electroporation in a hypothetical cell located along the centerline (r=0 mm) in the middle of the fat (z=0) and skin (z=5.75 mm) layers of the FEM described in EXAMPLE 1. The equations for TMP are derived under the assumption that there is no influence on the microscopic electric field from neighboring cells. Therefore, the macroscopic electric field in the bulk tissue predicted by the FEM dictates the microscopic electric field experienced by the cell. The vertical z-component of the electric field was imported from the specific locations within FEM into Mathematica to account for polarity changes. The radial r-component was neglected due to the fact that it never surpassed 3 V/cm as current traveled primarily in the z-direction. Non-uniform electric field data was fit with a series of step functions (50 ns duration), such that the Laplace transform of the field could be performed and the solution for TMP could be obtained in the frequency domain as the summation of individual steps. The inverse Laplace transform of the data was taken to obtain the complete time courses. Measurements were taken at the pole (θ=0) to depict the maximum induced TMP around the cell.

With respect to the skin, as the frequency of the applied field increases, the maximum oscillation amplitude of the TMP decreases, as shown in FIGS. **11**A-B. This occurs for two reasons. First, as seen in FIGS. **8**A-B, the electric field in the skin decreases with increasing frequency. Second, as seen in FIG. **5**, even with constant field amplitude, the TMP decreases with increasing frequency, because the time during which the membrane has to charge before the polarity switches is less at higher frequencies. In the case of the fat, the behavior is slightly more complex. At lower frequencies, a majority of the voltage drop occurs across the skin as demonstrated in FIGS. **9**A-D, resulting in a reduced electric field in the fat. This shielding effect is best shown in FIGS. **10**A-B along the 250 kHz trace. According to FIG. **5**, at 250 kHz, the maximum TMP should be reached. However, due to the shielding effect from the skin, a reduction in the TMP prior to the polarity change is seen. This reduction in TMP can be alleviated by increasing the frequency of the applied field. However, the tradeoff between increased frequency and reduced TMP is still evident at a frequency of 2 MHz (FIGS. **11**A-B).

As mentioned, there is a balance between employing pulses that are delivered on a short enough timescale to flow through epithelial cells but are long enough to induce electroporation in underlying cells. The time constant of 345 ns, predicted by the analytical model for TMP, falls between

the 2 MHz (250 ns pulse duration) and 1 MHz (500 ns pulse duration) bursts. Further, the 500 kHz burst (1 μs pulse duration) is close to the time it takes the TMP to reach steady state. Table 4 summarizes the results based on the time that the TMP on a hypothetical cell at the center of the fat layer is above 0.5 V. This amplitude was chosen such that even the highest frequency burst was above the set voltage level for a certain amount of time. The results would hold if the applied field was doubled and the voltage level was set to the 1 V threshold for pore formation, due to the linear dependence of TMP on the electric field. Based on this criterion, a frequency of 500 kHz is best suited to treat cells in the fat layer, followed by 1 MHz and 250 kHz. As frequency is increased, the dielectric properties and electric field distribution in the skin and fat become more macroscopically homogeneous, but above 1 MHz, the pulse duration is not adequate for the cell to charge.

EXAMPLE 4

System for Implementing High-Frequency, Bipolar Pulses for Tissue Electroporation

The electronic drive system for delivering bipolar electroporation signals is schematically depicted in FIG. **12**. The system relies upon both commercially available components and circuits built by the inventors. An arbitrary function generator (Tektronix AFG 3011) is programmed to output a tri-state square waveform. The AFG 3011 is capable of generating 20 V peak-to-peak into a 50 ohm load and has an effective analog bandwidth of 8 MHz. The burst width, interval between bursts, and total number of bursts is externally controlled by a microcontroller (Arduino Duemilanove) through the general purpose input/output (GPIO) pins. The output signal for a 1 MHz waveform with a burst width of 10 μs and amplitude of 6 V peak is given in FIG. **12**. This signal is simultaneously fed through both positive polarity and negative polarity high voltage MOSFET switches (IXYS Colorado HV 1000). The signal into the negative polarity HV 1000 is inverted using an LM 7171 op amp with a slew rate of 4100 V/ρs in order to properly sequence the amplification of the positive and negative polarity pulses without delay. The maximum output of each HV 1000 is 17 A and +/−850 V into a 50 ohm load. Additionally the pulse rise time is 10 ns or less. This results in an amplification of the AFG 3011 trigger signal up to 1700 V peak-to-peak, which is capable of inducing electroporation when the electrodes are spaced approximately 3 cm apart or less. The input power to each HV 1000 is maintained by a high voltage sequencer (LabSmith HVS 448), which can regulate voltage up to +/−3000 V and current up to 100 mA. In order to increase current storage up to 17 A, an external capacitor bank was included between the HVS 448 and HV 1000. The total capacitance of the bank can be adjusted depending on the desired voltage and current output or electrode spacing. This system allows for a flexible treatment program that may be tailored to meet a patient's individual needs.

Other systems are available in the literature for generating bipolar pulses, and the invention should not be limited to the system described above. For example, De Vuyst et al. built a generator around an NE555 timer configured as an astable multivibrator capable of producing up to 50 kHz bipolar pulses. De Vuyst, E., M. De Bock, E. Decrock, M. Van Moorhem, C. Naus, C. Mabilde, and L. Leybaert, In situ bipolar *Electroporation for localized cell loading with reporter dyes and investigating gap junctional coupling*. Biophysical Journal, 2008. 94(2): p. 469-479. However, the frequency of the pulses administered according to embodiments of the invention are an order of magnitude greater, which is easily met by the bandwidth of the AFG 3011. Additionally, the MOSFET switches provide an excellent means to produce high-frequency pulses for high voltage switching. However, MOSFETs are not the only semiconductor devices that can be utilized to produce a pulse. Bipolar Junction Transistors (BJTs), Insulated Gate Bipolar Transistors (IGBTs), and Junction Field Effect Transistors (JFETs) are examples of some of the semiconductor devices that may be used to produce an output pulse.

EXAMPLE 5

Experimental Results of High-Frequency, Bipolar Pulses for Electroporation of Cells

A chemical reaction technique was performed to fabricate parallel silver electrodes on glass microscope slides with 100 μM spacing. Briefly, a commercially available mirroring kit was used to deposit pure silver onto the microscope slides (Angel Gilding Stained Glass Ltd, Oak Park, Ill.). A negative thin film photoresist (#146DFR-4, MG Chemicals, Surrey, British Colombia, Canada) was laid on top of the slide and passed through an office laminator (#4, HeatSeal H212, General Binding Corporation, Lincolnshire, Ill.). A photomask printed at 20 k DPI on a transparent film (Output City, Cad/Art Services Inc, Bandon, Oreg.) was placed ink side down onto the photoresist, and slides were exposed to UV light for 45 seconds. After exposure, the slides were placed in a 200 mL bath containing a 10:1 DI water to negative photo developer (#4170-500ML, MG Chemicals, Surrey, British Colombia, Canada). The slides were placed in a beaker containing DI water to stop the development process and gently dried using pressurized air. Electrode structures on the microscope slides were fabricated by removing all silver not covered by the patterned photoresist. A two part silver remover was included in the mirroring kit used to deposit the silver. The photoresist was then removed by placing the slide in a bath of acetone.

Microfluidic channels were fabricated using the patterned photoresist on a microscope slide that had not undergone the silvering process. Liquid phase polydimethylsiloxane (PDMS) in a 10:1 ratio of monomers to curing agent (Sylgrad 184, Dow Corning, USA) was degassed under vacuum prior to being poured onto the photoresist master and cured for 1 hour at 100° C. After removing the cured PDMS from the mold, fluidic connections to the channels were punched in the devices using 1.5 mm core borers (Harris Uni-Core, Ted Pella Inc., Redding, Calif.). The PDMS mold was then bonded over the glass slides containing the patterned electrodes by treating with air plasma for 2 minutes in a PDC-001 plasma cleaner (Harrick Plasma, Ithaca, N.Y.).

High voltage electrical wires were taped to the glass slide with exposed wire placed in direct contact with the electrical pads. A drop of high purity silver paint (Structure Probe Inc., West Chester, Pa.) was placed on the pad and allowed to dry for one hour creating a solid electrical connection. A drop of 5 minute epoxy (Devcon, Danvers, Mass.), used to secure the electrical connections, was placed on top of each electrode pad and allowed to cure for 24 hours. Pulses were delivered across the electrodes as described in EXAMPLE 4 prior to the amplification stage. No amplification was needed as the gap between the electrodes was only 100 μm. Therefore, the output signal of a function generator (GFG-

3015, GW Instek, Taipei, Taiwan) +/−10 V can be used to generate an electric field capable of inducing electroporation, as shown in FIGS. **13**A-B.

Following culture in DMEM-F12 (supplemented with 10% FBS and 1% penicillin streptomycin) MDA-MB-231 cells were resuspended in a PBS solution 1:1 with Trypan Blue (0.4%). Trypan Blue is a determinant of cell membrane integrity, and stains electroporated cells blue, whereas non-electroporated cells remain transparent. Cells at a concentration of 10^6/ml were injected into the microfluidic channel using a syringe. The function generator was triggered by the microcontroller to deliver 80, 50 kHz bursts with a width of 1 ms and an amplitude of 500 V/cm. Results shown in FIGS. **14**A-B, which shows that 60% transfection efficiency was obtained when starting with cells that are 92% viable. This efficiency of reversible electroporation could be improved by either increasing the number of pulses or the burst width. Additionally, IRE could be performed by increasing the applied voltage.

EXAMPLE 6

Alternate Waveforms for Performing High-Frequency Electroporation

The analytical model for TMP described in the detailed description of the invention was utilized to investigate electroporation of a spherical cell subject to alternative waveforms. As mentioned, the critical TMP (Φ_{cr}) across the plasma membrane required to induce IRE is approximately 1 V. Belehradek, J., S. Orlowski, L. H. Ramirez, G. Pron, B. Poddevin, and L. M. Mir, *Electropermeabilization of Cells in Tissues Assessed by the Qualitative and Quantitative Electroloading of Bleomycin*. Biochimica Et Biophysica Acta-Biomembranes, 1994. 1190(1): p. 155-163. This threshold is illustrated in FIGS. **15**A-C by the dashed, horizontal line on the TMP profiles. Characteristic waveforms of IRE with unipolar pulses and high-frequency IRE with the corresponding TMP development across the plasma membrane (Φ_{pm}). All results are presented at the cell pole (θ=0) to show the maximum TMP around the cell. Further, results are only shown for TMP across the plasma membrane, as the TMP across the nuclear envelope never approached the permeabilizing threshold. For an electric field of 1500 V/cm, results indicate that a unipolar pulse (FIG. **15**A), a 250 kHz bipolar burst (FIG. **15**B), and 250 kHz bipolar burst that includes delays between the pulses (FIG. **15**C) are all capable of inducing IRE. However, the time above the threshold TMP varies between the different cases. The 1500 V/cm unipolar pulse causes the TMP to rise above the critical threshold for IRE (1 V, dashed line). The 1500 V/cm bipolar burst without a delay and with a delay causes the TMP to oscillate around the same critical threshold. This is investigated further in FIG. **16** for center frequencies of 0, 100, 250, 500, and 1000 kHz, with the 0 kHz case representing the unipolar pulse, and electric fields of 1000 V/cm and 1500 V/cm. FIG. **16** provides a comparison of time above the critical threshold (Φ_{cr}) for IRE at various center frequencies. The burst width of the bipolar waveform that included delays was twice as long (40 μs) as the corresponding burst with no delays in order to generate an equivalent pulse on-time (20 μs). The amount of time that the TMP was above the critical value was normalized by the on-time and converted to a percentage. FIG. **16** illustrates that, for a given frequency, as the electric field is increased from 1000 V/cm to 1500 V/cm, the percentage of the burst above the critical TMP also increases. At 250 kHz, IRE is possible during all waveforms, but at 500 kHz, only the waveforms with amplitudes of 1500 V/cm are capable of inducing IRE. As the center frequency of the burst increases, the percentage of the burst above the critical TMP decreases. However, with the inclusion of delays between the pulses, this characteristic dispersion is shifted towards higher frequencies. At 1 MHz, only the 1500 V/cm waveform with delays can theoretically cause IRE.

The theoretical model of TMP suggests that IRE should be possible up to 1 MHz for an electric field of 1500 V/cm. Including a delay between the positive and negative pulses comprising the bipolar burst offers a therapeutic advantage in addition to protecting the MOSFETs in the pulse generation system (see EXAMPLE 4) from ringing. By not forcing a discharge of the TMP with an immediate reversal of polarity, the cell is allowed to return to the resting TMP according to its characteristic time constant. As a result, the TMP is maintained above the critical voltage required for IRE for a longer amount of time. This metric has been recognized as a potential indicator of treatment outcomes in electroporation based therapies with bipolar waveforms. Garcia, P. A., J. H. Rossmeisl, R. E. Neal, T. L. Ellis, J. D. Olson, N. Henao-Guerrero, J. Robertson, and R. V. Davalos, *Intracranial Nonthermal Irreversible Electroporation: In Vivo Analysis*. Journal of Membrane Biology, 2010. 236(1): p. 127-136.

Other potential waveforms for performing high-frequency electroporation are shown in FIGS. **17**A-C, which provide characteristic waveforms of IRE with unipolar pulses and high-frequency IRE with the corresponding TMP development across the plasma membrane (Φ_{pm}). A unipolar pulse with an amplitude of 1500 V/cm is shown for comparison (FIG. **17**A). A waveform without delays between polarity reversals (FIG. **17**B) can maintain a positive TMP throughout the entire treatment if the duration of positive polarity is tuned to be slightly longer than the duration of negative polarity. Similarly, for a waveform that includes delays (FIG. **17**C), a train of positive ultra-short pulses could be used to gradually increase the TMP up to the critical permeabilizing threshold, and a single ultra-short pulse of negative polarity could follow the train without causing the TMP to go negative. In both examples, the ultra-short negative going pulse is designed to maintain the predicted benefits of high-frequency electroporation. Namely, it is predicted that the negative going pulse will prevent action potential generation and still permit a degree of capacitive coupling across epithelial layers. FIG. **18** is a chart showing an exemplary output from an in vivo treatment of the brain with high-frequency, bipolar pulses, where the snapshot is taken within a single burst.

EXAMPLE 7

Experimental Results of High-Frequency IRE (H-FIRE) of Brain Tissue

H-FIRE was performed using a custom pulse generator as described in EXAMPLE 4 with minor modifications. An unregulated DC power supply was constructed to replace the both the high voltage sequencer and external capacitor in order to maintain a sufficient level of charge to deliver 20 A over a 100 μs burst. A center tapped 400 VA transformer (AS-4T320, Antek, Inc., North Arlington, N.J., USA) was rectified and smoothed by a capacitor bank to provide positive and negative power rails to the HV1000P and HV1000N, respectively. The voltage rails were controlled by adjusting the input voltage using a variable transformer,

and the maximum output rating of the system was +/−450 V. A delay equal to the duration of single polarity was included between the pulses in order to protect the MOSFETs from ringing. A unity gain inverting amplifier (AD844, Analog Devices, Norwood, Mass., USA) was used to invert this signal and appropriately trigger the negative pulse generator. The outputs of the two monopolar pulse generators were terminated into a 50Ω load in parallel with the electrodes. This load was used to maintain appropriate pulse characteristics and as a safety to ensure the system was never triggered without an attached load. For comparison, the IRE treatments were performed using the BTX ECM 830 electroporation system (Harvard Apparatus, Holliston, Mass., USA).

All study procedures were conducted following Institutional Animal Care and Use Committee approval and performed in a GLP compliant facility. Four, Fischer 344 male rats weighing 200-240 g were anesthetized by intraperitoneal injection of 10 mg/kg xylazine and 60 mg/kg ketamine hydrochloride, and a surgical plane of anesthesia was assessed by loss of the tail pinch reflex. To monitor muscle contractions, a 3-axis accelerometer breakout board (ADXL335, Adafruit Industries, New York, N.Y., USA) with a sensing range of ±3 g's was sutured to the dorsum of each rat in the interscapular region at the cervicothoracic junction using 5-0 monocryl suture. Low-pass filter capacitors (0.1 μF) were included at the x, y, and z outputs of the accelerometer for noise reduction. The hair of the skull was clipped and aseptically prepared using povidone-iodine and alcohol solutions. Anesthetized rats were placed in a small animal stereotactic head frame (Model 1350M, David Kopf Instruments, Tungisten, Calif., USA). A routine lateral rostrotentorial surgical approach to the skull was made, and 6 mm by 3 mm rectangular parieto-occipital craniectomy defects were created in the right and left aspects of the skull of each rat using a high-speed electric drill. Custom electrodes were inserted into the center of the forelimb area of the sensorimotor cortex of each rat (coordinates relative to Bregma: 1 mm anterior, 2.5 mm lateral, 2 mm dorsoventral) and advanced to a depth of 2 mm beneath the surface of the exposed dura. The electrodes were fashioned by blunting stainless steel acupuncture needles (0.45 mm diameter, Kingli Medical Appliance Co., Wuxi, China) with high grade sandpaper. Exposure length was set to 1 mm by insulating the electrodes with miniature polyimide tubing (25 AWG, Small Parts, Seattle, Wash., USA), and the edge-to-edge electrode spacing was set to 1 mm by molding the electrodes in liquid phase polydimethylsiloxane (PDMS) cured in a 10:1 ratio with Sylgard 184 (Dow Corning Corp., Midland, Mich., USA) at 150° C. for 30 min.

Pulse parameters were chosen based on the results from the analytical and numerical models to ensure the greatest potential for non-thermal tissue ablation. Following electrode insertion, pulses were applied to the right and left cerebral hemispheres, resulting in two treatments per rat (Table 5).

TABLE 5

Pulse parameters of various treatment protocols.

Rat Number	Treatment	Hemisphere	Frequency (kHz)	Voltage (V)
1	IRE	Left	—	100
	H-FIRE	Right	250	100
2	IRE	Left	—	200
	H-FIRE	Right	250	200
3	H-FIRE	Left	250	300
	H-FIRE	Right	250	400
4	IRE	Left	—	50
	H-FIRE	Right	500	400

H-FIRE experiments were performed using 180 bursts with a pulse on-time of 200 μs within each burst, and bursts were delivered at a rate of one per second. In Rat #1 and Rat #2, H-FIRE was applied at voltages of 100 V and 200 V, respectively, to the right hemisphere with a center frequency of 250 kHz (duration of single polarity equal to two microseconds). The left hemisphere of Rat #1 and Rat #2 were treated with 180 IRE pulses (200 μs duration) of equivalent energy. In Rat #3, H-FIRE was applied to the left and right hemispheres at voltages of 300 V and 400 V, respectively, with a frequency of 250 kHz. In Rat #4, H-FIRE was applied at a voltage of 400 V to the right hemisphere with a frequency of 500 kHz (duration of single polarity equal to one microsecond). The left hemisphere of Rat #4 was treated with 90 IRE pulses (200 μs) and an applied voltage of 50V. This lower energy scenario was designed to compare H-FIRE treatment outcomes to traditional IRE protocols in the brain. Kotnik, T. and D. Miklavcic, *Theoretical evaluation of voltage inducement on internal membranes of biological cells exposed to electric fields*. Biophysical Journal, 2006. 90(2): p. 480-491.

Immediately following treatment, Rats #3 and #4 were subjected to MRI examinations of the brain while under general anesthesia. The MRI was performed with a 0.2 T MRI scanner using a dual phased array hand/wrist coil for RF signal transmission and reception. Sequence acquisition parameters were as follows: T1-weighted images were acquired using spin echo pulse sequence (TR=200 ms, TE=16 ms, FOV=6 cm, matrix=256×196, slice thickness=2 mm), and T2-weighted images were acquired using a gradient echo pulse sequence (TR=3000 ms, TE=90 ms, FOV=6 cm, matrix=256×196, slice thickness=3 mm) T1-weigthed images were obtained following intraperitoneal injection of 0.1 mmol/kg of gadopentetate dimeglumine (Magnevist, Berlex Laboratories, NJ, USA). In all rats, humane euthanasia was performed by cervical dislocation approximately 1 hr post-treatment, and the brain was removed and fixed intact in 10% neutral buffered formalin. Following fixation for 48 hours, an adult rat brain matrix slicer (Zivic Instruments, Pittsburgh, Pa.) was used to obtain contiguous 2 mm coronal brain sections from each animal Brain and sections were embedded routinely in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E).

Treatments evaluated in this study produced ablative lesions in brain tissue, as evaluated with MRI examinations (FIGS. **21**A-F) and pathologic preparations (FIGS. **20**A-D). In Rats #3 and #4, the MRI characteristics of both H-FIRE and IRE lesions were similar. The MRI appearance of lesions in rat brain appeared as focal, ovoid to elliptical, T1 iso- to hypo-intense, uniformly and markedly contrast enhanced (FIGS. **21**A, B, C, D, F) and T2 hyper-intense (FIG. **21**E). In all panels, lesions appear as focal hyperintense regions (white) compared to adjacent untreated cerebrocortical tissue (gray). Top Panels (A-C) obtained from Rat #3, in which both the left and right cerebral

hemispheres were treated with high-frequency waveforms at 300 V/250 kHz and 400 V/250 kHz, respectively. Bottom Panels (D-F), Rat #4, which underwent high-frequency, bipolar pulses in the right cerebrum at 400 V/500 kHz, and conventional IRE with unipolar pulses at 50 V in the left cerebrum. Panels A and D, post-gadolinium T1-weighted MRI sequences in the axial plane. Panel B, post-gadolinium T1-weighted MRI sequences in the right parasagittal plane. Panels C and F, post-gadolinium T1-weighted MRI sequences in the dorsal plane. Panel D, T2-weighted MRI sequence in the transverse plane. In all panels, the right side of the brain is on the left side of the panel.

All lesions were well demarcated from adjacent, normal brain tissue and appeared similar in size. Compared to untreated brain (FIGS. **20**A and B), histopathologic examination of brain sections from all treatments demonstrated clear areas of ablation indicated by pallor of the treated tissue that was sharply delineated from adjacent normal brain (FIG. **20**C). H-FIRE and IRE lesions were predominantly characterized by areas of complete obliteration of cerebrocortical architecture by an eosinophilic, vacuolated amorphous debris (FIGS. **20**C and D). In Rat #1, the H-FIRE ablation zone was confined to regions of elevated electric field surrounding the electrodes, whereas all other pulsing protocols resulted in ablation zones spanning the entire region between the electrodes. Cavitary cerebrocortical defects were induced with H-FIRE in Rat #1 and IRE in Rat #4. Variably sized regions of intraparenchymal hemorrhage were most pronounced immediately adjacent to and within electrode insertion tracks. The morphology of remnant neuronal and glial elements within H-FIRE ablated regions demonstrated features of both apoptosis and necrosis, including shrunken and hypereosinophilic cytoplasm, nuclear chromatin condensation, and nuclear pyknosis and karyolysis (FIG. **20**D). Free glial and neuronal nuclei in various states of degeneration were scattered throughout ablation zones. Inflammation was not a significant feature of IRE or H-FIRE lesions at the time point brains were examined.

EXAMPLE 8

Elimination of Muscle Contractions During High-Frequency IRE (H-FIRE) of Brain Tissue

Muscle contractions were monitored throughout the procedure described in EXAMPLE 7 with the accelerometer located in the interscapular region at the cervicothoracic junction. All IRE pulsing protocols were associated with macroscopic muscular contractions of the cervicothoracic junction, which were also palpable to the neurosurgeon, while no visual or tactile evidence of muscular contraction was seen during any of the H-FIRE bursts. These results were quantitatively confirmed by the data recordings from the accelerometer (FIGS. **22**A-D). Peak acceleration was determined during the first 90 bursts of the highest energy H-FIRE protocol (400 V/250 kHz) and the first 90 pulses of each IRE protocol (50 V, 100 V, 200 V). A one-way ANOVA was used to investigate the effects of each protocol on the ranks of peak acceleration at the cervicothoracic junction. In the event of a significant main effect, pairwise comparisons were completed using Tukey's Honestly Significant Difference (HSD). All statistical analyses were conducted using JMP 7 (Cary, N.C., USA) with a significance level of $p<0.05$. Results indicate that, even in the highest energy H-FIRE protocol, there are no detectable peaks in acceleration above the inherent noise of the system. However, in all IRE protocols, peaks in acceleration associated with each pulse are detectable above the baseline noise. Further, pairwise comparisons between the various IRE protocols indicated that the mean peak acceleration during each treatment was energy dependent. Specifically, the mean peak acceleration decreased as the applied voltage decreased (FIG. **23**).

EXAMPLE 9

Experimental Results of High-Frequency IRE (H-FIRE) of Liver Tissue

All study procedures were conducted following Institutional Animal Care and Use Committee approval and performed in a GLP compliant facility. Two, Fischer 344 male rats weighing 200-240 g were anesthetized by intraperitoneal injection of 10 mg/kg xylazine and 60 mg/kg ketamine hydrochloride, and a surgical plane of anesthesia was assessed by loss of the tail pinch reflex. A routine laparotomy surgical approach to the abdomen was made in order to expose the liver. Custom electrodes were inserted into the liver parenchyma and advanced to a depth of 2 mm beneath the surface. The electrodes were fashioned from steel pins (Dritz, 0.5 mm diameter), and the edge-to-edge electrode spacing was set to 1 mm by inserting the electrodes in a custom polycarbonate spacer.

In Rat #1, H-FIRE was applied at 1000 V/cm with 80 unipolar bursts at a center frequency of 2 MHz and, 50% duty cycle, and 50 μs burst width. In Rat #2 IRE was applied at an equivalent energy using 80 unipolar pulses with a duration of 50 μs and amplitude of 1000 V/cm. In all rats, humane euthanasia was performed by cervical dislocation approximately 1 hr post-treatment, and the liver was removed and fixed intact in 10% neutral buffered formalin. Following fixation for 48 hours, 5 mm sections from each animal were obtained and embedded routinely in paraffin, sectioned at 5 pm, and stained with hematoxylin and eosin (H&E).

Histologically, in both treatments, there is evidence of necrosis and sinusoidal congestion (FIGS. **19**A-B). Additionally, the lesions are well demarcated with cell scale resolution between treated and untreated tissue. Both of these features are common to IRE ablation of liver. No evidence of muscle contraction was observed visually. These results supplement those presented in EXAMPLE 7 and confirm that H-FIRE ablation can be achieved in multiple tissue types.

EXAMPLE 10

The Electric Field Distribution During High-Frequency Electroporation can be Approximated by the Laplace Equation

A 2D axisymmetric FEM representative of a slab of non-infiltrated fat adjacent to dry skin was simulated using COMSOL 4.2a (Burlington, Mass.). An energized and grounded electrode were modeled as infinite fins (0.5 mm diameter) separated 0.5 cm from the skin-fat interface, for a total spacing of 1 cm. The electric potential distribution within the tissue was obtained by transiently solving Equation 7 (see Example 1). Additionally, the homogeneous solution was solved according to the Laplace equation:

$$-\nabla\cdot(\nabla\Phi)=0 \tag{11}$$

For the heterogeneous case, the dielectric properties of various tissues were chosen from data generated by Gabriel

et al. available at (http://niremf.ifac.cnr.it/docs/dielectric/home.html). Gabriel, S., R. W. Lau, and C. Gabriel, *The dielectric properties of biological tissues* 0.2. Measurements in the frequency range 10 Hz to 20 GHz. Physics in Medicine and Biology, 1996. 41(11): p. 2251-2269. The data was interpolated in Mathematica 7 (Wolfram Research, Inc.) in order to estimate the dielectric properties at 1 kHz and 1 MHz. For the homogeneous case, the electric field distribution is independent of the dielectric properties. The energized and grounded electrodes were subtracted from the skin and fat subdomains, and treated purely as boundary conditions at 1000 V and 0V, respectively.

FIGS. **24**A and B show the electric field distribution during a bipolar burst with the frequencies given in TABLE 6.

TABLE 6

Dielectric properties of skin and fat tissue at various frequencies.

		Tissue	
Frequency	Property	Skin	Fat
1 kHz	σ [S/m]	0.000180	0.0246
	ε_r	1170	20800
1 MHz	σ [S/m]	0.0119	0.0267
	ε_r	792	25

From the surface contour map, at 1 kHz, which is representative of a 500 μs traditional electroporation pulse, the electric field is highly non-uniform. A majority of the voltage drop occurs within the skin layer, and the fat layer remains untreated. However, at 1 MHz, which is representative of a 500 ns high-frequency electroporation pulse, the voltage drop is distributed more uniformly throughout the entire domain. As a result, both the skin and fat layers can be treated. Additionally, the electric field distribution at 1 MHz closely resembles that of the homogenous solution. Therefore, knowledge of dielectric properties and intricate geometrical arrangements of heterogeneous tissues can be neglected during treatment planning for high-frequency electroporation. This greatly reduces treatment planning protocols and produces more predictable outcomes.

The present invention has been described with reference to particular embodiments having various features. It will be apparent to those skilled in the art that various modifications and variations can be made in the practice of the present invention without departing from the scope or spirit of the invention. One skilled in the art will recognize that these features may be used singularly or in any combination based on the requirements and specifications of a given application or design. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention. Where a range of values is provided in this specification, each value between the upper and lower limits of that range is also specifically disclosed. The upper and lower limits of these smaller ranges may independently be included or excluded in the range as well. As used in this specification, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. It is intended that the specification and examples be considered as exemplary in nature and that variations that do not depart from the essence of the invention are intended to be within the scope of the invention. Further, the references cited in this disclosure are incorporated by reference herein in their entireties.

The invention claimed is:

1. A method of ablating tissue cells by non-thermal irreversible electroporation comprising:

positioning one or more electrodes near a target area containing target tissue cells to be ablated;

applying a plurality of electrical pulses to the target area through the positioned electrodes by applying pulses at a frequency rate of 50 kHz or higher with each electrical pulse having a pulse width of 10 microseconds or less, so as to ablate the target tissue cells by non-thermal irreversible electroporation.

2. The method of claim **1**, wherein the step of applying includes applying the plurality of electrical pulses at the frequency rate of between 50 kHz and 2 MHz, inclusive.

3. The method of claim **1**, wherein the step of applying includes applying the plurality of electrical pulses at the frequency rate of between 250 kHz and 2 MHz, inclusive.

4. The method of claim **1**, wherein the step of positioning includes non-invasively positioning the one or more electrodes near the target area.

5. The method of claim **1**, wherein the pulse width of a single electrical pulse is at least 100 nanoseconds.

6. The method of claim **1**, wherein the pulse width of a single electrical pulse is in the range of 250 nanoseconds to 2 microseconds, inclusive.

7. The method of claim **1**, wherein the step of applying includes applying a plurality of bipolar electrical pulses.

8. The method of claim **1**, wherein the step of applying includes applying a plurality of bipolar bursts of electrical pulses with multiple pulses in a single phase before a polarity switch.

9. The method of claim **1**, wherein the electrical pulses are square, ramp, sinusoidal, exponential, or trapezoidal.

10. The method of claim **1**, wherein the step of applying includes applying a burst of electrical pulses which is sufficient to cause a transmembrane potential (TMP) of the target tissue cells to rise above a critical threshold (CT) for inducing electroporation while a single electrical pulse in the burst of electrical pulses is insufficient to cause the TMP of the target tissue cells to rise above the CT.

11. The method of claim **1**, wherein the step of applying includes applying a plurality of electrical pulses wherein each electrical pulse is sufficient to cause a transmembrane potential (TMP) of the target tissue cells to rise above a critical threshold (CT) for inducing electroporation.

12. The method of claim **1**, wherein the step of applying includes applying a burst of electrical pulses which is sufficient to cause a transmembrane potential (TMP) of the target tissue cells to rise above a critical threshold for inducing irreversible electroporation (CTIRE) while a single electrical pulse in the burst of electrical pulses is insufficient to cause the TMP of the target tissue cells to rise above the CTIRE.

13. The method of claim **12**, wherein the CTIRE is about 1 Volt.

14. The method of claim **1**, wherein the step of applying includes applying a plurality of electrical pulses wherein each electrical pulse is sufficient to cause a transmembrane potential (TMP) of the target tissue cells to rise above a critical threshold (CT) for inducing irreversible electroporation.

15. The method of claim **1**, wherein the step of applying includes applying the electrical pulses at 2500 V/cm or lower.

16. The method of claim **1**, wherein the step of applying includes controlling voltage and pulse width of the electrical

pulses to reduce tissue stimulation sufficiently to perform tissue ablation without using general anesthesia.

* * * * *