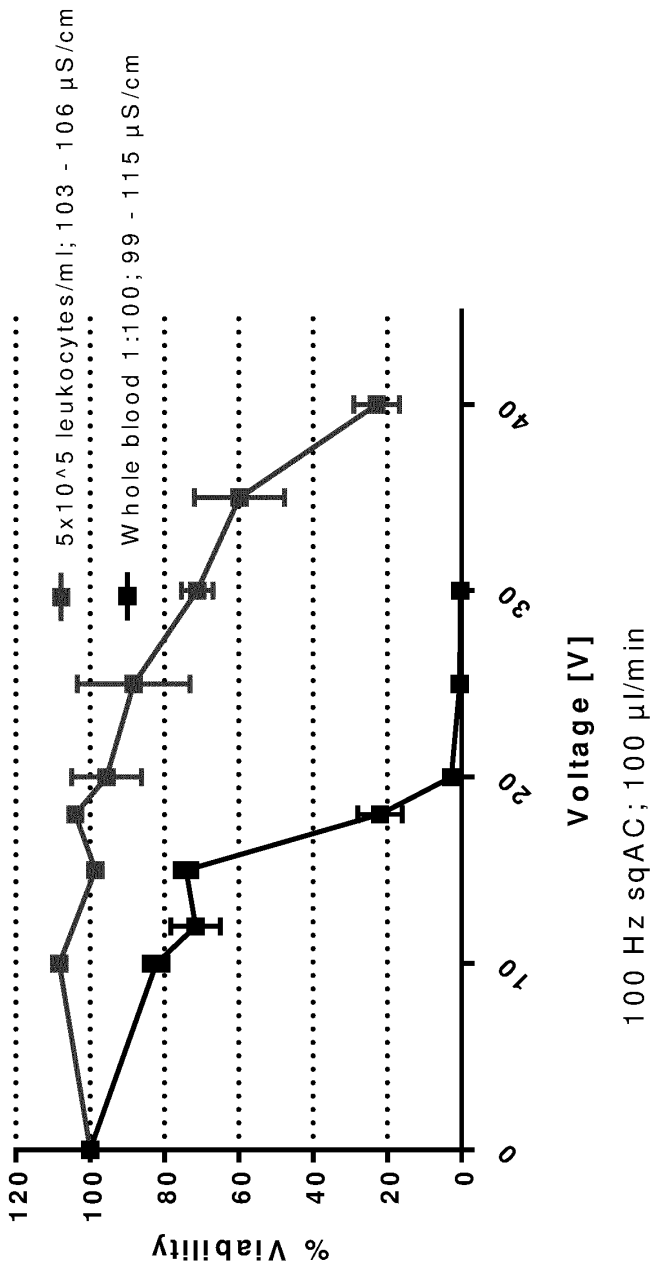

5x10^5 leukocytes/ml; 103 - 106 µS/cm
Whole blood 1:100; 99 - 115 µS/cm
% Viability
120
100
80
60
40
20
0
0
10
20
30
40
Voltage [V]
100 Hz sqAC; 100 µl/min

Fig. 1

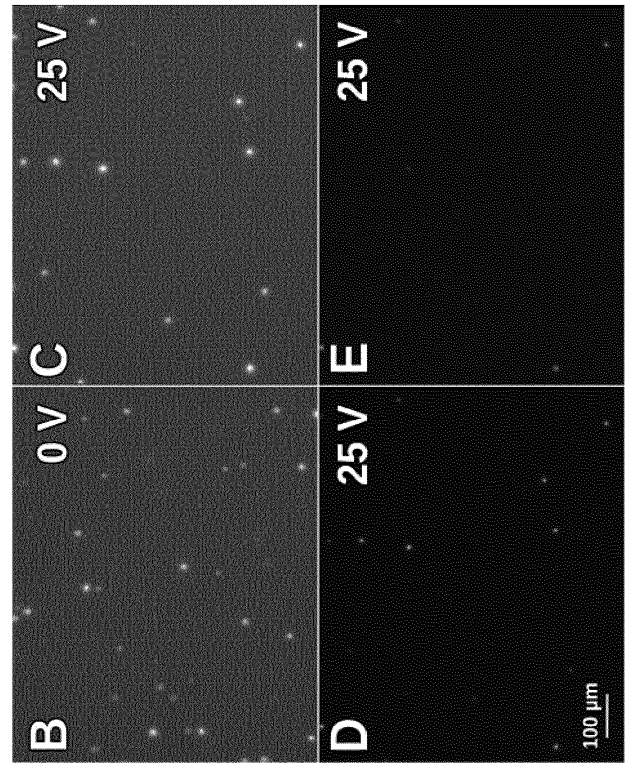

B
0 V
C
25 V
D
25 V
E
25 V
100 µm

Fig. 3

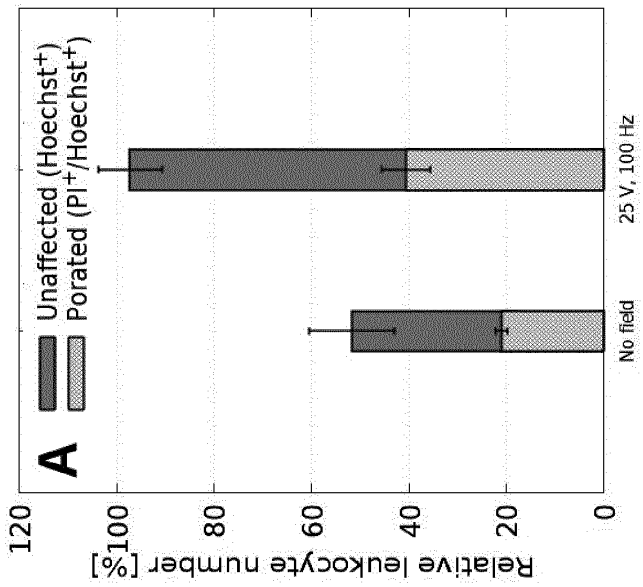

A
Unaffected (Hoechst+)
Porated (PI+/Hoechst+)
Relative leukocyte number [%]
120
100
80
60
40
20
0
No field
25 V, 100 Hz

Fig. 2

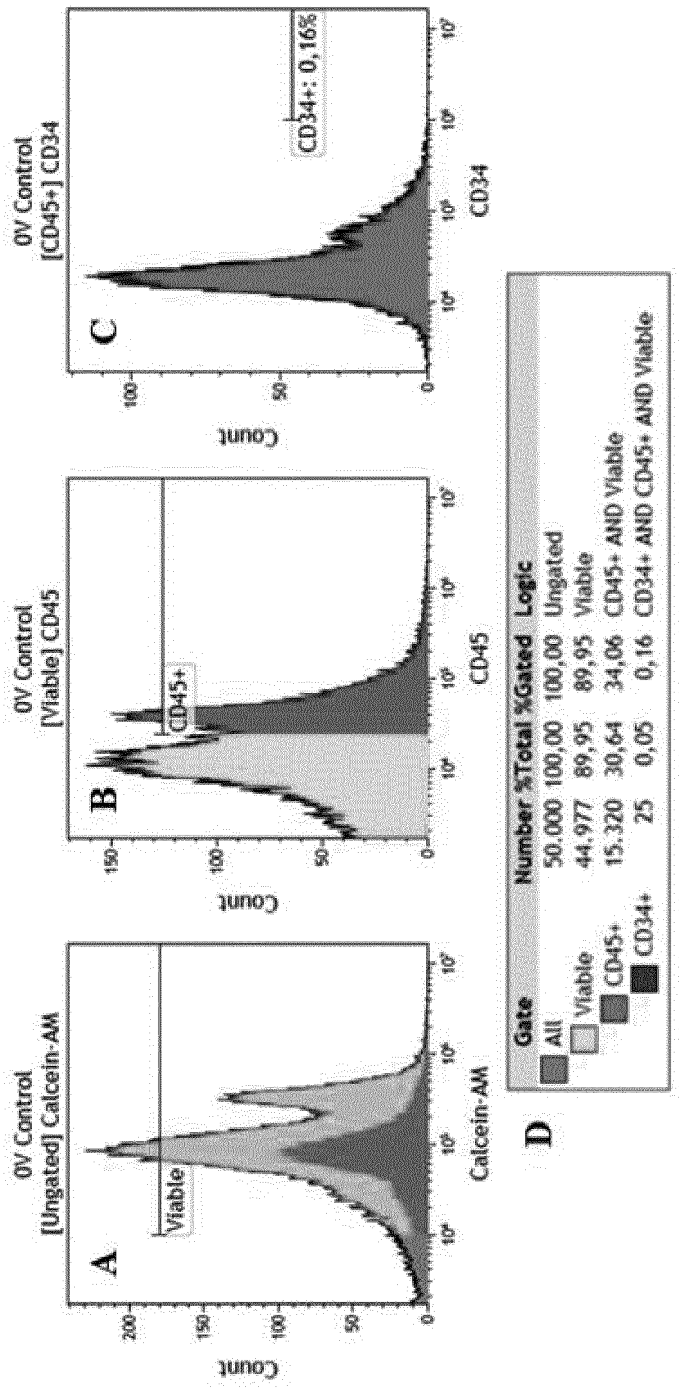

A
0V Control
[Ungated] Calcein-AM
Viable
Count
Calcein-AM
B
0V Control
[Viable] CD45
CD45+
Count
CD45
C
0V Control
[CD45+] CD34
CD34+: 0,16%
Count
CD34
D
Gate Number %Total %Gated Logic
All 50.000 100,00 100,00 Ungated
Viable 44.977 89,95 89,95 Viable
CD45+ 15.320 30,64 34,06 CD45+ AND Viable
CD34+ 25 0,05 0,16 CD34+ AND CD45+ AND Viable

Fig. 4

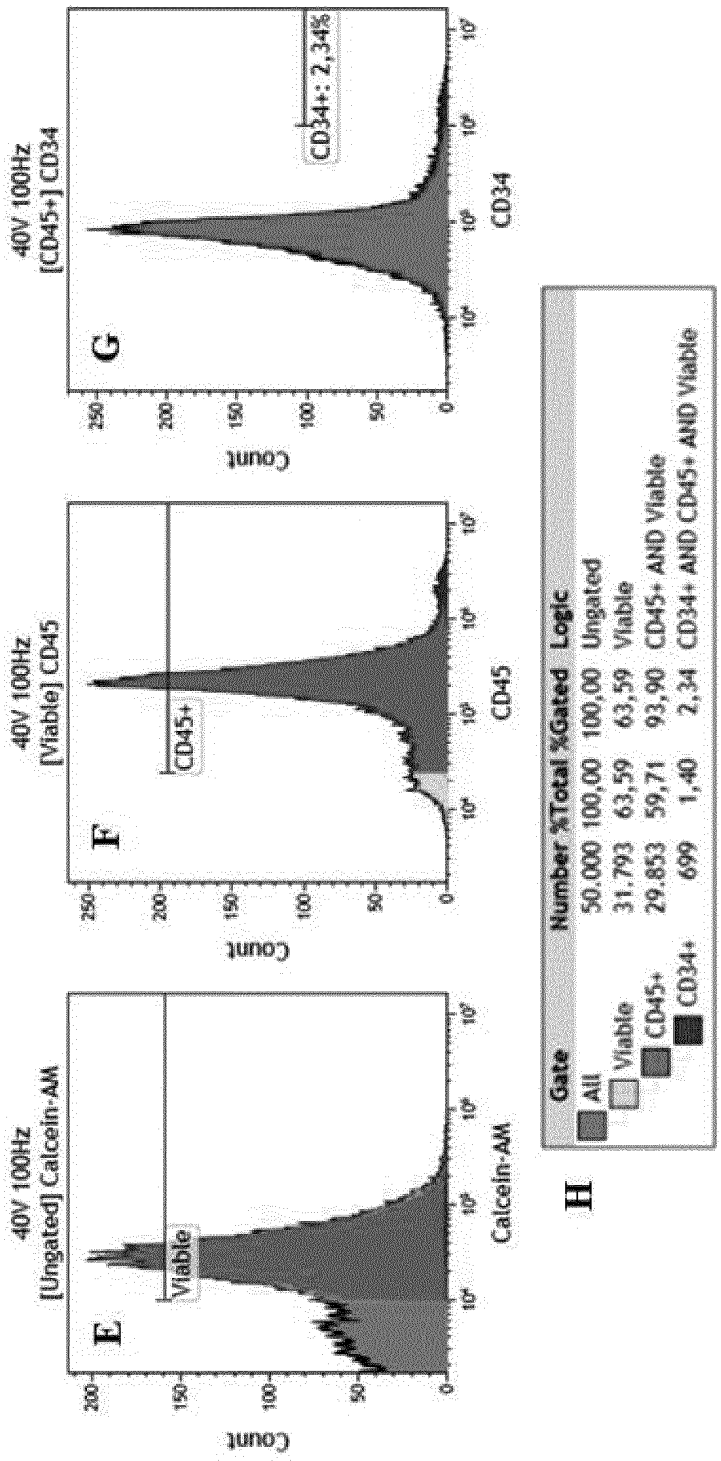

E
40V 100Hz
[Ungated] Calcein-AM
Viable
Count
Calcein-AM
F
40V 100Hz
[Viable] CD45
CD45+
Count
CD45
G
40V 100Hz
[CD45+] CD34
CD34+: 2,34%
Count
CD34
H
Gate Number %Total %Gated Logic
All 50,000 100,00 100,00 Ungated
Viable 31,793 63,59 63,59 Viable
CD45+ 29,853 59,71 93,90 CD45+ AND Viable
CD34+ 699 1,40 2,34 CD34+ AND CD45+ AND Viable

Fig. 4

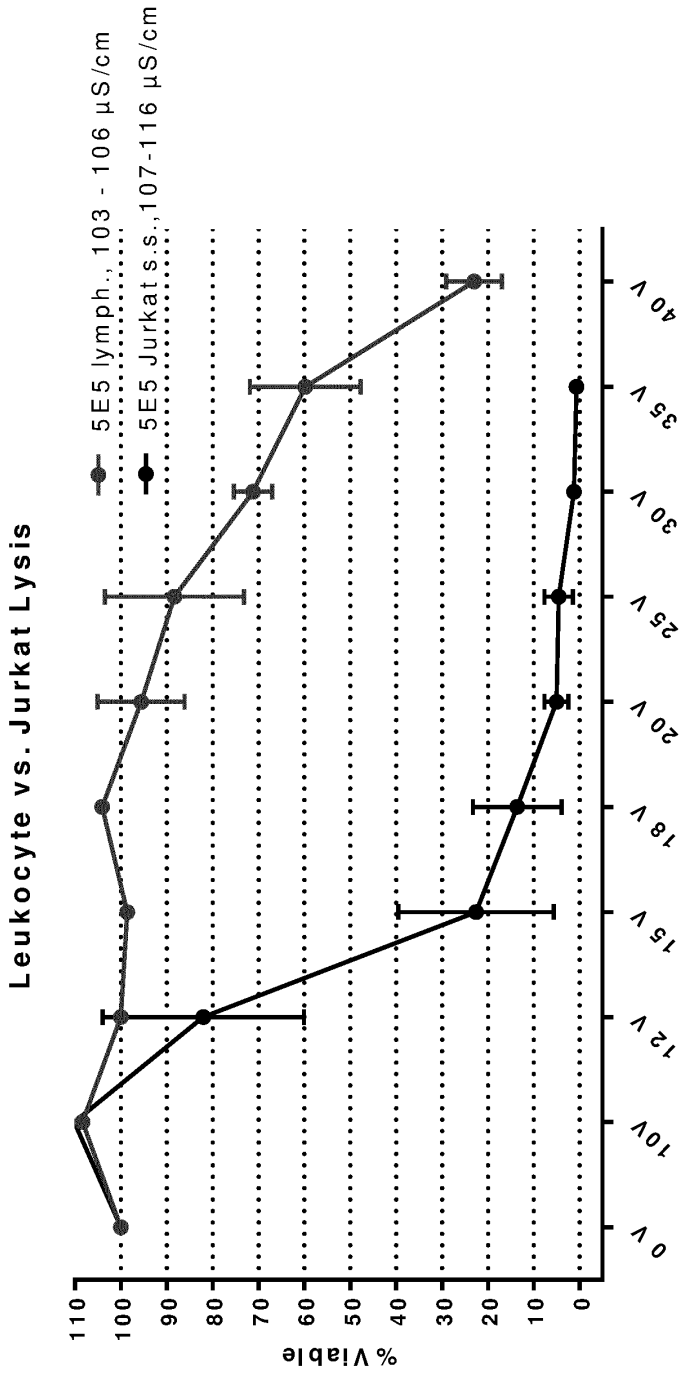

Leukocyte vs. Jurkat Lysis
5E5 lymph.,,103 - 106 µS/cm
5E5 Jurkat s.s.,107-116 µS/cm
% Viable
110
100
90
80
70
60
50
40
30
20
10
0
0 V
10 V
12 V
15 V
18 V
20 V
25 V
30 V
35 V
40 V

Fig. 5

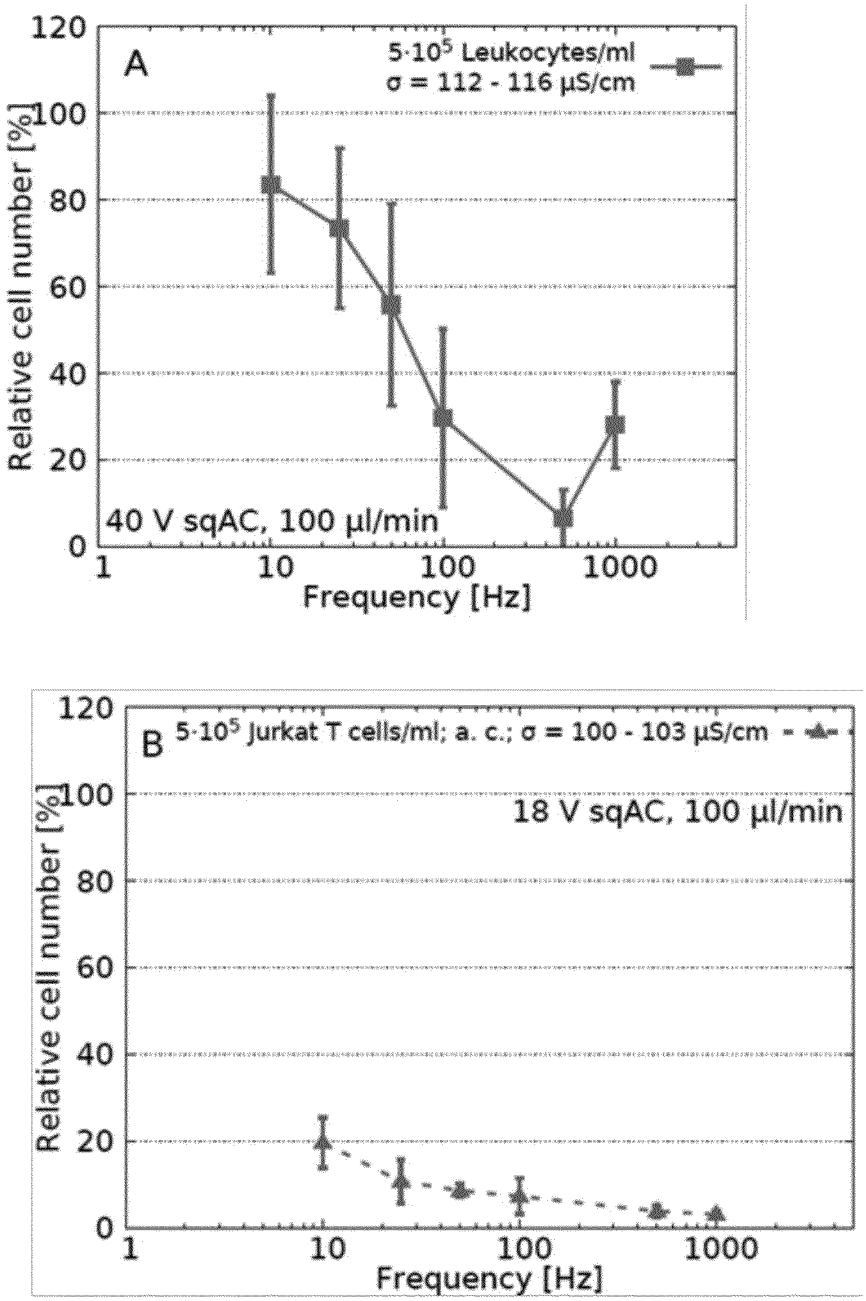

A
5·10^5 Leukocytes/ml
σ = 112 - 116 µS/cm
40 V sqAC, 100 µl/min
Relative cell number [%]
Frequency [Hz]
B
5·10^5 Jurkat T cells/ml; a. c.; σ = 100 - 103 µS/cm
18 V sqAC, 100 µl/min
Relative cell number [%]
Frequency [Hz]

Fig. 6

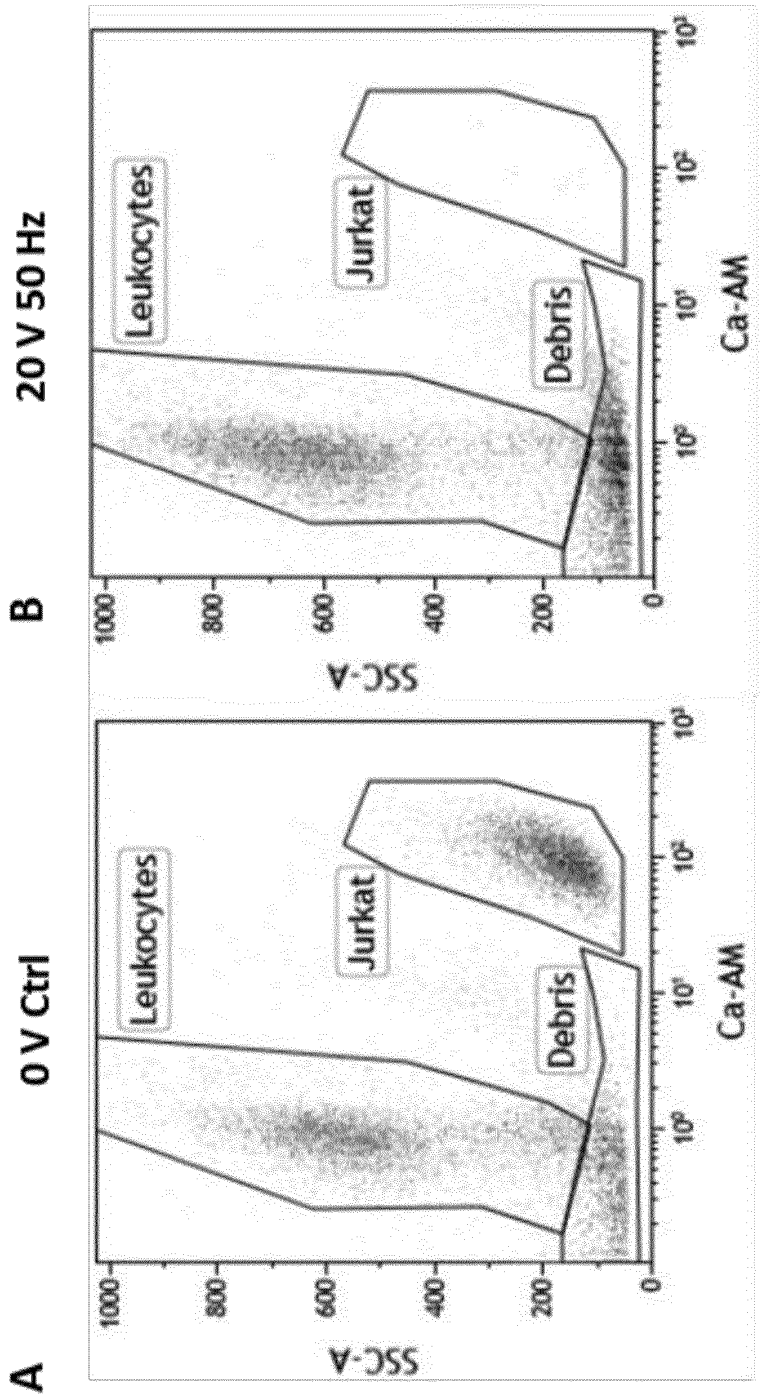

A
0 V Ctrl
B
20 V 50 Hz
Leukocytes
Jurkat
Debris
SSC-A
Ca-AM
1000
800
600
400
200
0
10^0
10^1
10^2
10^3

Fig. 7

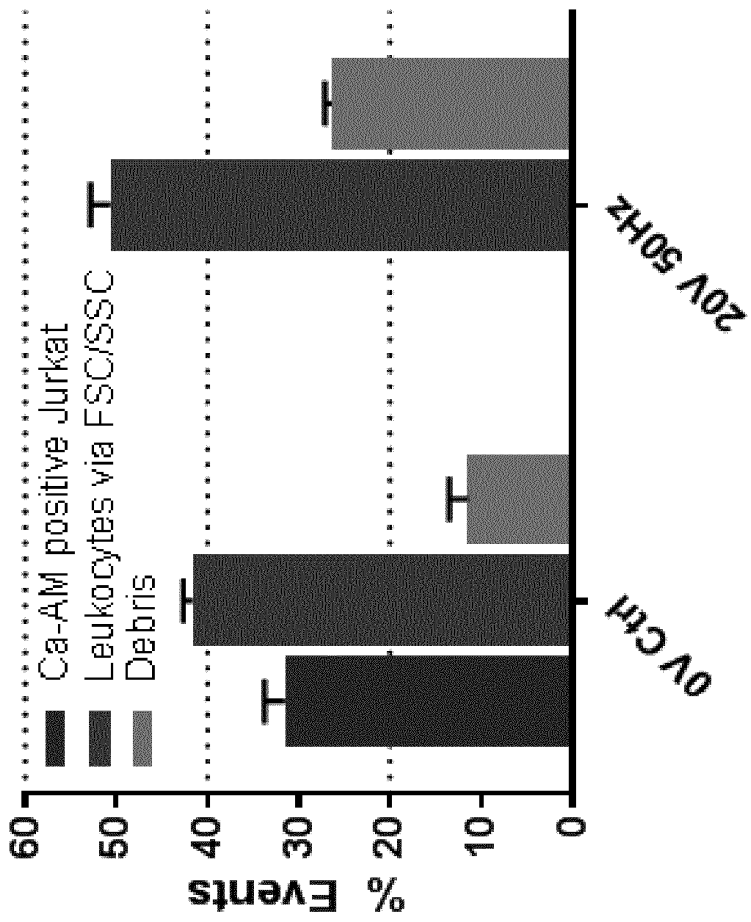

C
% Events
60
50
40
30
20
10
0
Ca-AM positive Jurkat
Leukocytes via FSC/SSC
Debris
0V Ctrl
20V 50Hz

Fig. 7

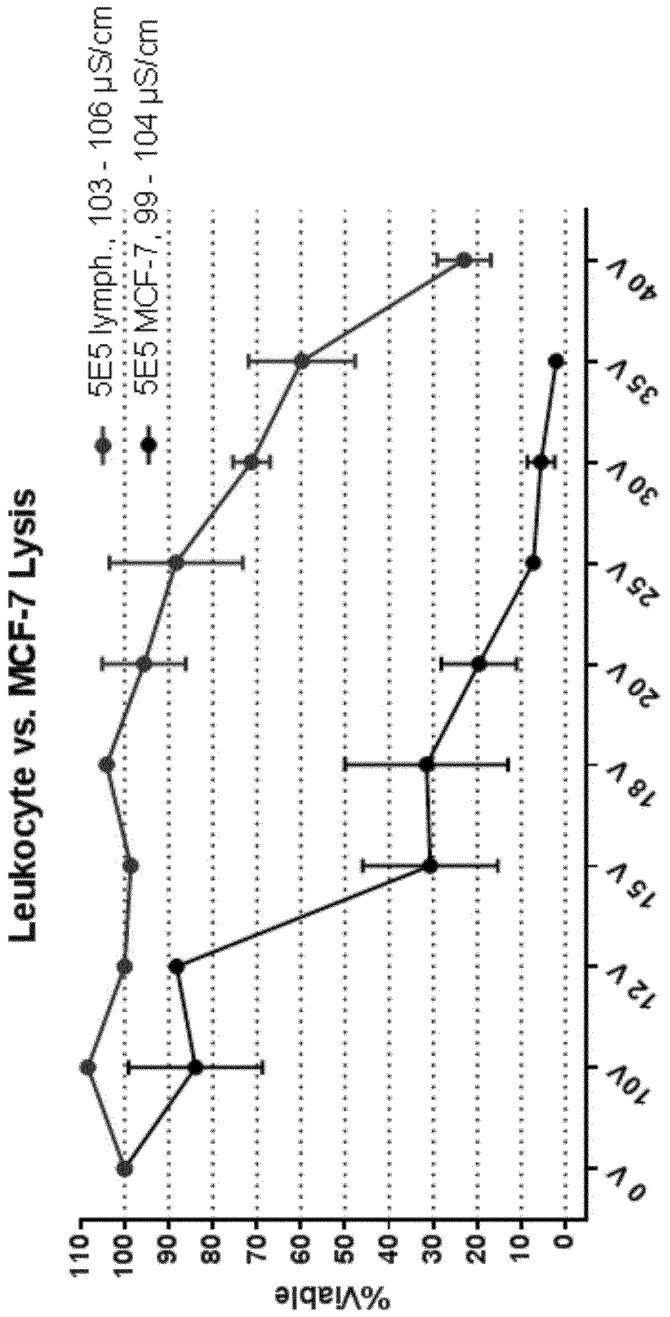

Leukocyte vs. MCF-7 Lysis
5E5 lymph., 103 - 106 µS/cm
5E5 MCF-7, 99 - 104 µS/cm
%Viable
110
100
90
80
70
60
50
40
30
20
10
0
0 V
10 V
12 V
15 V
18 V
20 V
25 V
30 V
35 V
40 V

Fig. 8

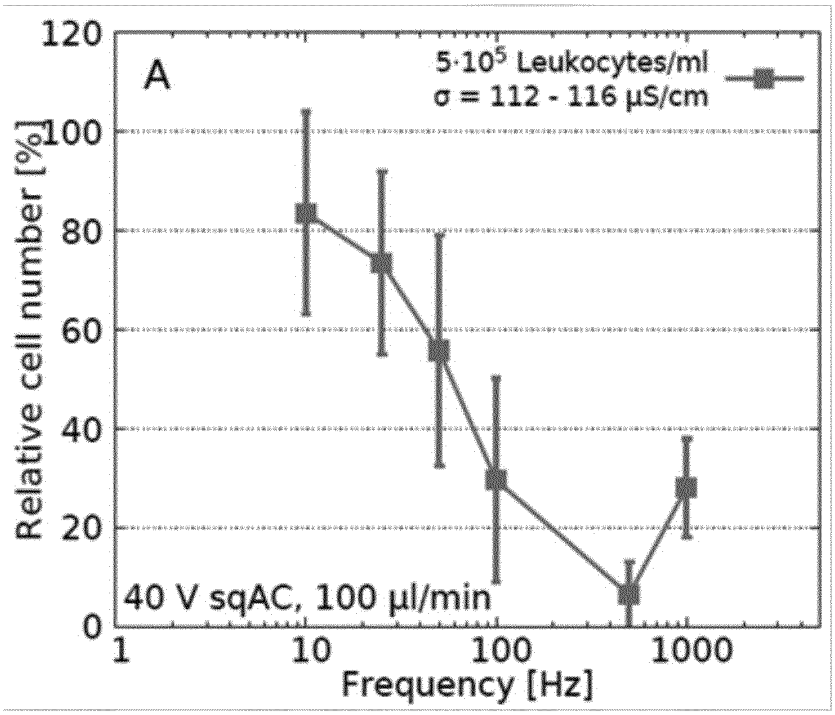

A
5·10^5 Leukocytes/ml
σ = 112 - 116 µS/cm
Relative cell number [%]
120
100
80
60
40
20
0
40 V sqAC, 100 µl/min
1
10
100
1000
Frequency [Hz]

Fig. 9

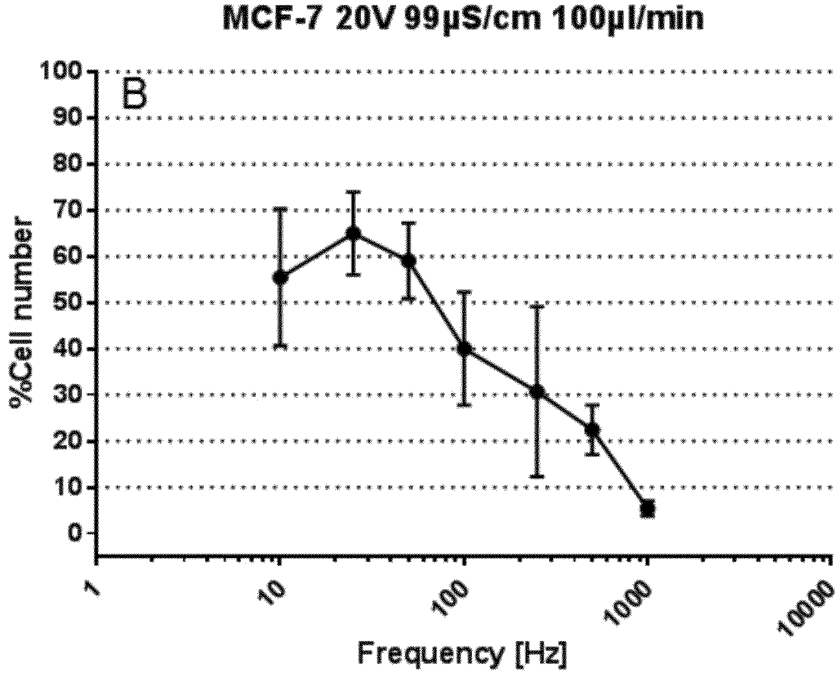

MCF-7 20V 99µS/cm 100µl/min
B
%Cell number
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
10000
Frequency [Hz]

Fig. 9

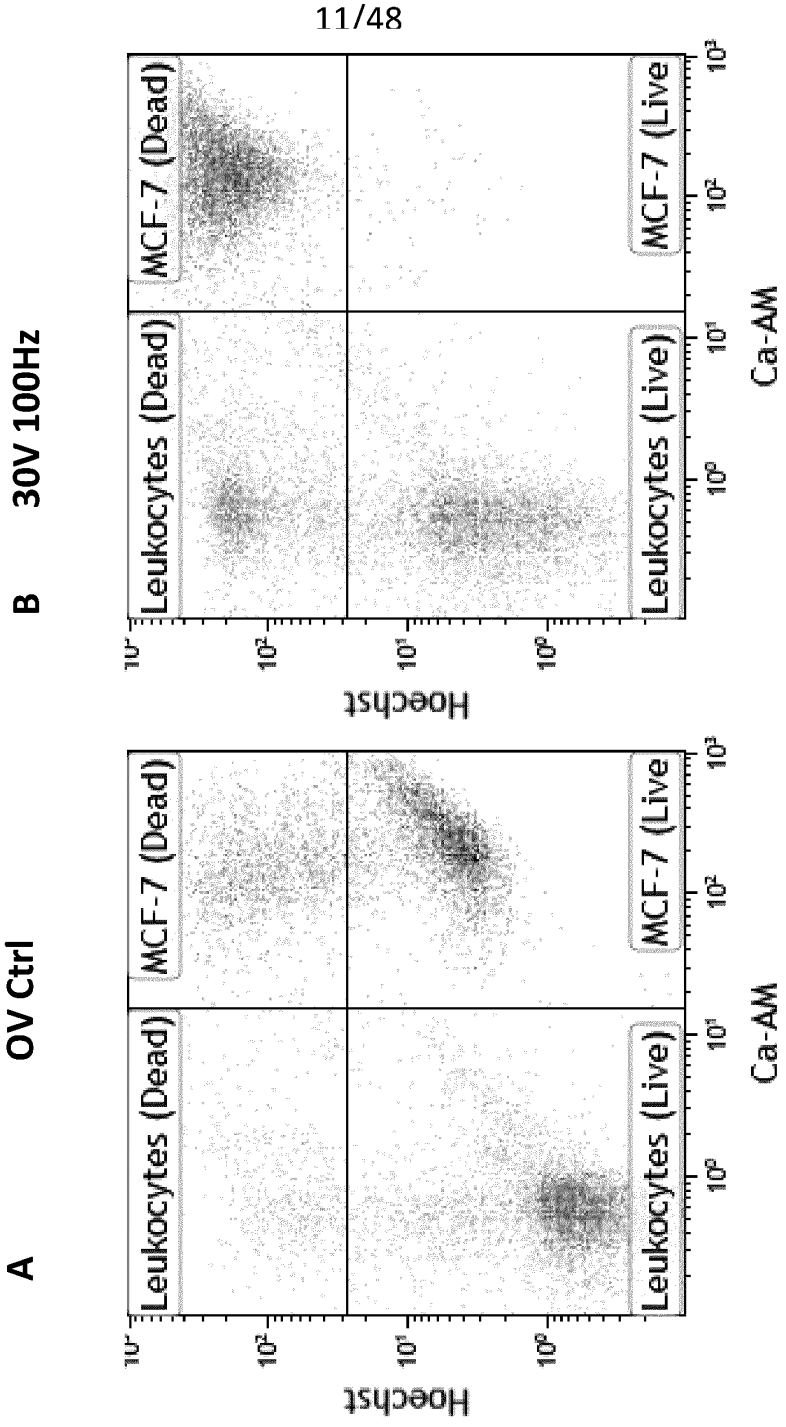

A
OV Ctrl
B
30V 100Hz
Leukocytes (Dead)
MCF-7 (Dead)
Leukocytes (Live)
MCF-7 (Live
Hoechst
Ca-AM

Fig. 10

C

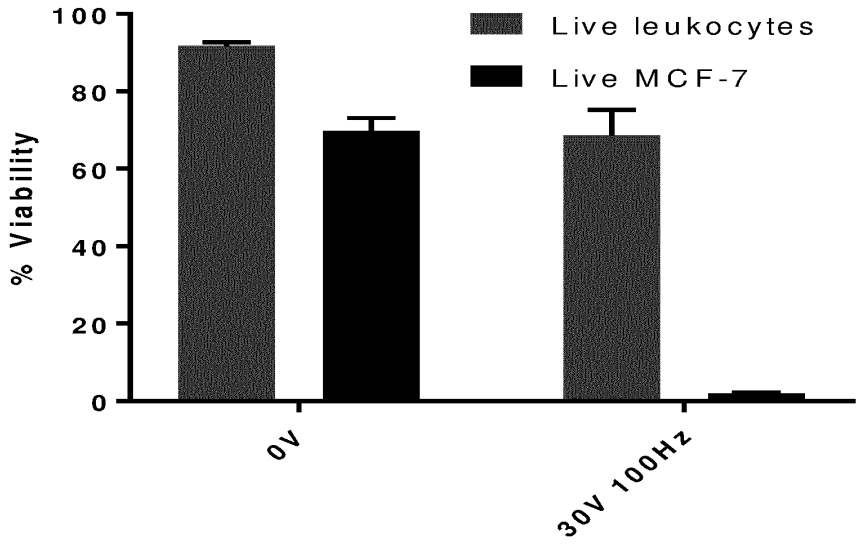
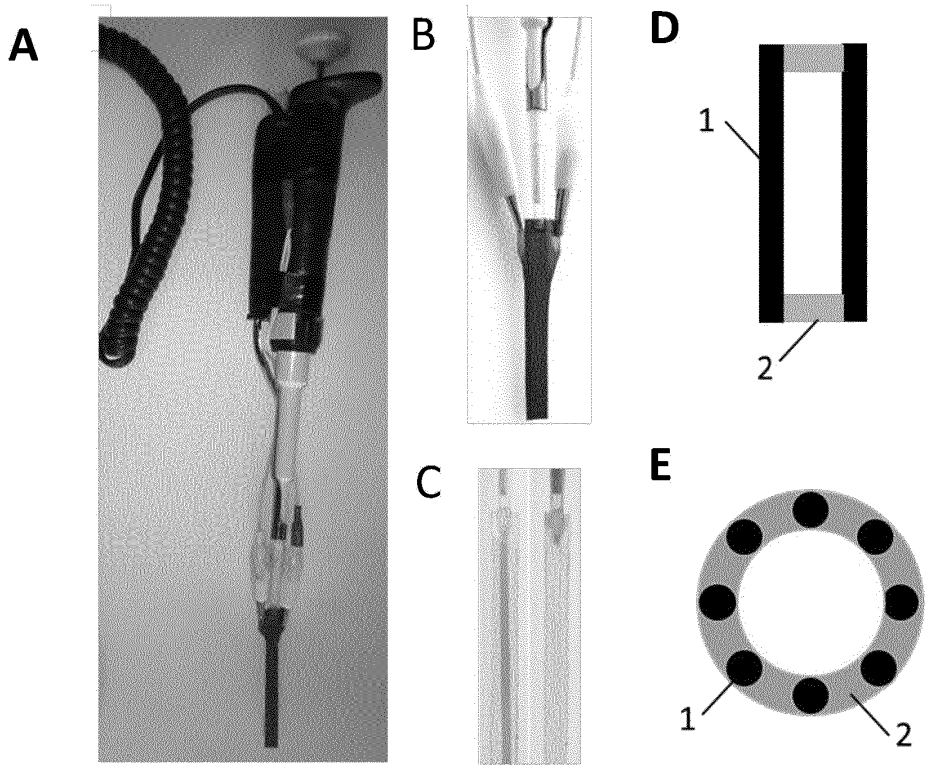

100
80
60
40
20
0
% Viability
Live leukocytes
Live MCF-7
0V
30V 100Hz

Fig. 10

Fig. 11



A

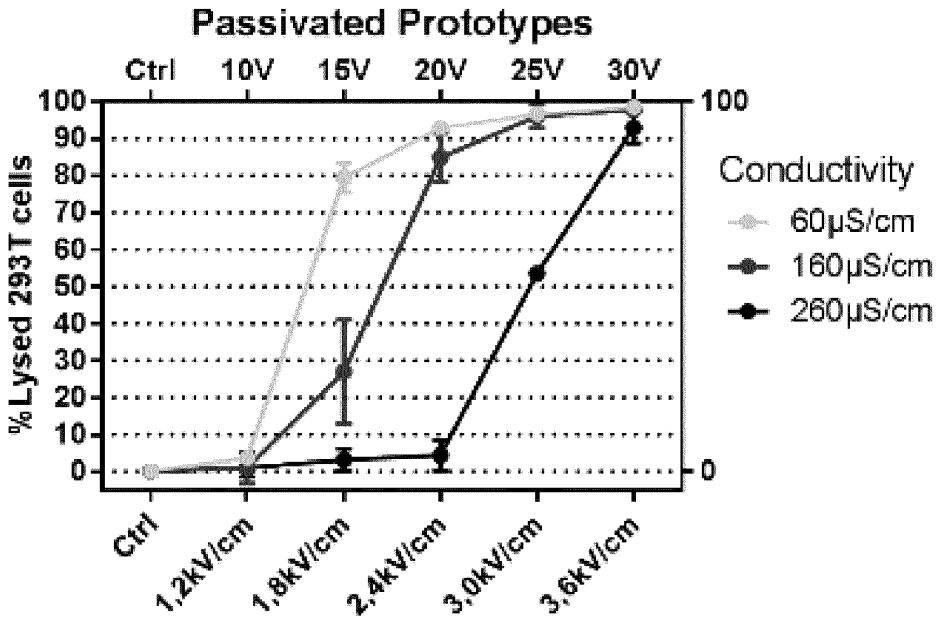
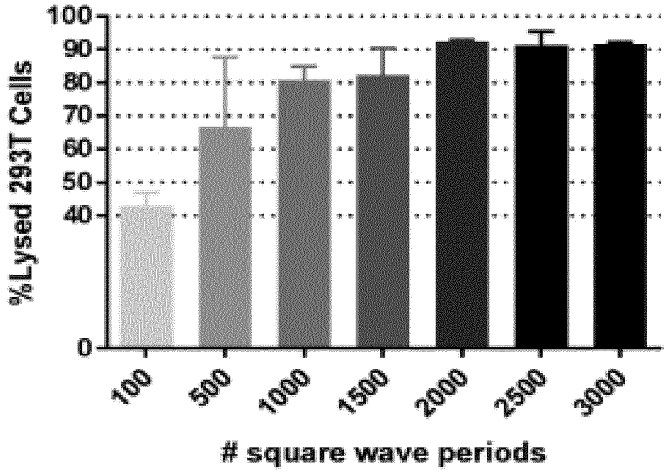

Passivated Prototypes
Ctrl
10V
15V
20V
25V
30V
% Lysed 293T cells
100
90
80
70
60
50
40
30
20
10
0
100
0
Conductivity
60µS/cm
160µS/cm
260µS/cm
Ctrl
1,2kV/cm
1,8kV/cm
2,4kV/cm
3,0kV/cm
3,6kV/cm

Fig. 12

Fig. 12

B


100
90
80
70
60
50
40
0
%Lysed 293T Cells
100
500
1000
1500
2000
2500
3000
square wave periods

C

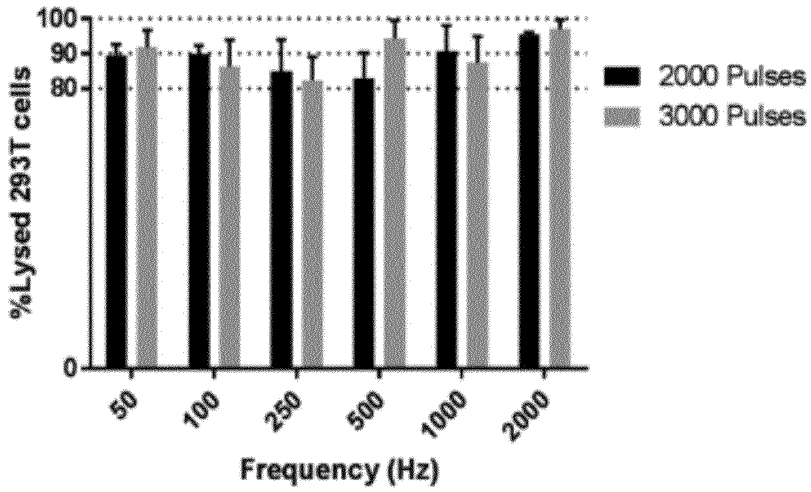
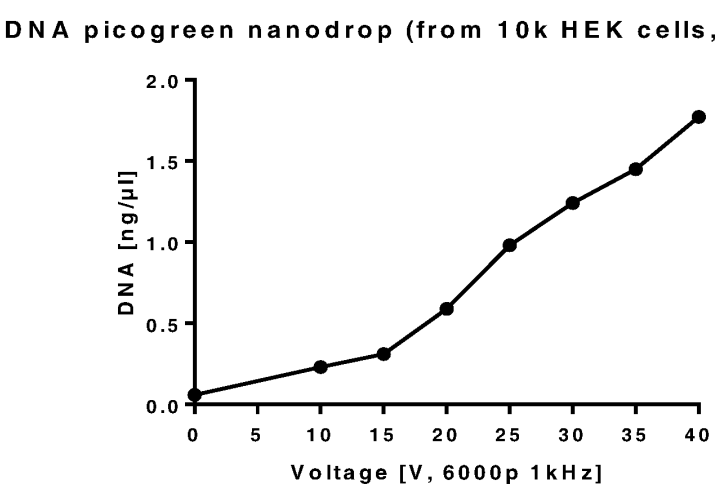

100
90
80
0
%Lysed 293T cells
2000 Pulses
3000 Pulses
50
100
250
500
1000
2000
Frequency (Hz)

Fig. 12

D


DNA picogreen nanodrop (from 10k HEK cells, 160µS/cm)
DNA [ng/µl]
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
Voltage [V, 6000p 1kHz]

E

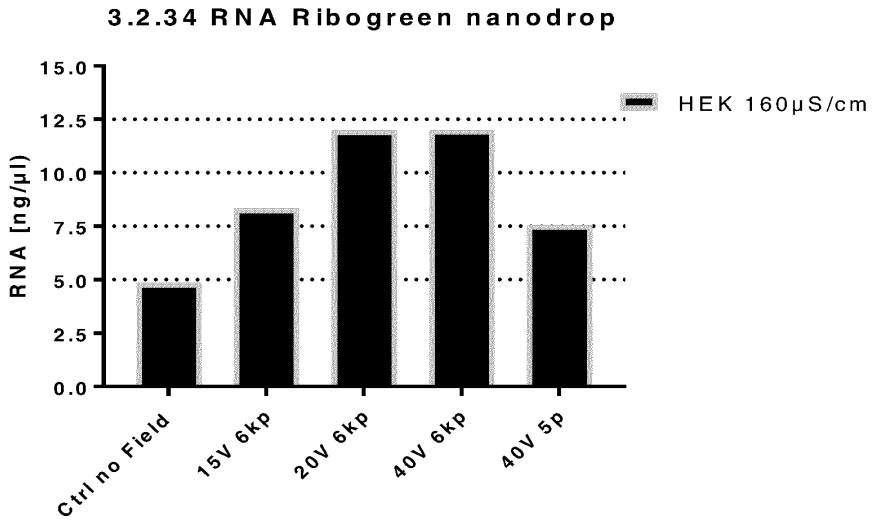

3.2.34 RNA Ribogreen nanodrop
RNA [ng/µl]
15.0
12.5
10.0
7.5
5.0
2.5
0.0
HEK 160µS/cm
Ctrl no Field
15V 6kp
20V 6kp
40V 6kp
40V 5p

Fig. 13

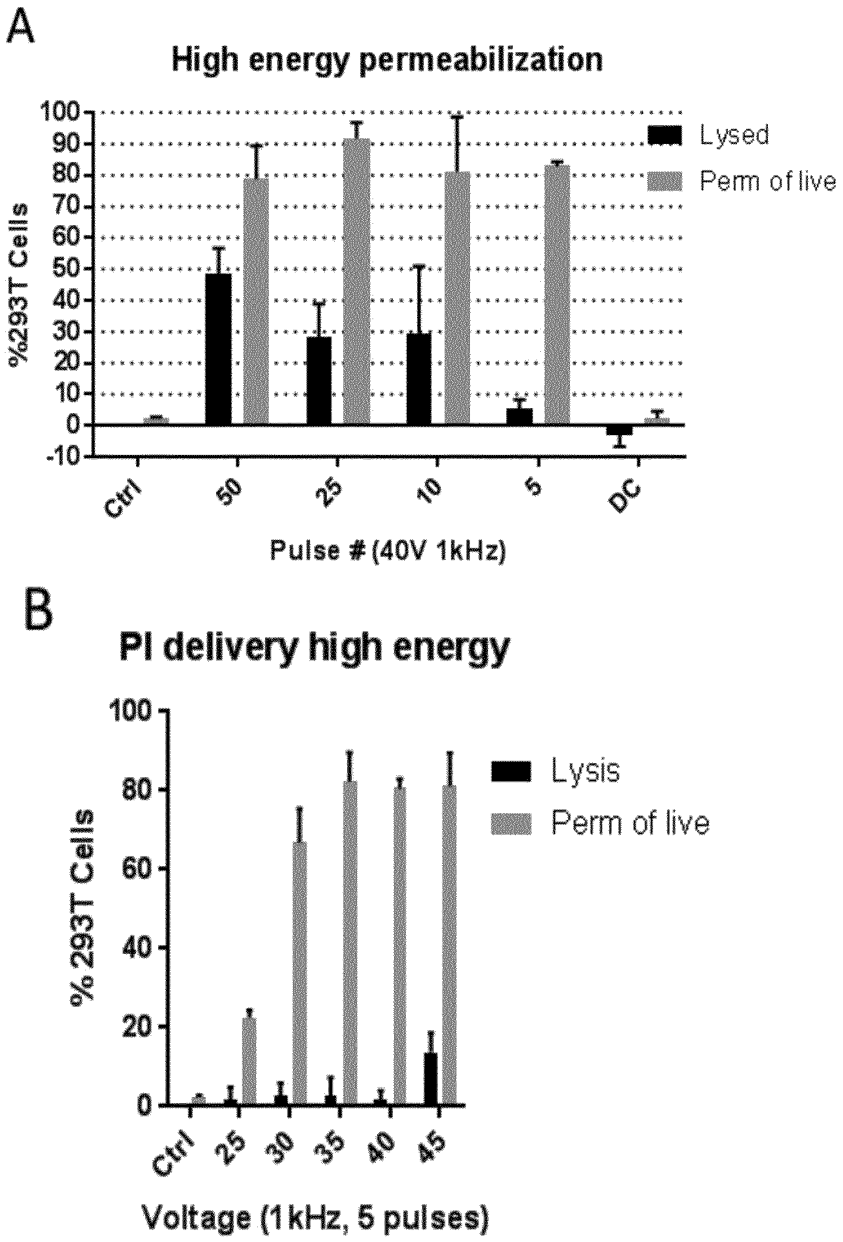
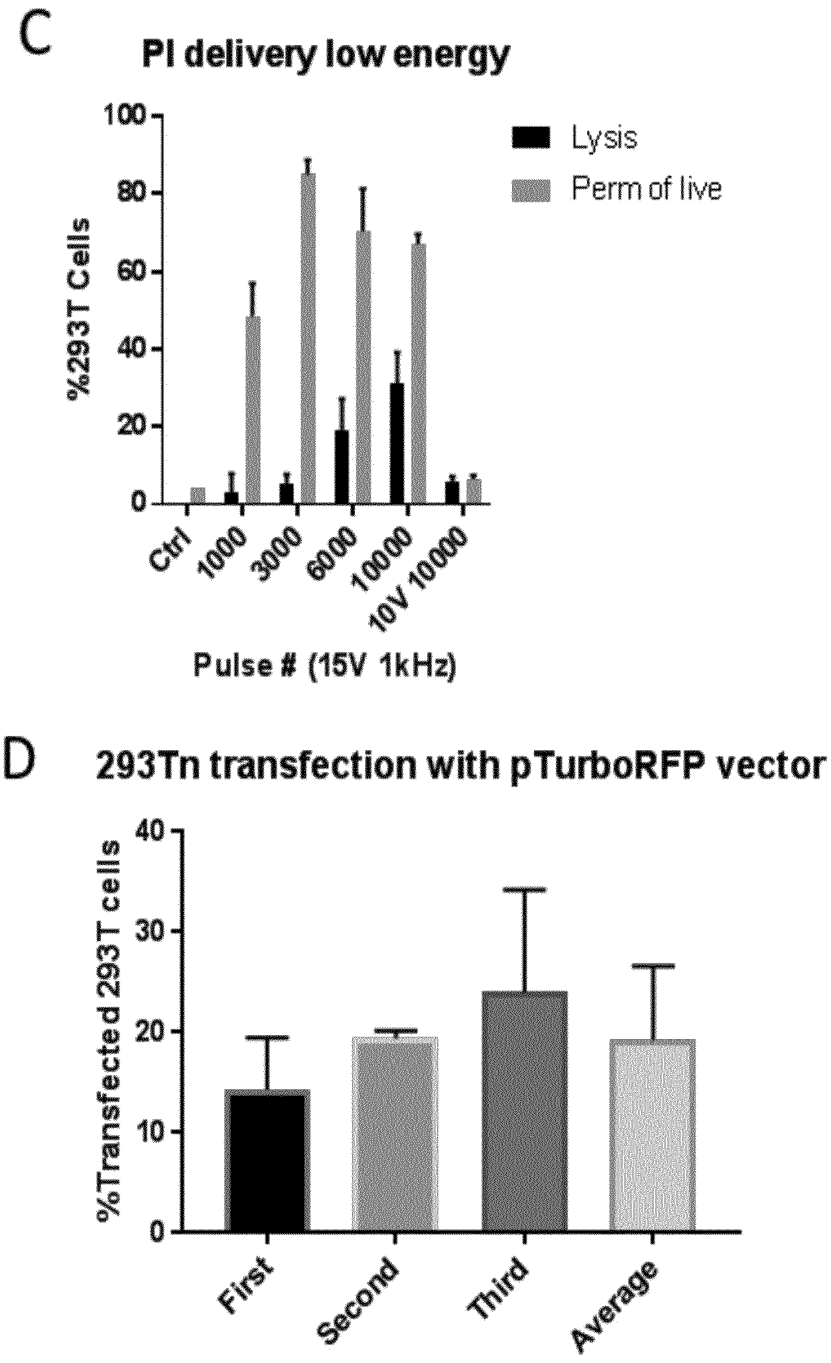

A
High energy permeabilization
%293T Cells
100
90
80
70
60
50
40
30
20
10
0
-10
Ctrl
50
25
10
5
DC
Pulse # (40V 1kHz)
Lysed
Perm of live
B
PI delivery high energy
% 293T Cells
100
80
60
40
20
0
Ctrl
25
30
35
40
45
Voltage (1kHz, 5 pulses)
Lysis
Perm of live

Fig. 13


C
PI delivery low energy
Lysis
Perm of live
%293T Cells
100
80
60
40
20
0
Ctrl
1000
3000
6000
10000
10V 10000
Pulse # (15V 1kHz)
D
293Tn transfection with pTurboRFP vector
%Transfected 293T cells
40
30
20
10
0
First
Second
Third
Average

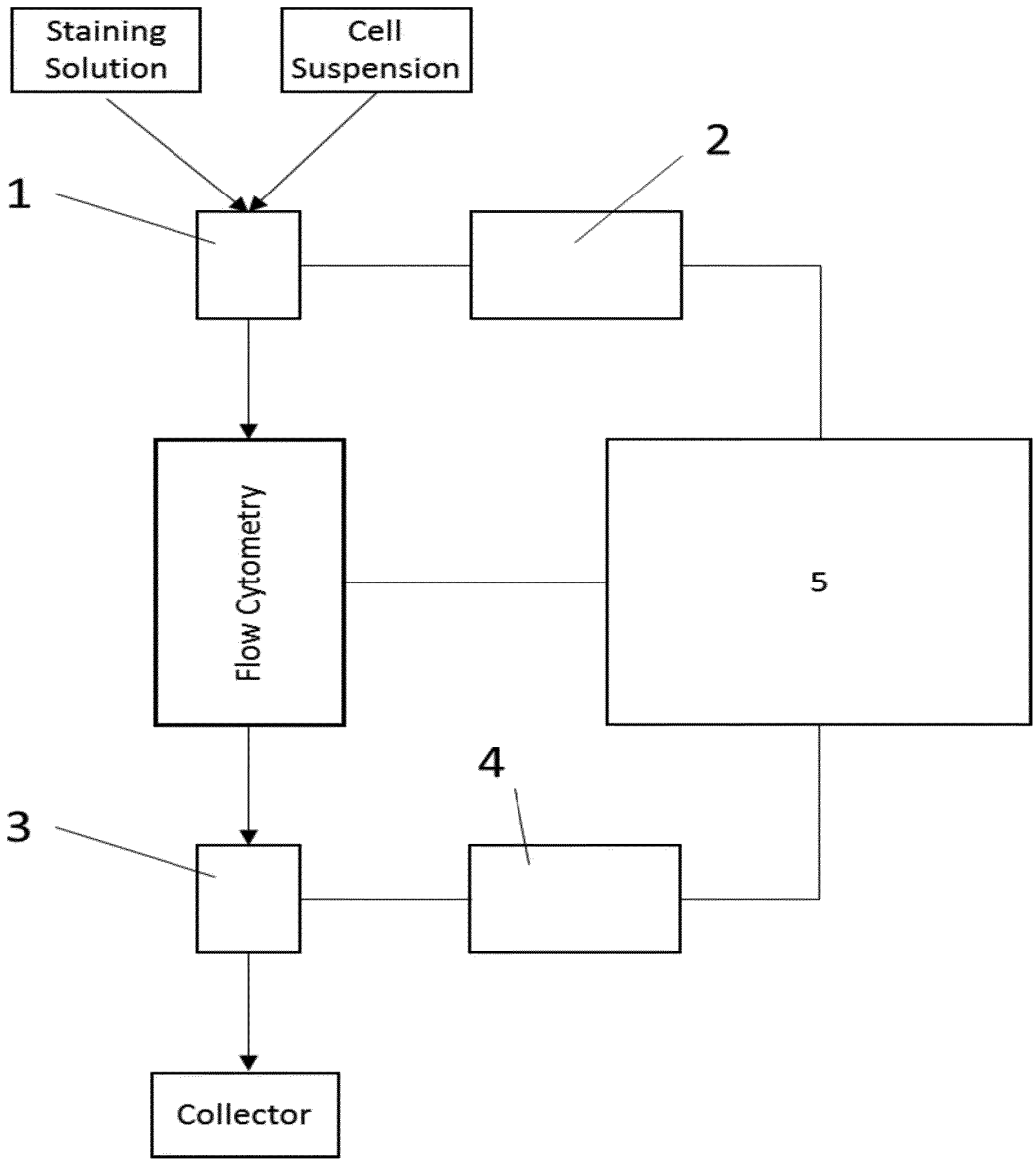

Staining Solution
Cell Suspension
1
2
Flow Cytometry
5
4
3
Collector

Fig. 14

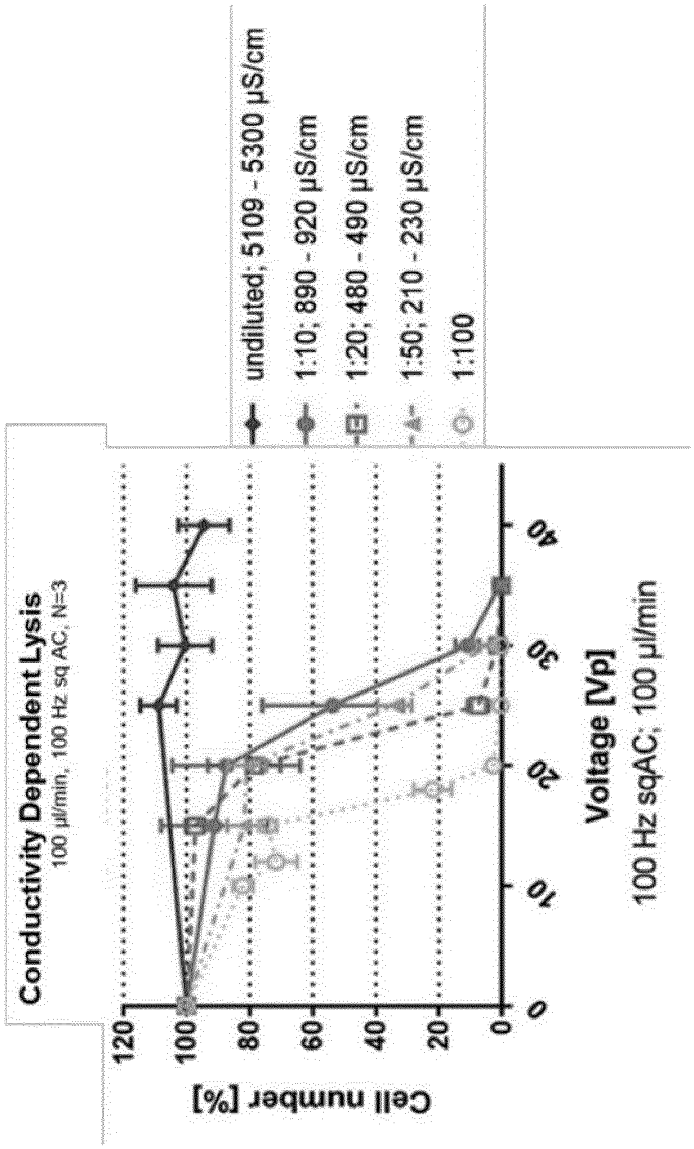
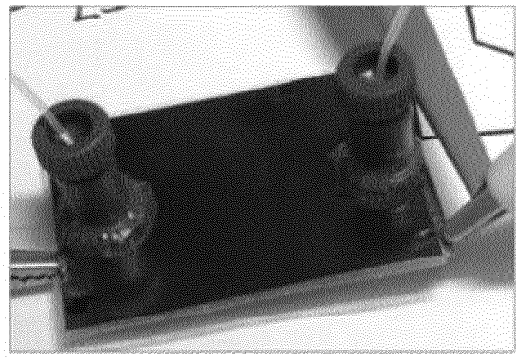

Conductivity Dependent Lysis
100 µl/min, 100 Hz sq AC, N=3
undiluted; 5109 - 5300 µS/cm
1:10; 890 - 920 µS/cm
1:20; 480 - 490 µS/cm
1:50; 210 - 230 µS/cm
1:100
Cell number [%]
120
100
80
60
40
20
0
0
10
20
30
40
Voltage [Vp]
100 Hz sqAC; 100 µl/min

Fig. 15

Fig. 16

a



b

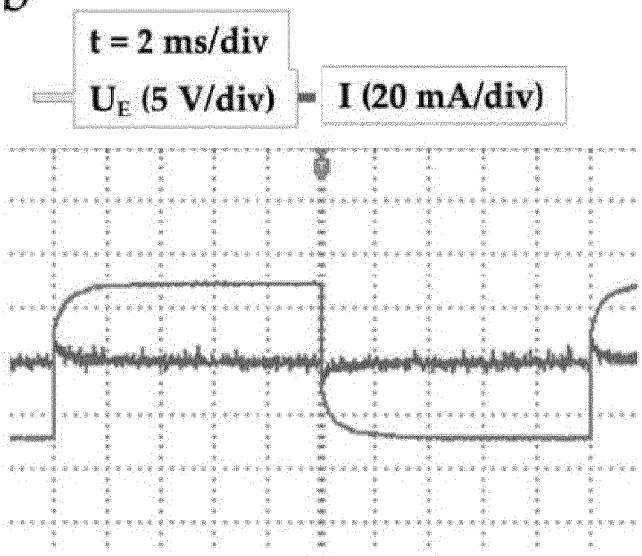
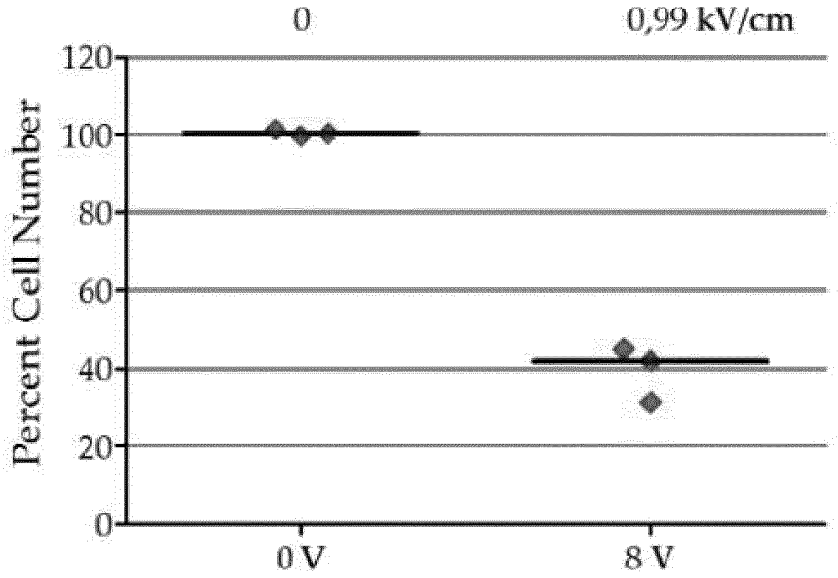


Fig. 16

C



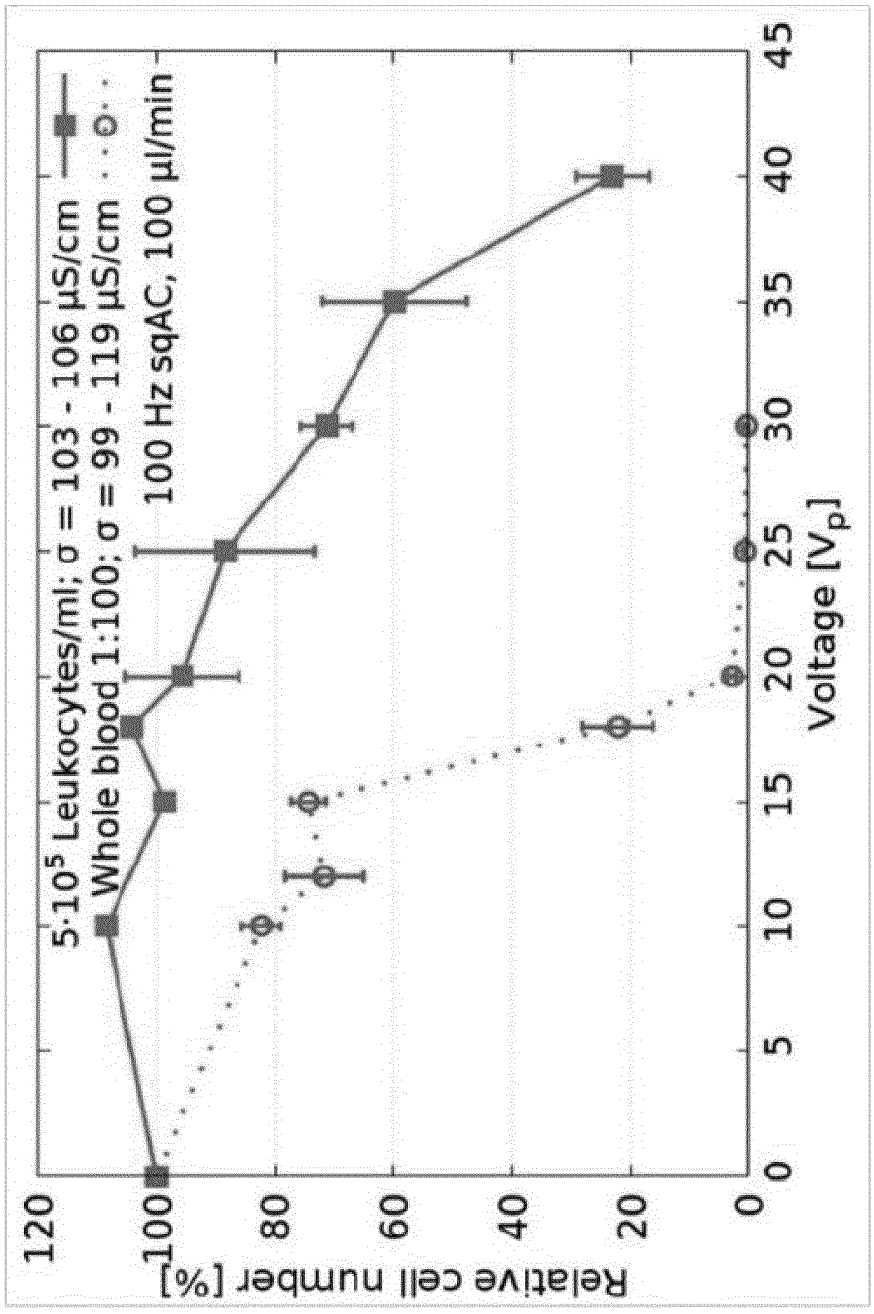
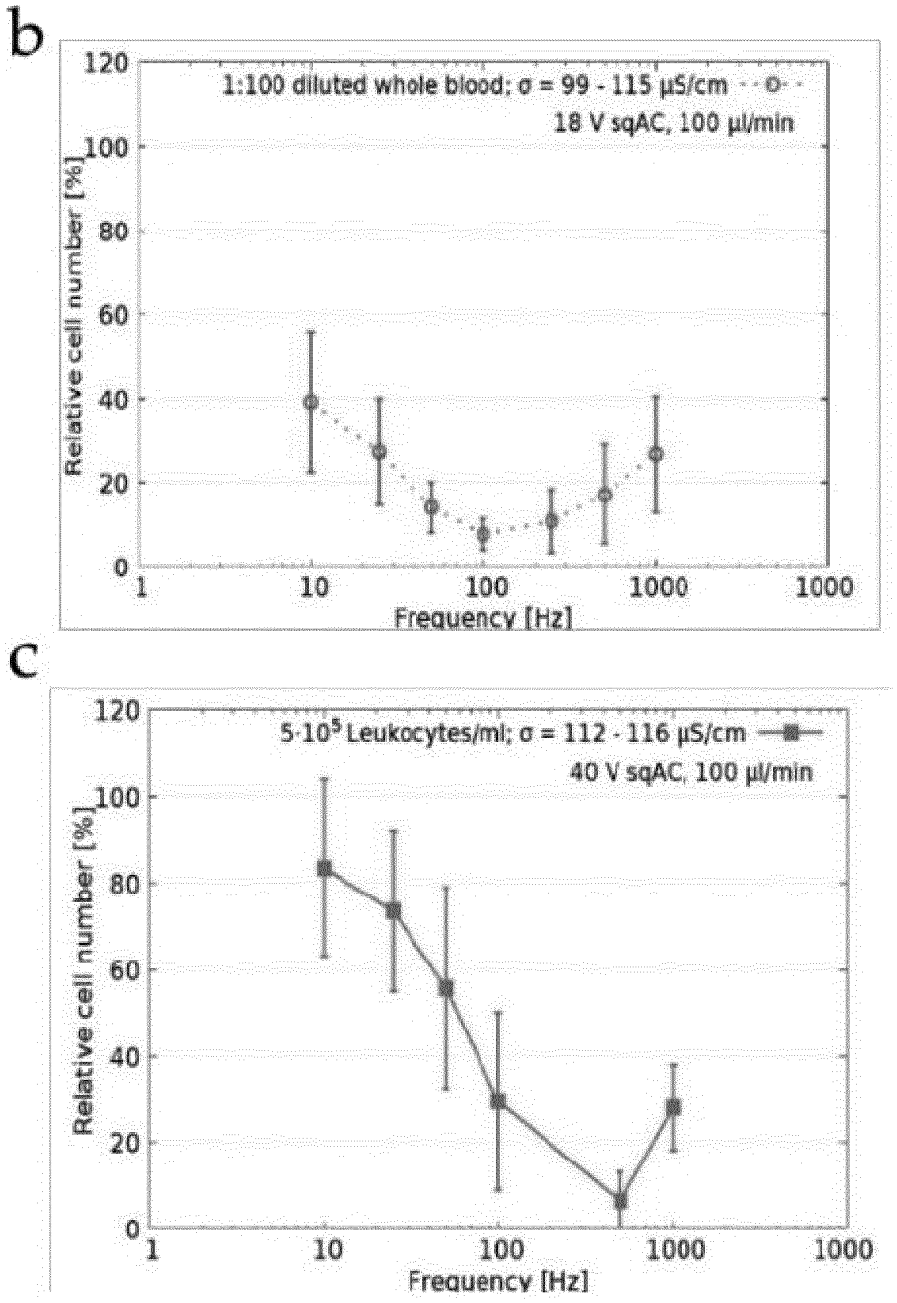

a
Relative cell number [%]
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Voltage [Vp]
5·10⁵ Leukocytes/ml; σ = 103 - 106 µS/cm
Whole blood 1:100; σ = 99 - 119 µS/cm
100 Hz sqAC, 100 µl/min

Fig. 17

Fig. 17


b
1:100 diluted whole blood; σ = 99 - 115 µS/cm
18 V sqAC, 100 µl/min
Relative cell number [%]
Frequency [Hz]
c
5·10⁵ Leukocytes/ml; σ = 112 - 116 µS/cm
40 V sqAC, 100 µl/min
Relative cell number [%]
Frequency [Hz]

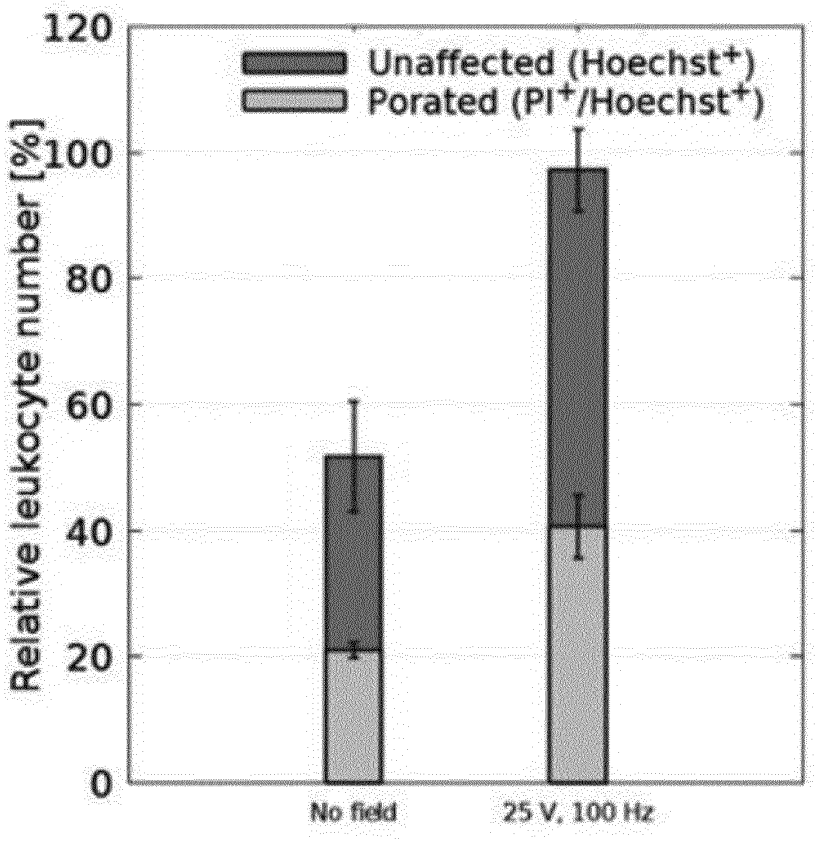

120
100
80
60
40
20
0
Relative leukocyte number [%]
Unaffected (Hoechst+)
Porated (PI+/Hoechst+)
No field
25 V, 100 Hz

Fig. 18

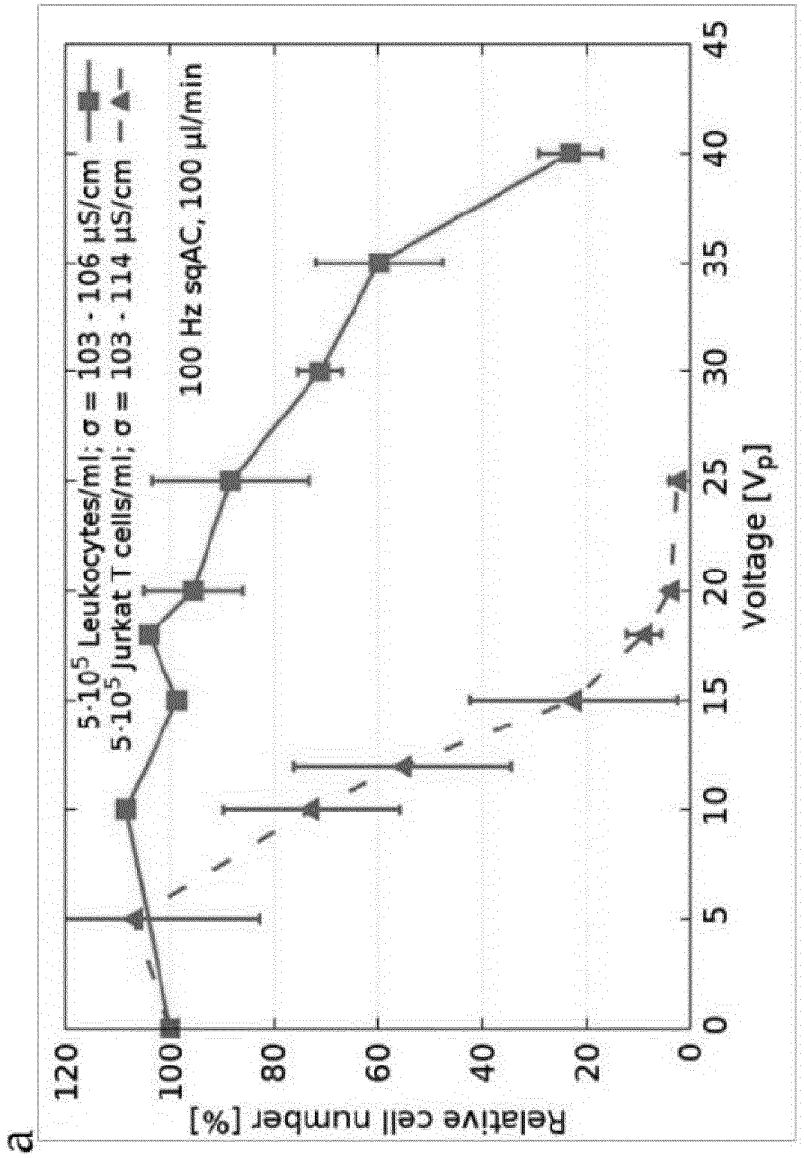
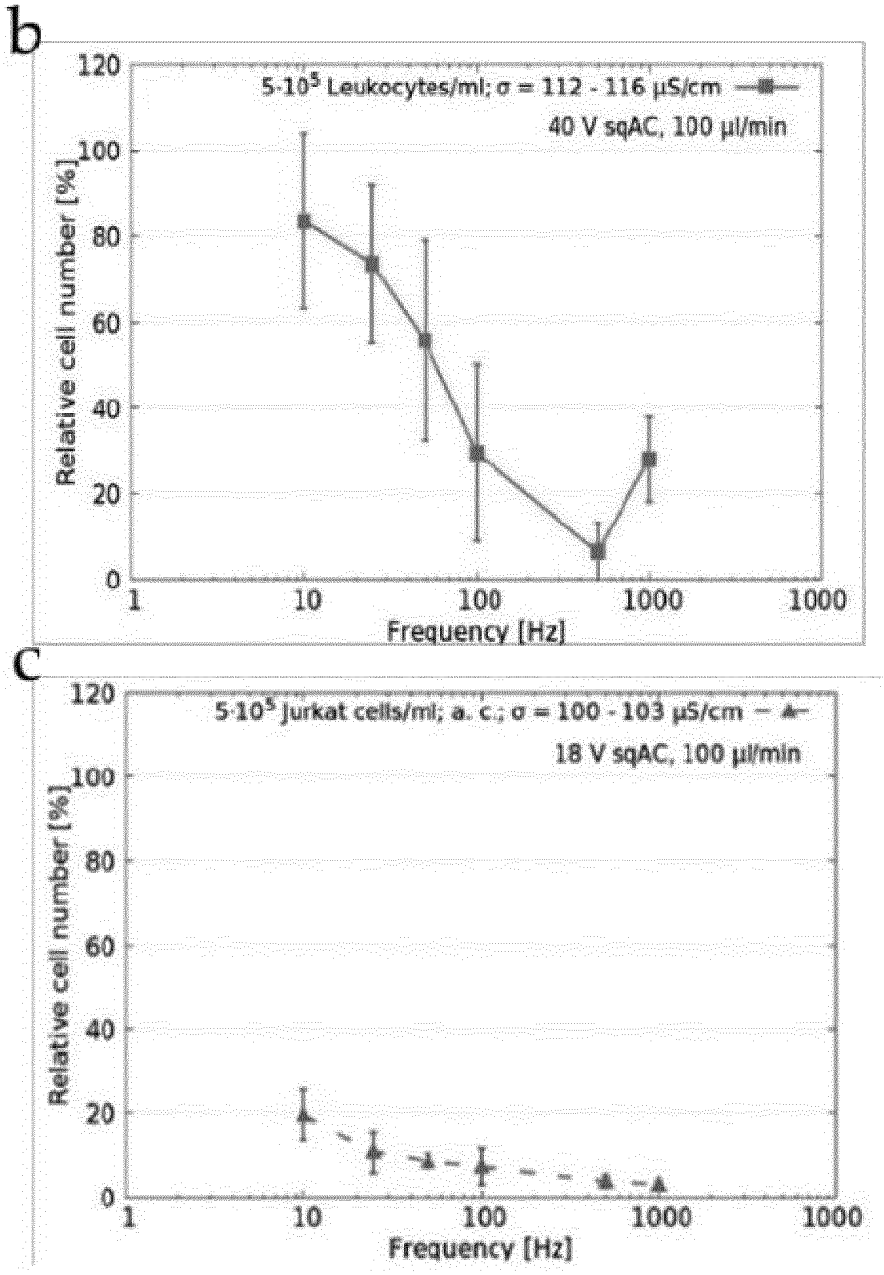

a
Relative cell number [%]
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Voltage [Vp]
5·10^5 Leukocytes/ml; σ = 103 - 106 µS/cm
5·10^5 Jurkat T cells/ml; σ = 103 - 114 µS/cm
100 Hz sqAC, 100 µl/min

Fig. 19

Fig. 19


b
5·10^5 Leukocytes/ml; σ = 112 - 116 µS/cm
40 V sqAC, 100 µl/min
Relative cell number [%]
Frequency [Hz]
c
5·10^5 Jurkat cells/ml; a. c.; σ = 100 - 103 µS/cm
18 V sqAC, 100 µl/min
Relative cell number [%]
Frequency [Hz]

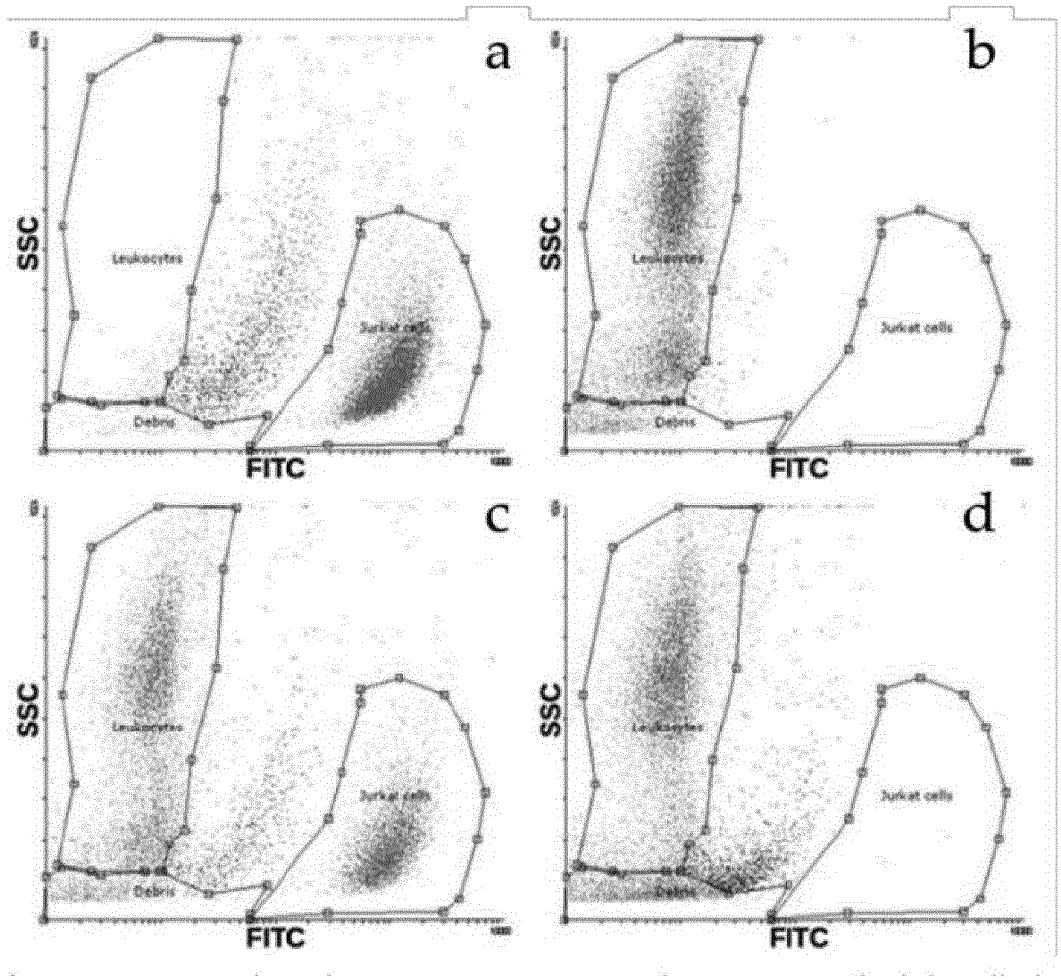


Fig. 20

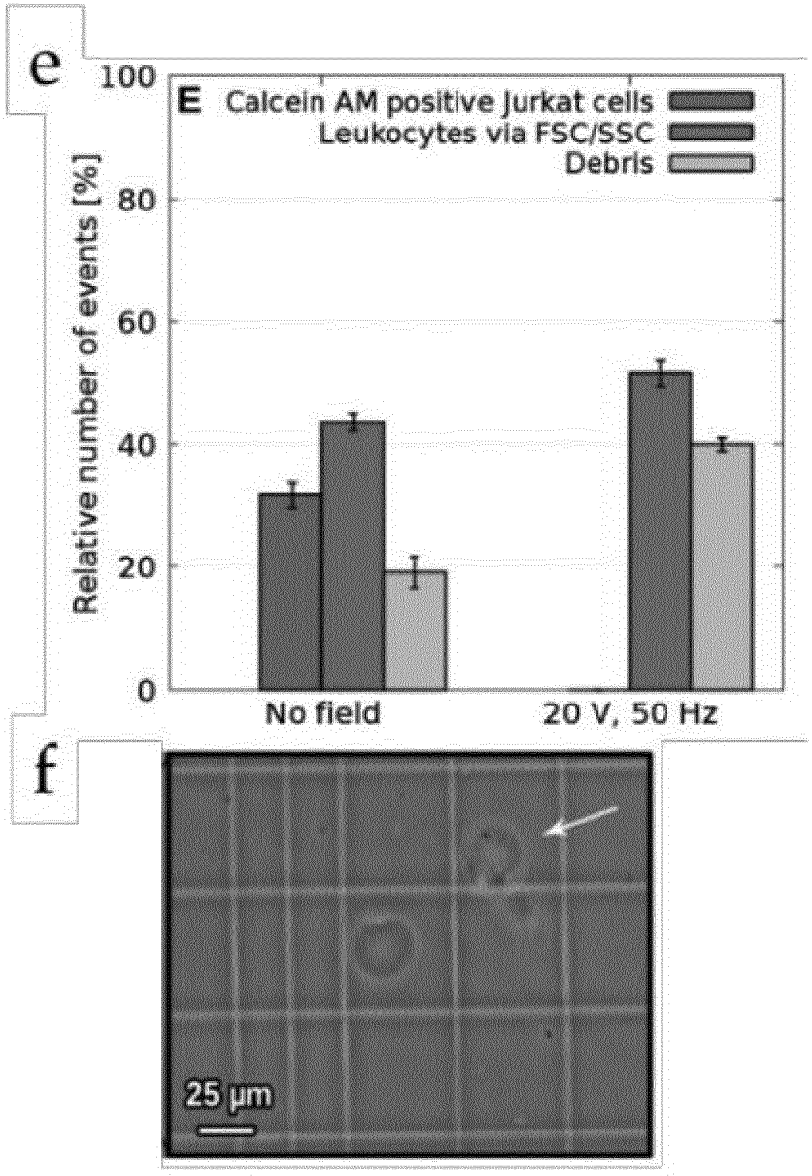

e
E
Calcein AM positive Jurkat cells
Leukocytes via FSC/SSC
Debris
Relative number of events [%]
100
80
60
40
20
0
No field
20 V, 50 Hz
f
25 µm

Fig. 20

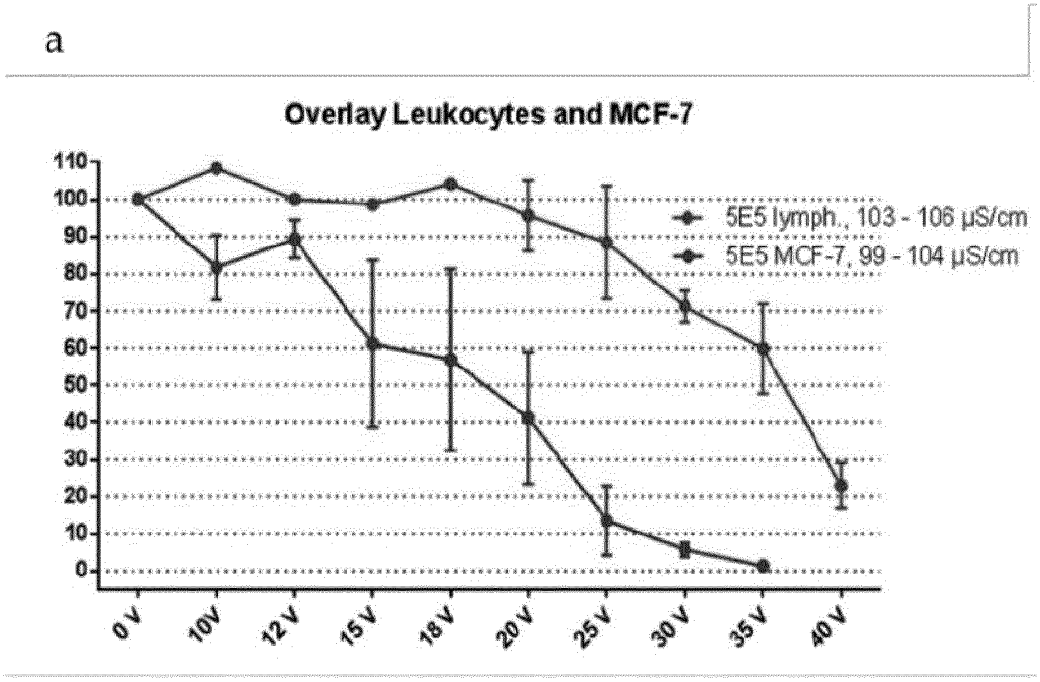
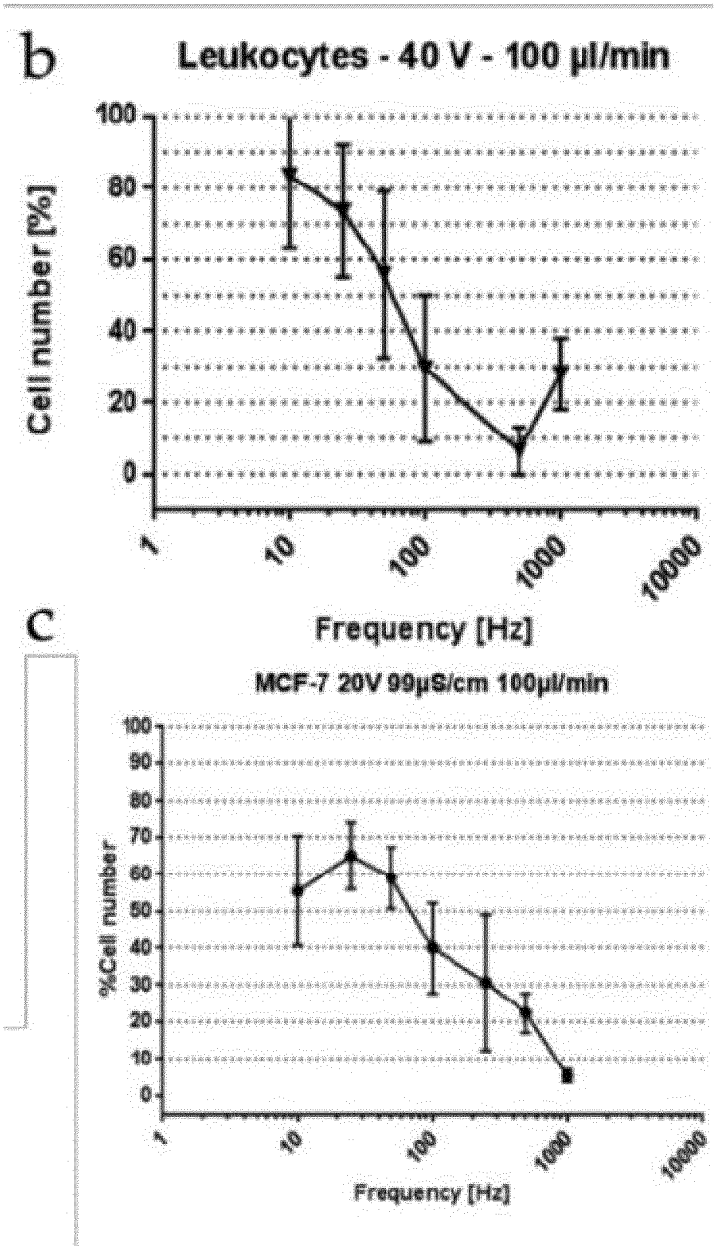

a
Overlay Leukocytes and MCF-7
110
100
90
80
70
60
50
40
30
20
10
0
0 V
10V
12 V
15 V
18 V
20 V
25 V
30 V
35 V
40 V
5E5 lymph., 103 - 106 µS/cm
5E5 MCF-7, 99 - 104 µS/cm

Fig. 21

Fig. 21


b
Leukocytes - 40 V - 100 µl/min
Cell number [%]
100
80
60
40
20
0
1
10
100
1000
10000
Frequency [Hz]
c
MCF-7 20V 99µS/cm 100µl/min
%Cell number
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
10000
Frequency [Hz]

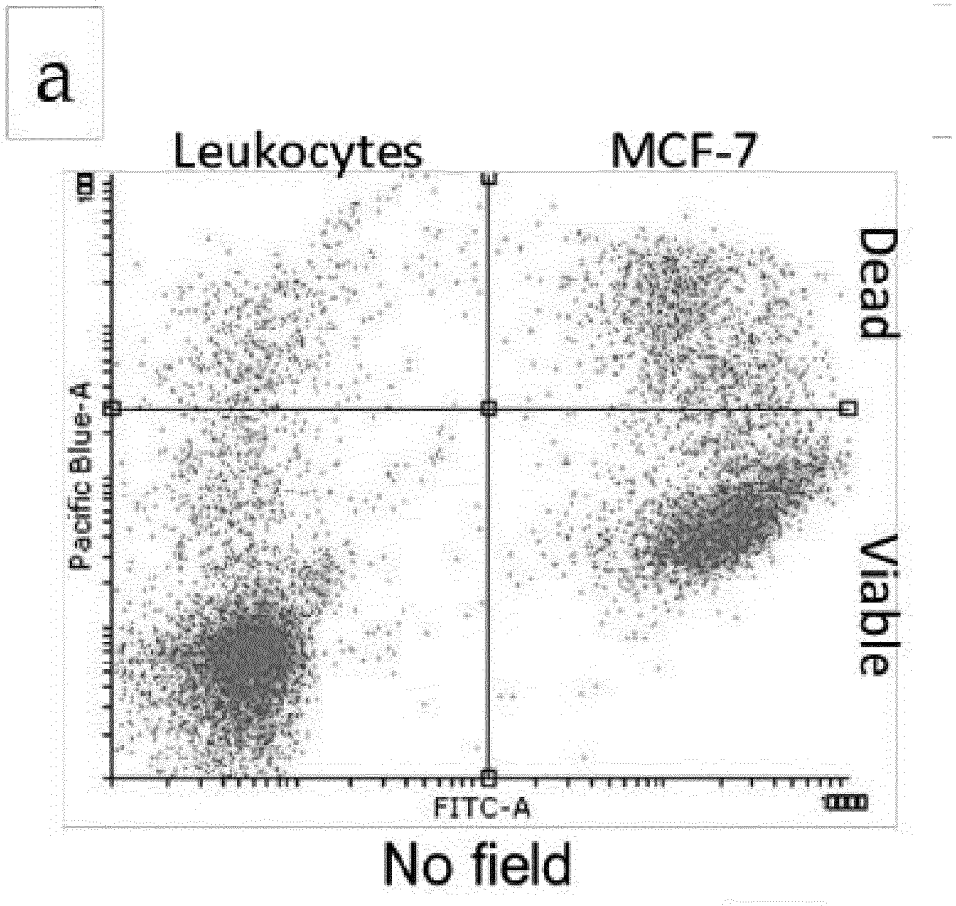


Fig. 22

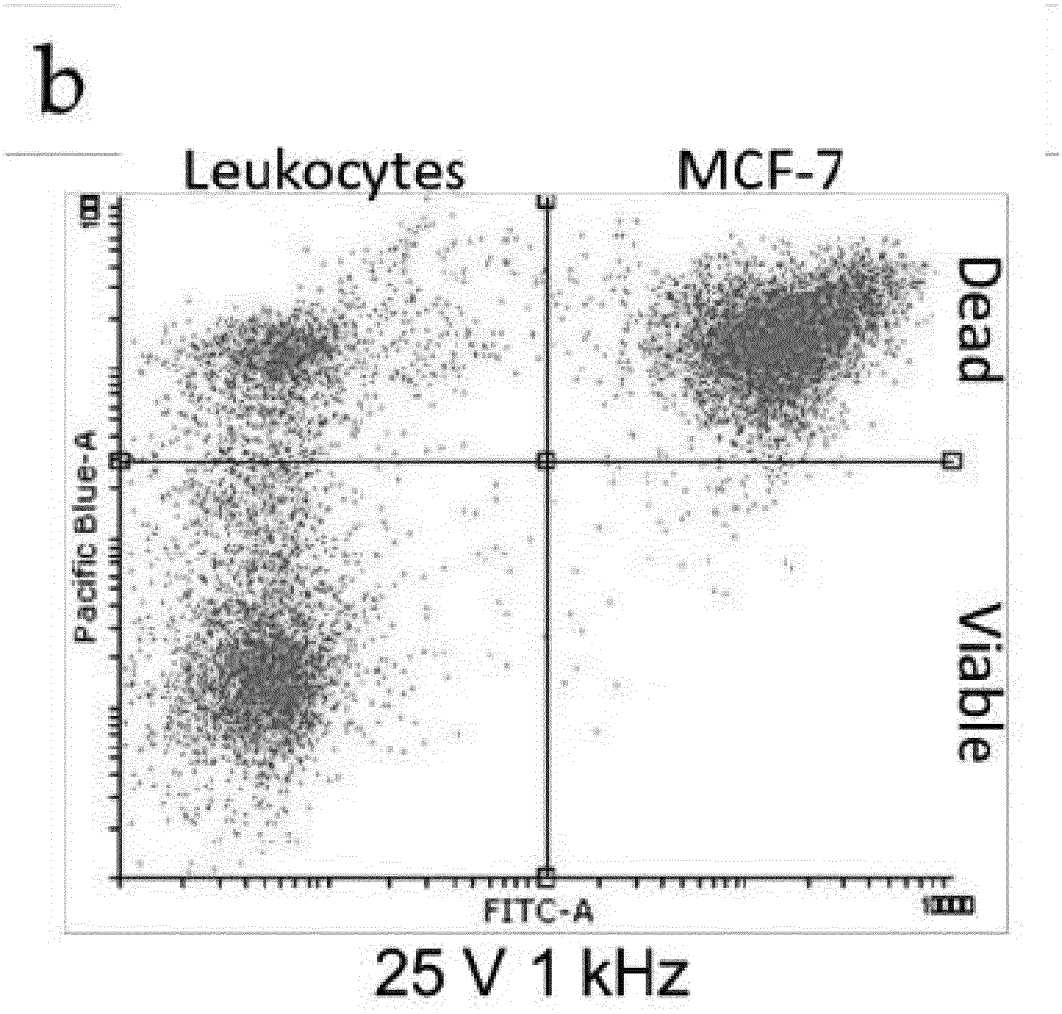


Fig. 22

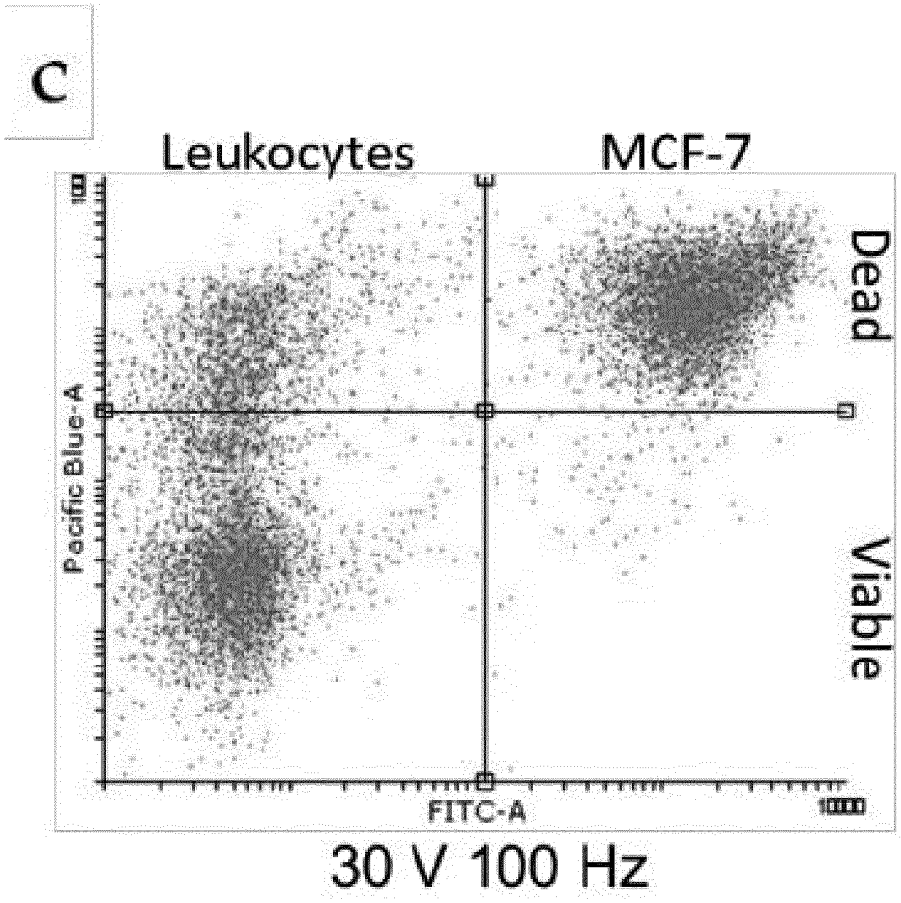

C
Leukocytes
MCF-7
Dead
Viable
Pacific Blue-A
FITC-A
30 V 100 Hz

Fig. 22

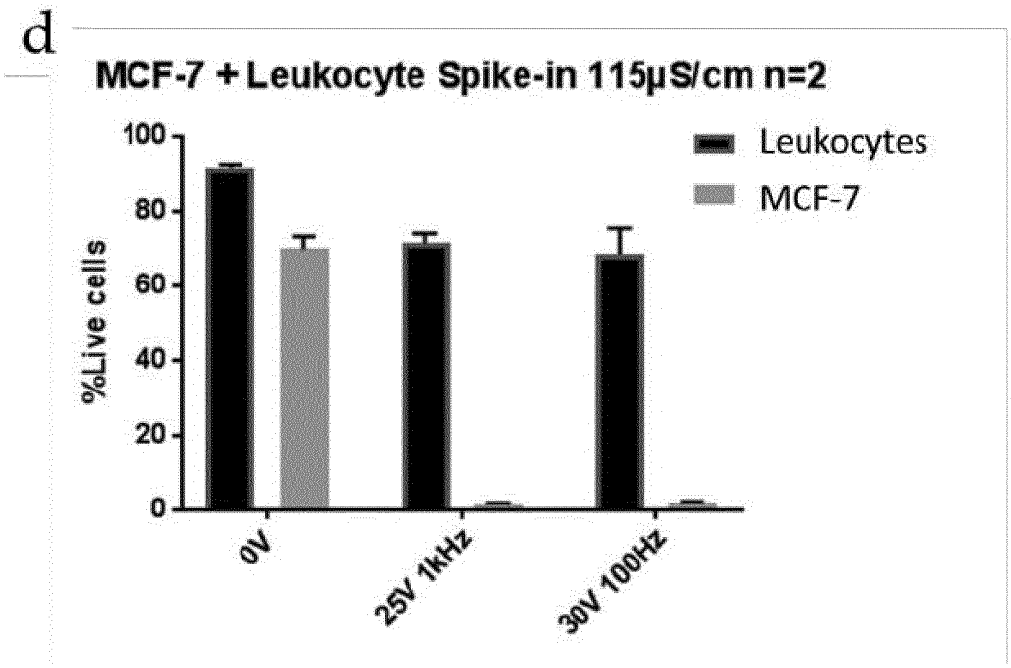

d
MCF-7 + Leukocyte Spike-in 115µS/cm n=2
Leukocytes
MCF-7
%Live cells
100
80
60
40
20
0
0V
25V 1kHz
30V 100Hz

Fig. 22

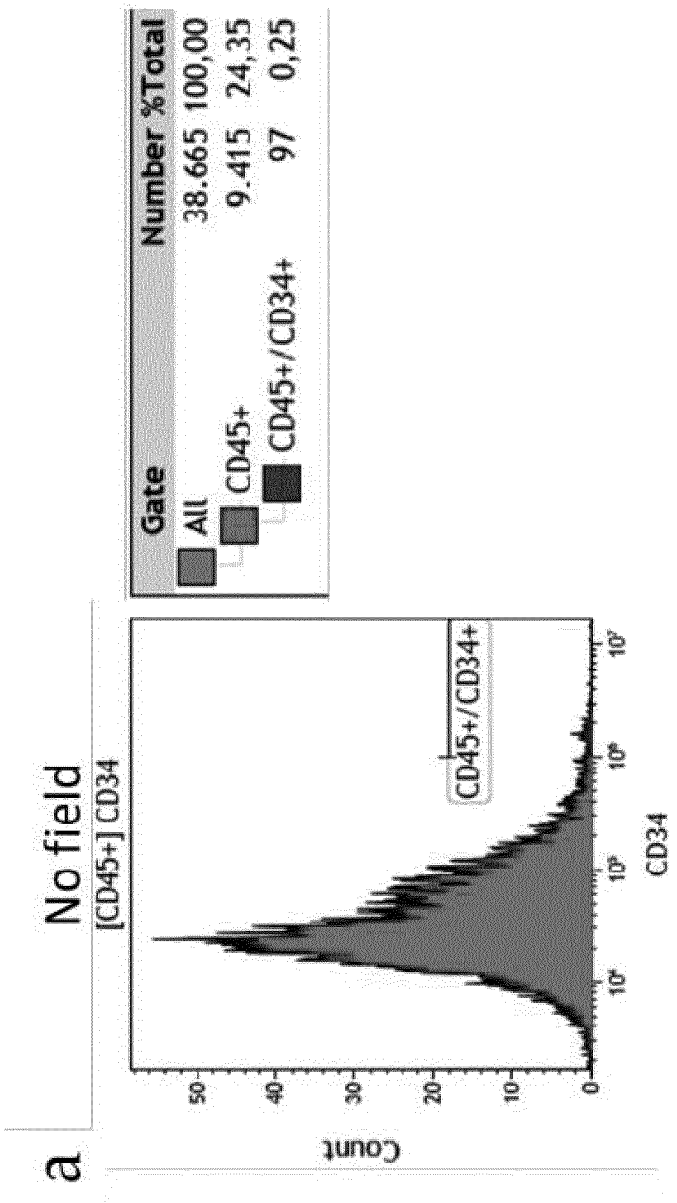

a
No field
[CD45+] CD34
Count
CD34
CD45+/CD34+
Gate
Number %Total
All 38.665 100,00
CD45+ 9.415 24,35
CD45+/CD34+ 97 0,25

Fig. 23

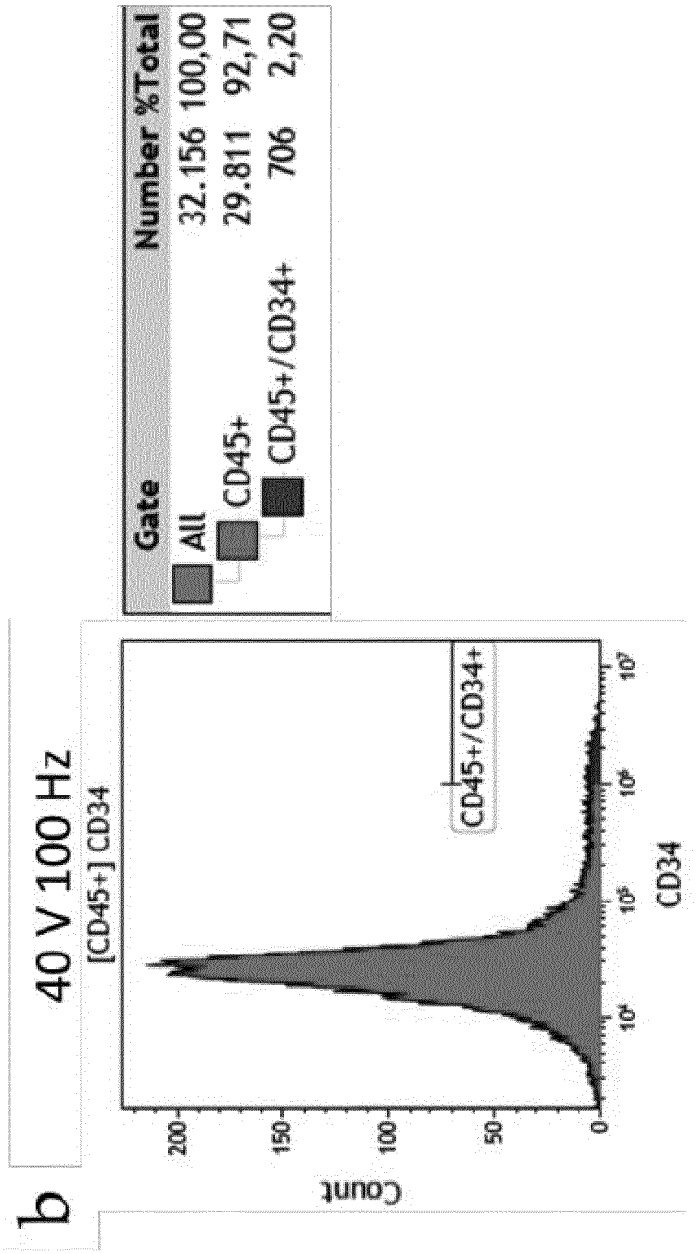

b
40 V 100 Hz
[CD45+] CD34
Count
CD34
CD45+/CD34+
Gate
Number %Total
All 32.156 100,00
CD45+ 29.811 92,71
CD45+/CD34+ 706 2,20

Fig. 23

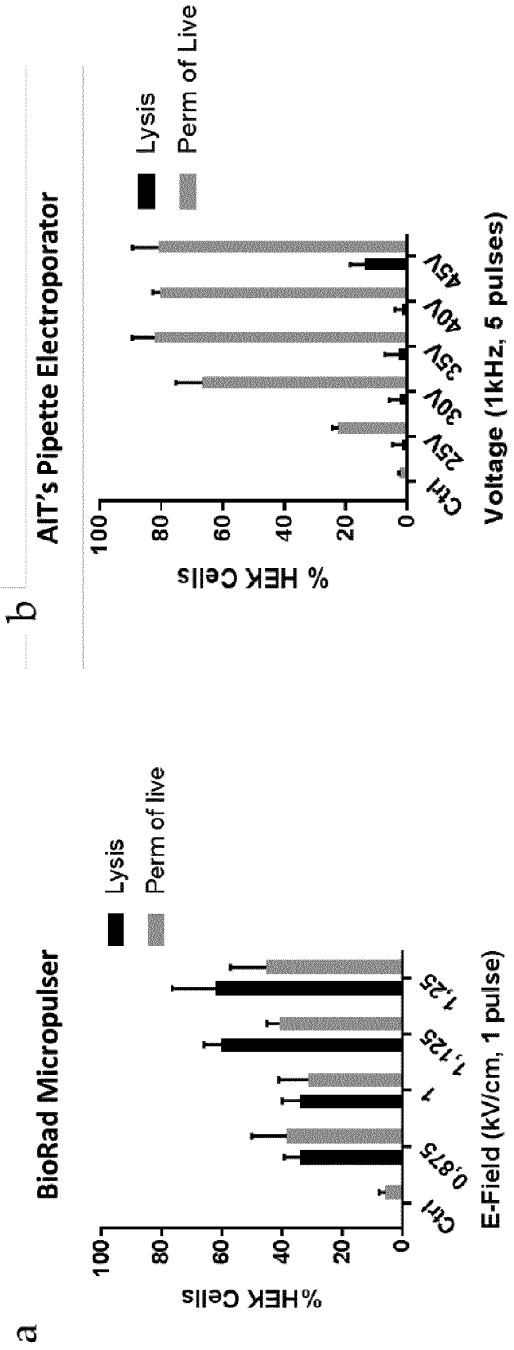
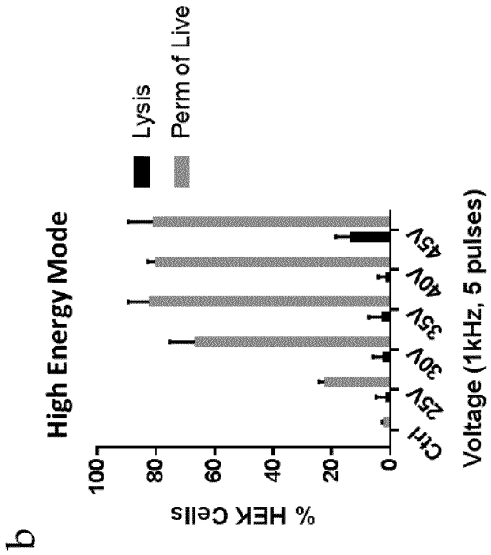

a
BioRad Micropulser
Lysis
Perm of live
%HEK Cells
100
80
60
40
20
0
Ctrl
0,875
1
1,125
1,25
E-Field (kV/cm, 1 pulse)
b
AIT's Pipette Electroporator
Lysis
Perm of Live
% HEK Cells
100
80
60
40
20
0
Ctrl
25V
30V
35V
40V
45V
Voltage (1kHz, 5 pulses)

Fig. 24


b
High Energy Mode
Lysis
Perm of Live
% HEK Cells
100
80
60
40
20
0
Ctrl
25V
30V
35V
40V
45V
Voltage (1kHz, 5 pulses)

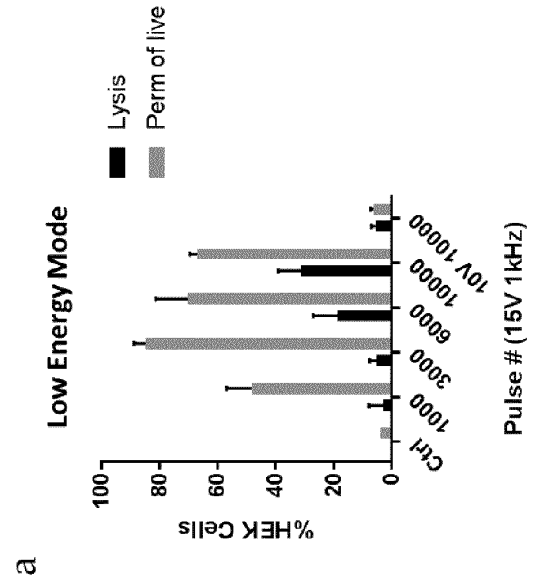
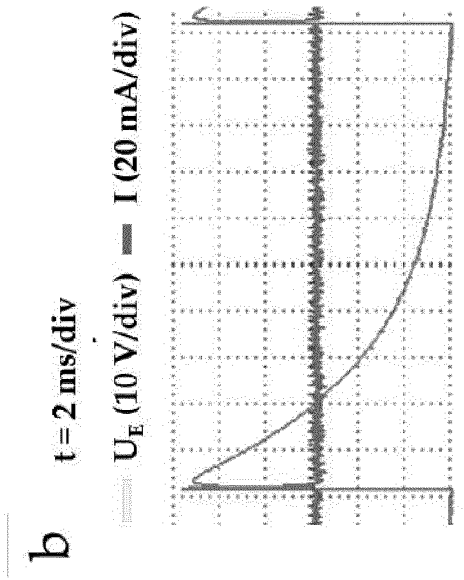

a
Low Energy Mode
Lysis
Perm of live
%HEK Cells
100
80
60
40
20
0
Ctrl
1000
3000
6000
10000
10V 10000
Pulse # (15V 1kHz)

Fig. 25



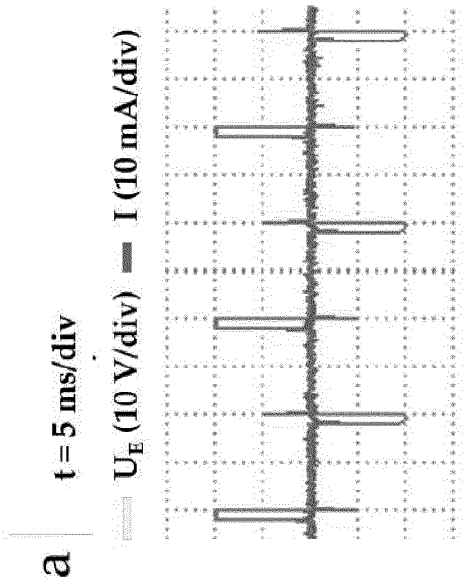


Fig. 26

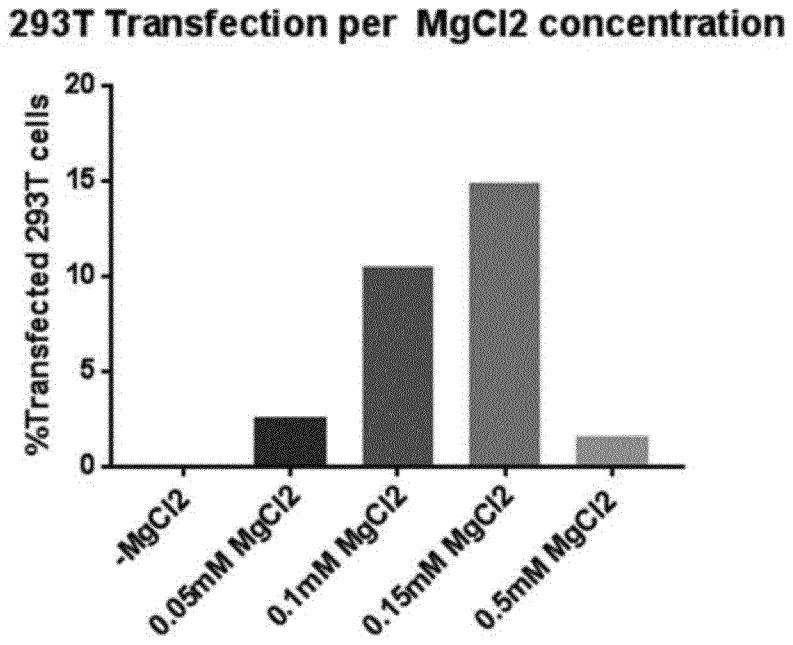

293T Transfection per MgCl2 concentration
%Transfected 293T cells
20
15
10
5
0
-MgCl2
0.05mM MgCl2
0.1mM MgCl2
0.15mM MgCl2
0.5mM MgCl2

Fig. 27

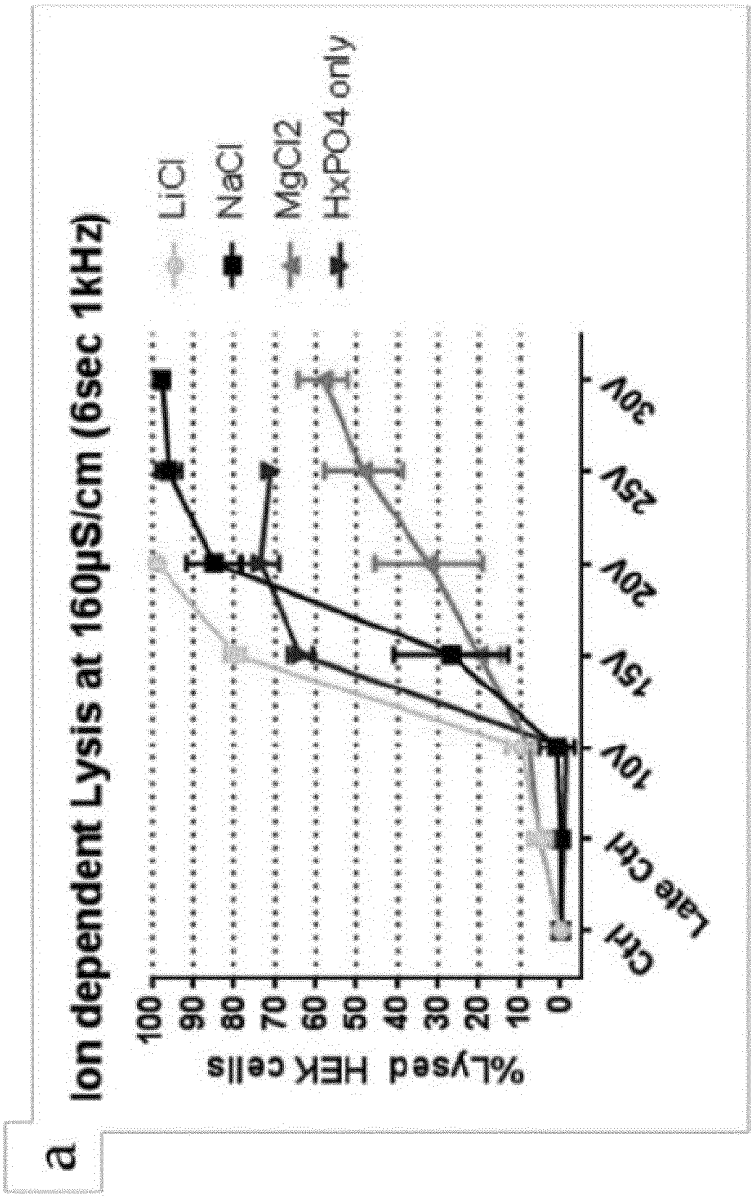

a
Ion dependent Lysis at 160µS/cm (6sec 1kHz)
LiCl
NaCl
MgCl2
HxPO4 only
%Lysed HEK cells
100
90
80
70
60
50
40
30
20
10
0
Ctrl
Late Ctrl
10V
15V
20V
25V
30V

Fig. 28

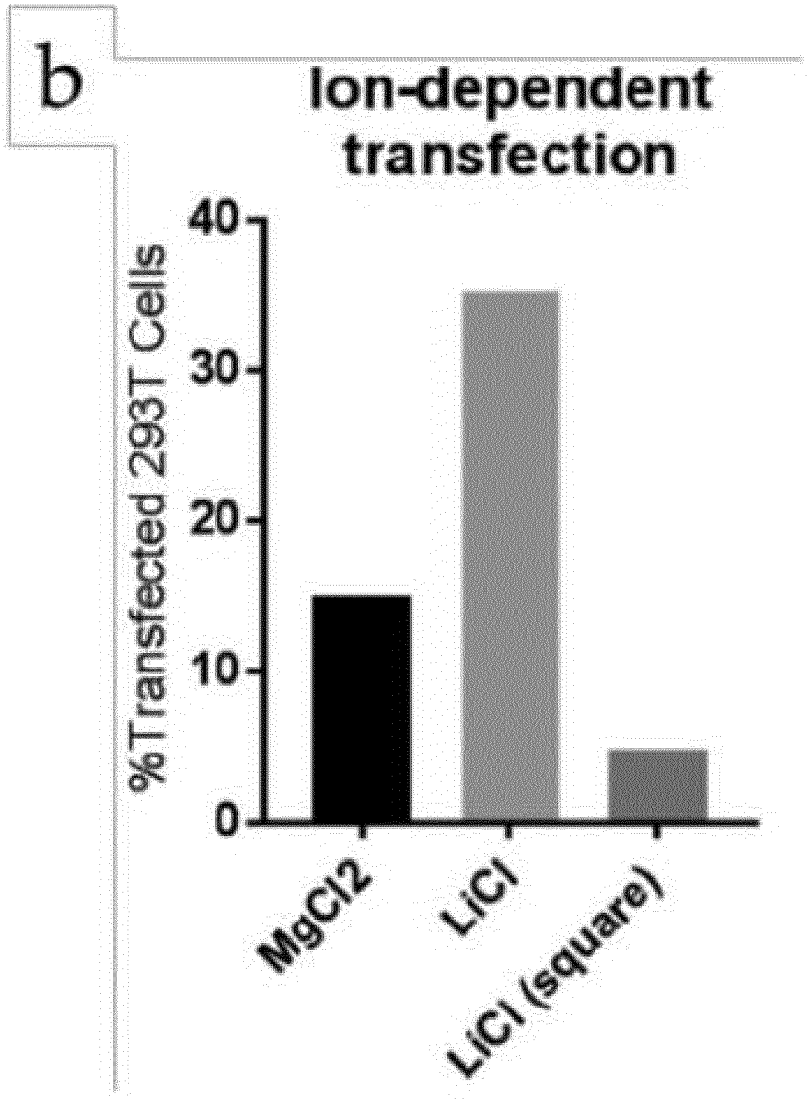

b
Ion-dependent transfection
% Transfected 293T Cells
40
30
20
10
0
MgCl2
LiCl
LiCl (square)

Fig. 28

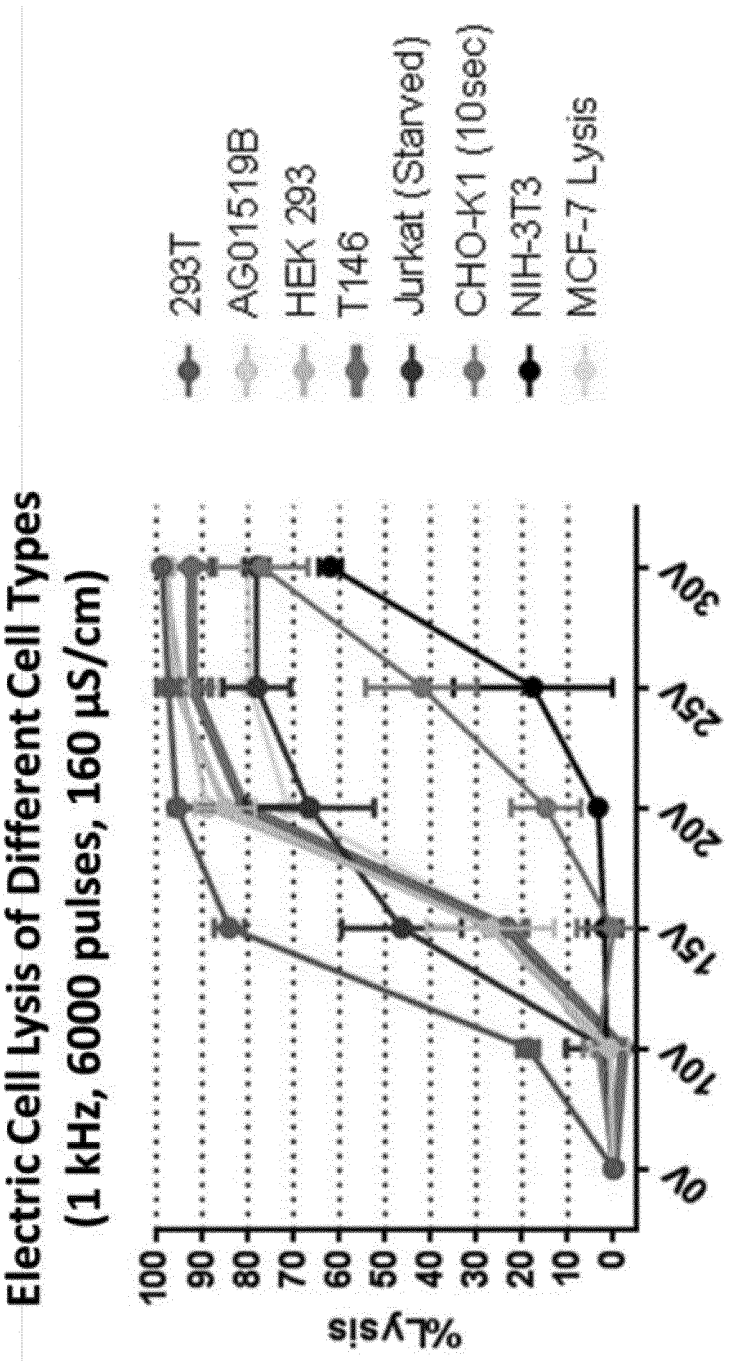

Electric Cell Lysis of Different Cell Types
(1 kHz, 6000 pulses, 160 µS/cm)
293T
AG01519B
HEK 293
T146
Jurkat (Starved)
CHO-K1 (10sec)
NIH-3T3
MCF-7 Lysis
%Lysis
100
90
80
70
60
50
40
30
20
10
0
0V
10V
15V
20V
25V
30V

Fig. 29

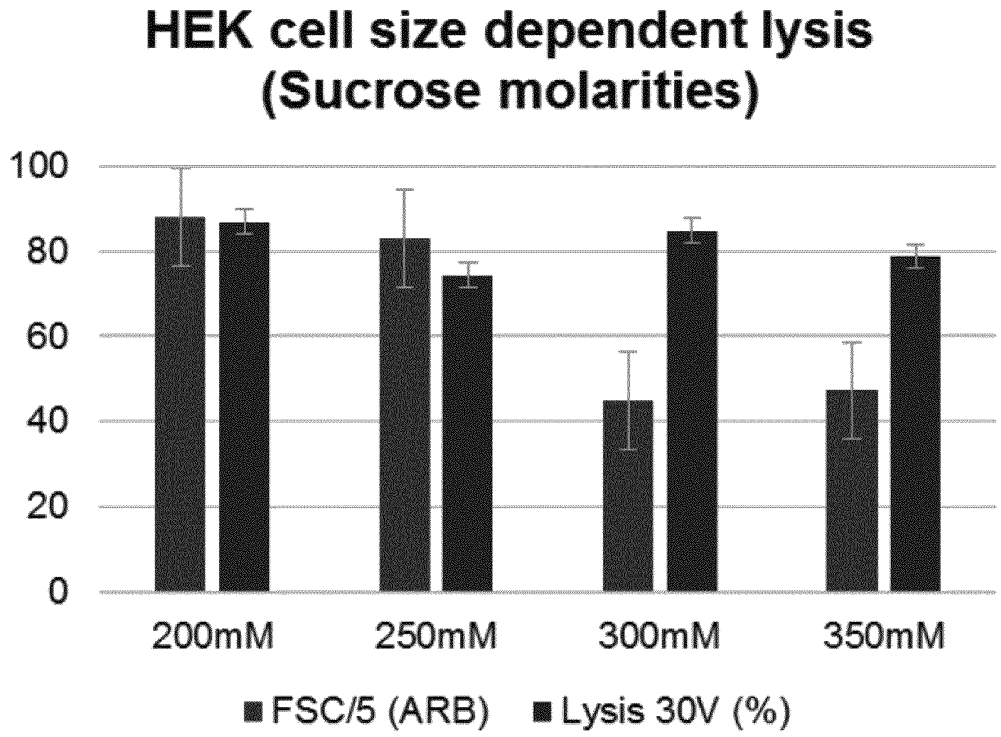

HEK cell size dependent lysis
(Sucrose molarities)
100
80
60
40
20
0
200mM
250mM
300mM
350mM
FSC/5 (ARB)
Lysis 30V (%)

Fig. 30

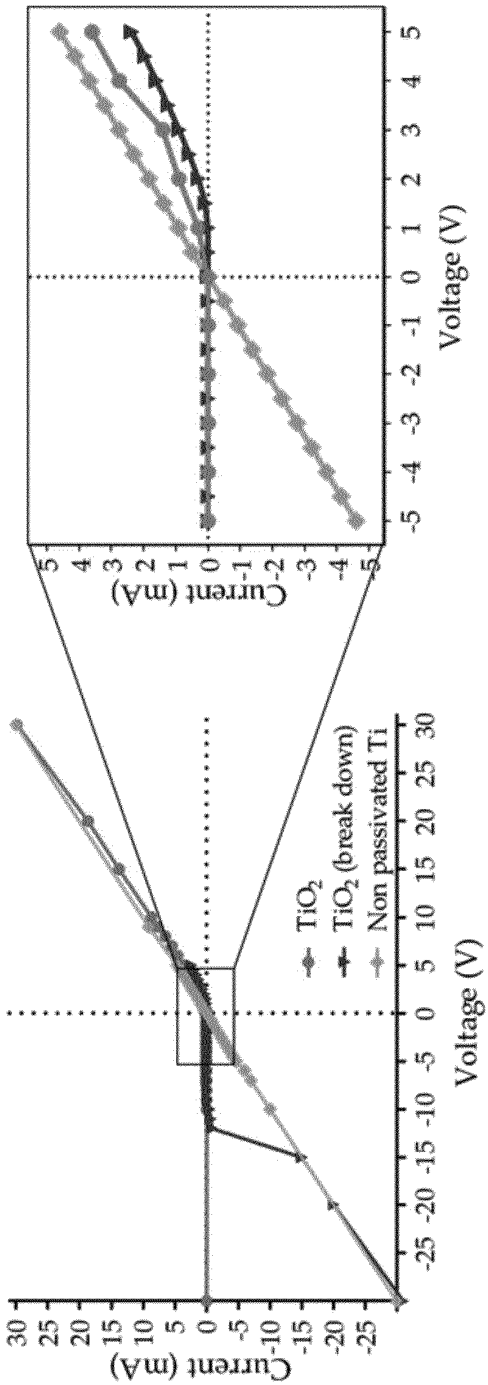

Current (mA)
Voltage (V)
TiO2
TiO2 (break down)
Non passivated Ti
Current (mA)
Voltage (V)

Fig. 31

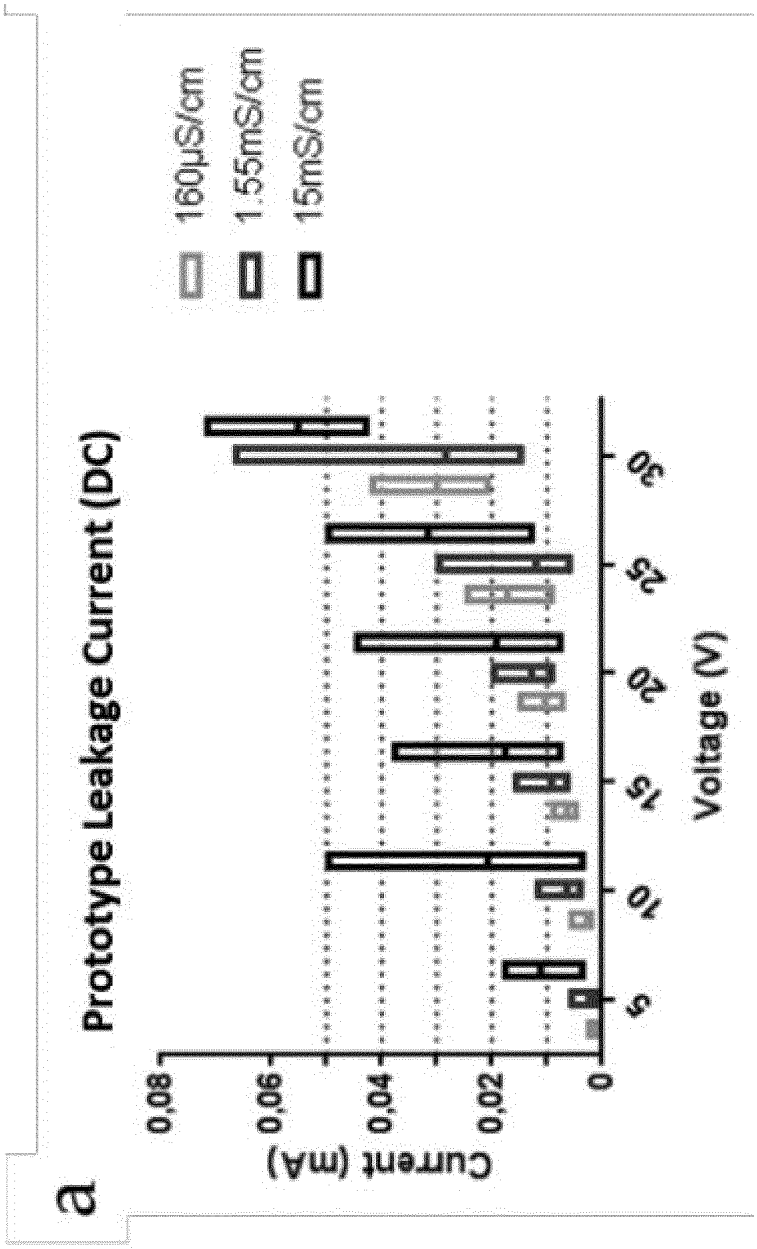

a
Prototype Leakage Current (DC)
160µS/cm
1.55mS/cm
15mS/cm
Current (mA)
0,08
0,06
0,04
0,02
0
5
10
15
20
25
30
Voltage (V)

Fig. 32

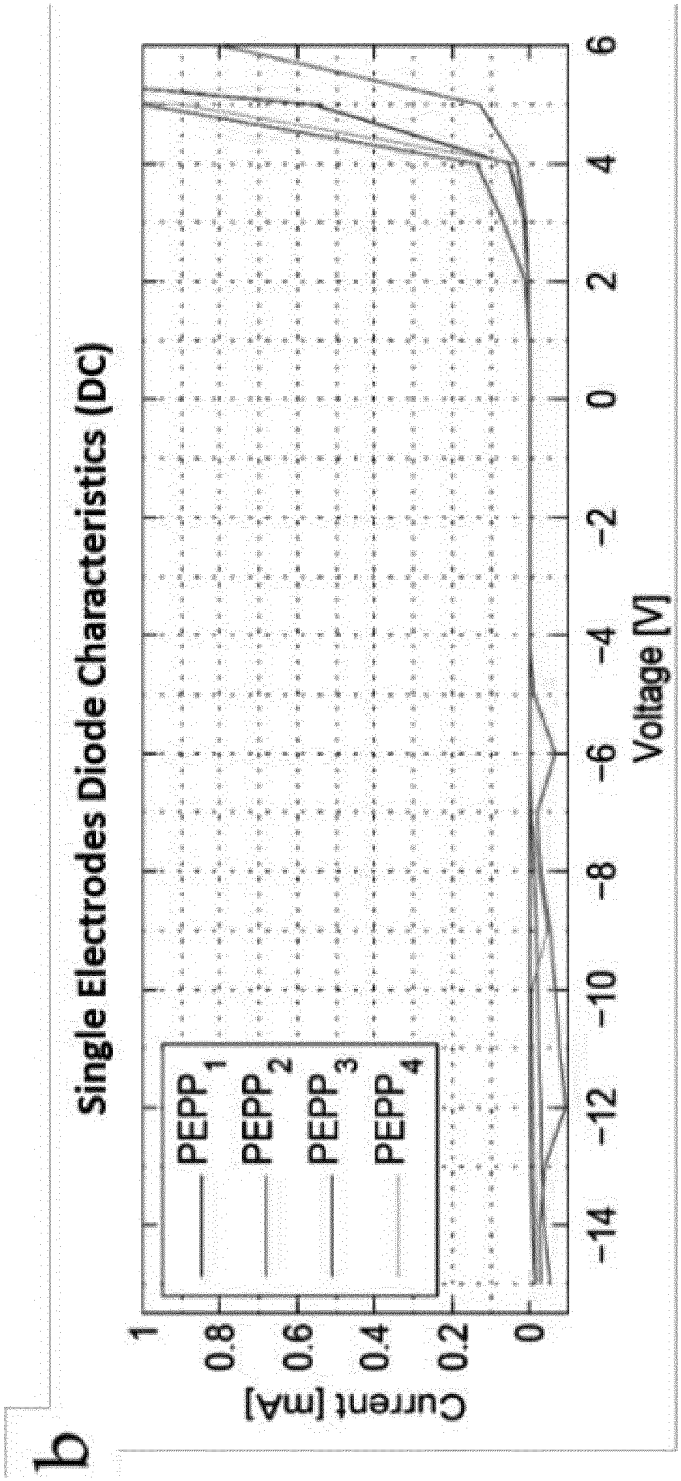

b
Single Electrodes Diode Characteristics (DC)
Current [mA]
Voltage [V]
PEPP1
PEPP2
PEPP3
PEPP4
1
0.8
0.6
0.4
0.2
0
6
4
2
0
−2
−4
−6
−8
−10
−12
−14

Fig. 32