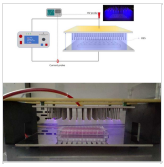

May 04, 2021

LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells

Forked from LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells

Almha Gilheany[1], Sebnem Gunes[1], Laurence Scally[1], Patrick J Cullen[2], Brijesh Tiwari[3], James F Curtin[1], Andressa Maria Aguiar de Carvalho[1]

[1]Technological University Dublin; [2]University of Sydney; [3]Teagasc

1 Works for me dx.doi.org/10.17504/protocols.io.buqznvx6

Technological University Dublin

James Curtin
Technological University Dublin

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

ABSTRACT

Plasma can be described as the fourth state of matter, and as a neutral ionised gas composed of neutral and charged particles, along with electrons, UV-radiation, radicals and electric fields. CAP is generated at temperatures below 40 °C and at near atmospheric pressures rendering it appropriate for use in biomedical applications such as an anti-cancer tool, that has already shown its cytotoxicity effects, selectivity potential and efficacy as a Glioblastoma (GBM) therapy.
How CAP interacts with cells depends on many chemical and physical factors, with CAP activity varying with differing plasma discharges, cell types and culture conditions. Physical factors such as UV and heat have been shown to have had little effect on DNA integrity. Chemical factors are reported to have a more substantial effect due to the production of reactive oxygen species during CAP treatment. Characterising a new device is important as different plasma discharge modes arising from plasma-self adaptation leads to different interactions between cells and plasma.

The novel device seen here in Figure 1, is a large Pin-to-Plate non-thermal atmospheric plasma generator. It consists of 88 pin electrodes which are powered by AC Supply (Leap100, PlasmaLeap Technologies, Dublin, Ireland) and has a resonance frequency of 30 to 125 kHz, a discharge frequency of 50 to 3000 Hz with a power range of 50 to 400 W and a discharge gap of a maximum of 55 mm. The electrodes/samples work area is encased in a fitted container to minimize the escape of Cold Atmospheric Plasma generated reactive species into the environment.
The position of pins and a plate electrode acts as a ground, reduces power consumption, increases stability and diffuses the discharge across the entire surface. The discharge has also been seen to diffuse to a larger area than just the pin tip meaning more interactions between reactive species and cells can occur.
This protocol describes the steps involved in assessing the cytotoxic effects of a novel large Pin-to-Plate non-thermal atmospheric plasma on mammalian cells.

PURPOSE: Step-by-step instructions for the use of the Pin-to-Plate device, non-thermal atmospheric plasma, for the treatment of mammalian cells.

SCOPE: To provide a standardized protocol for using the Pin-to-Plate device to generate Cold Atmospheric Plasma to treat mammalian cells for use by any laboratory using the Leap100 system device.

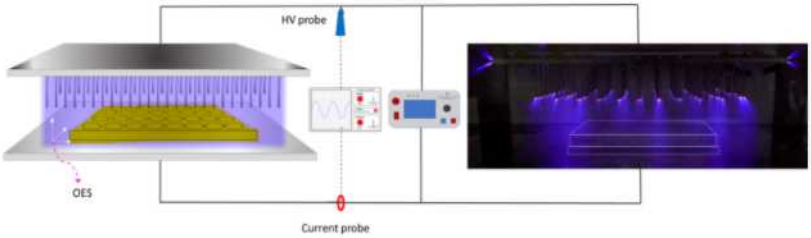

Figure 1: Schematic and photograph of the pin reactor, including a sample well plate (orange) in the discharge gap. Left: Schematic of the pin reactor. Right: Photograph of the atmospheric air discharge. (Scally et al, 2021)

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/2021.01.08.425903v1

DOI

dx.doi.org/10.17504/protocols.io.buqznvx6

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/2021.01.08.425903v1

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

PROTOCOL CITATION

Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho 2021. LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.buqznvx6

FORK NOTE

FORK FROM

Forked from LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells, Almha Gilheany

KEYWORDS

Pin-To-Plate Device, Non-thermal Atmospheric Plasma, Cold Atmospheric Plasma, Pin Electrode Reactor

IMAGE ATTRIBUTION

< Aguiar de Carvalho, A., Behan, S., Scally, L., Sarangapani, C., Malone, R., Cullen, P., Tiwari, B. and Curtin, J., 2021. Pin Electrode Reactor: A novel cold atmospheric plasma device and its potential in glioblastoma treatment. bioRxiv 2021.01.08.425903;> <Leap 100 User Manual, Plasma Leap Technologies, Version L2.0, 2018. >

CREATED

May 04, 2021

LAST MODIFIED

May 04, 2021

PROTOCOL INTEGER ID

49657

GUIDELINES

Considerations:

ENSURE CONTAINMENT BOX IS PLACED FIRMLY ON DEVICE. Cell viability is dependent on the containment of the plasma during treatment. In the absence of containment, no cytotoxicity will be observed.

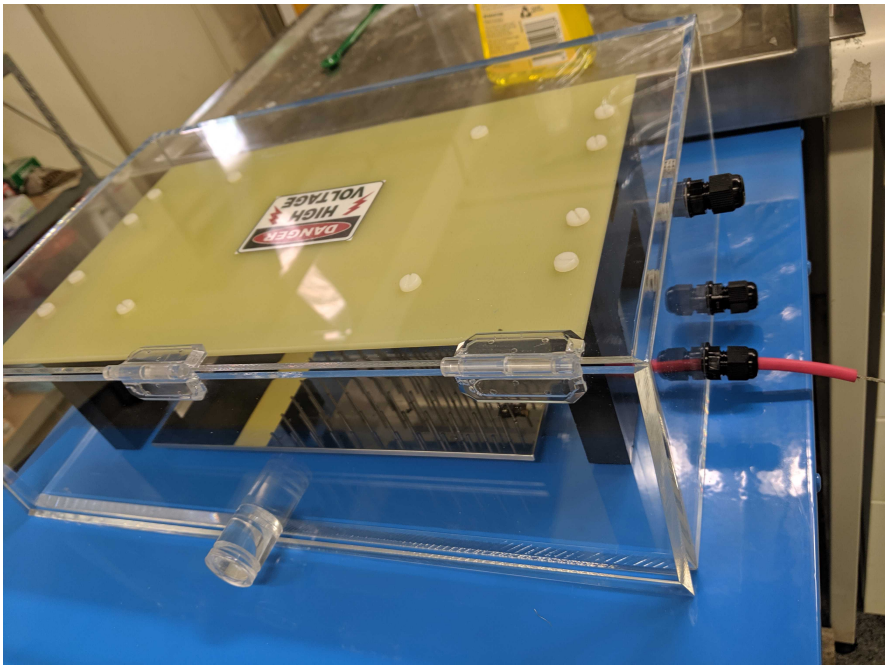

Figure 11: LEAP100 Pin-to-Plate device with container in place

Studies were carried out to assess the impact of temperature and humidity on the cytotoxicity results. No impact on cytotoxicity was observed.

After treatment, wait 5 seconds before removing the plate from the device.

Ensure that no bubbles form in the wells of the sample well plate to ensure stability during treatment.

Ensure that at least 20 microliters of media is left in the wells before treatment.

MATERIALS TEXT

Equipment: Pin-to-Plate device

Reagents: Dulbecco's modified eagle medium (DMEM) supplemented with 0.11% sodium pyruvate, phosphate buffered saline (PBS), DMEM high glucose in the absence of sodium pyruvate, mammalian cells.

Supplies: 96 well-plate, 60mm cell culturing dish

SAFETY WARNINGS

SAFETY INFORMATION

- The “Plasma power supply unit Leap100” (in text as the PSU) can only be used as laboratory equipment.
- This power supply must be used with caution.
- Only qualified personnel should operate the unit.
- The equipment cannot be used in the case of mechanical or electrical damage of any part of the equipment.
- A properly grounded electrical outlet of correct voltage and current handling capacity must be used.
- Before connecting the unit to the electrical supply, check the supply voltage is within the range stated on the

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

rating label and that the device is earthed.

- Before carrying out maintenance and servicing, disconnect from the power supply.
- Do not use this equipment in the presence of flammable or combustible material.
- **All inductive material which may be near the High Voltage transformer, the High Voltage wire or the electrodes must be grounded.**
- **Once the output of the PSU has been activated, do not touch the electrodes, HV transformer or HV wire.**
- **Keep a 30cm minimum distance from the high voltage parts (HV transformer, HV wire, electrodes) when the output of the PSU is activated.**
- **Switch OFF the PSU and discharge energy from all HV parts by connecting to ground before touching the electrodes.**
- **WARNING! This equipment can be harmful to pacemaker wearers!**
- **Operate under vented conditions or a fume hood if generating a plasma discharge in open atmospheric conditions.**

BEFORE STARTING

Before starting the system for the first time, ensure that all parts of the system/device are free from mechanical damage.

Installation and Connection of Pin-to-Plate Device

1 Install the Electrodes: Pin Electrodes

1.1 Connect the bottom flat electrode to the ground. The system will not work correctly without this connection.

1.2 Using the High Voltage (HV) wire, connect the bottom electrode to the blue connector of the HV transformer.

1.3 Place the bottom electrode on a flat surface.

1.4 Using the HV wire connect the TOP electrode to the red connector of the HV transformer.

1.5 HV wires must not come into contact with each other and the HV wire connected to the top electrode must not connect to any conductive material. The distance between these wires and the conductive material must be more than 70 mm.

1.6 Place the top electrode over the bottom electrode and check that both metal plates of the electrodes are parallel.

2 Install the HV Transformer

2.1 Remove the black plug from the top of the transformer and replace it with the orange air ventilation

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

plug. The black plug is used only in transport.

2.2 Connect the HV cable from the top electrode to the red connector (see picture below) at the HV transformer.

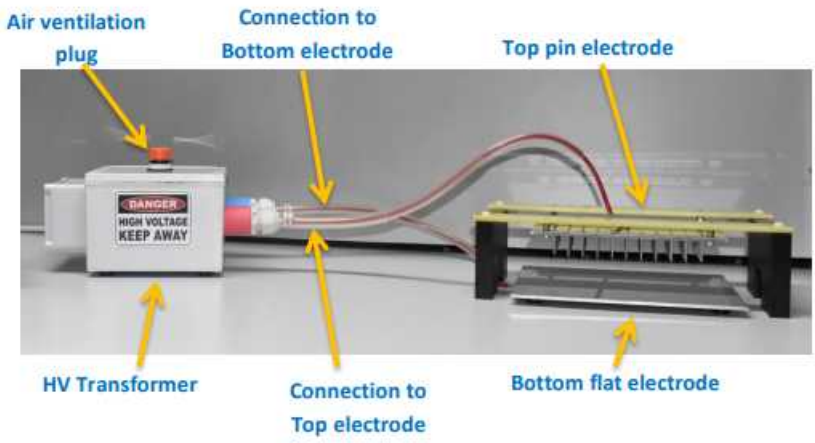

Figure 2: The correct set-up for connecting the HV transformer to electrodes (Leap100 User Manual)

2.3 Connect the HV cable from the bottom electrode to the blue connector.

2.4 Connect the input cable to the power supply unit (output).

3 Install Power Supply Unit (PSU)

3.1 Connect the cable from the HV transformer to the “OUTPUT” connector.

3.2 Check the range of input voltage and frequency on the production label and connect the input voltage cable if the power supply line is compatible with the version of PSU.

4 Check all connections are correct as described thus far.

5 Switch ON the main switch.

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

Figure 3: The front panel of the device displaying the different interfaces (Leap100 user manual)

6 The LCD will display the start screen and the version of software used for approximately 4 seconds, the Main Screen is then shown, the STATUS green LED will then slowly flash. If the ERROR red LED is ON, check the position of the Emergency Switch.

7 It is necessary to run an Initial sequence at each power-up or after an emergency stop or after a strong local plasma discharge.

8 If the system status is WAIT INIT, the start initialization sequence can be started by pressing INIT Itb (LCD touch button) and pressing the START button. The Max time between pressing the INIT Itb and the START button is approximately 8 seconds. INIT Itb will flash during this time.

9 Initialization will take around 3 seconds. If initialization is successful, the STATUS green LED is ON and the System Status is READY.

10 To display the RUN screen press RUN Itb. Before the first start, set low values for the Main parameters. For Voltage (about 100-150), Duty (30-75), Frequency Discharge (300-500) and Timer (000).

11 Check that all safety rules which are described in the Safety Information section are followed.

12 The output of PSU is activated by pressing START Itb (LCD touch button) and pressing the START mechanical button within 8 seconds.

13 If the system is running, the blue RUN LED is ON and the Status is Running.

14 To deactivate the output of the PSU, press the mechanical red STOP button or press the STOP LCD touch button. In the case of an emergency, the system can be stopped by pressing the EMERGENCY STOP button. This button should only

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

be used in an emergency.

Set the Desired Parameters

15 Set parameters and variables seen below.

Parameters:
(a) Time (Sec): Range of 0-900, this is used to set the timer in seconds of how long after starting the system the output will be activated. When this value is 000, the timer is not in use and the output is activated until the stop button is pressed.
(b) Voltage (V): Range of 500-325, this is the Output voltage of the PSU. It is the input value for the HV transformer circuit and is used to set requested plasma discharge.
(c) Duty cycle (µS): Range of 25 steps. This is the time in microseconds during which the energy from the PSU is transferred to the HV transformer resonance circuit. It is related to the resonance frequency.
(d) Frequency discharge (Hz): Range of 100-3000Hz. The discharge frequency is the time set for repeating discharge.
(e) Frequency resonance (kHz): Range of 30-125kHz. This is the resonance frequency of the resonance circuit. This value is set based on the configuration of the HV transformer and electrodes. It is not normally necessary to change this value when only one type of discharge electrode is being used.
(f) D.Time (µS): Range of 0.6-1.6. The default value is 0.800 µS. This value is the dead time between switching the top and bottom transistor of H bridge. It is only for internal use. This value will not influence the chemistry of the plasma and should be set to the default value at all times.
(g) P.shift: Range of 0-100. The default value is 100. This value is only for internal testing and should be set to the default value at all times.
(h) Tpfc Off: Not used.
(i) T12 Off: Range of 40-120 °C. The default value is 70 °C. This sets the temperature of the PSU when the output is deactivated.
(j) T3 Off: Not used.
(k) T12 Min: Range of 20-80 °C. The default value is 30 °C. This value is the temperature when the internal fans start to run at their minimal speed.
(l) T12 Max: Range of 30-120 °C. The default value is 65 °C. This value is the temperature when the internal fans start to run at their maximum speed.
(m) T3 Min: Not used.
(n) T3 Max: Not used.

Variables:
(o) 10-T1: The internal temperature of the system.
(p) 11-T2: The internal temperature of the system.
(q) 13-Time (s): The remaining time in seconds until the output is deactivated. This counts down the parameter **1 - Time sec**.

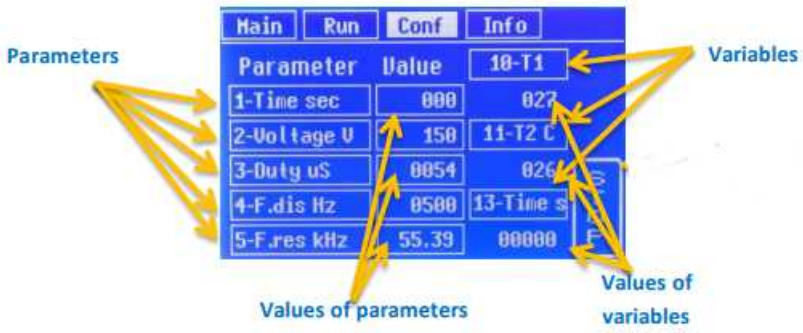

Figure 4: LCD Touch Screen displaying parameters and variables.

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

15.1 Each parameter value can be changed using the encoder.

15.2 The encoder is turned left or right to change the active value.

15.3 An active value can be chosen by pressing the touch LCD in the position of value.

15.4 When the value is active, the area is inverted (see Configuration on the picture below).

Figure 5: Touch LCD displaying Conf and other parameter values (Leap100 User Manual)

15.5 To save the value into the internal EEPROM, the encoder is pressed (activating the buzzer). This is also done after both power off and on.

15.6 When parameters are changed while the system is running, the new value will be used immediately and also when the value has not been saved in EEPROM (the encoder was not pressed).

15.7 The 4 most important parameters used to modify plasma discharge are shown on the RUN screen. These parameters can be changed on the RUN screen or on the CONF screen.

Tune the Plasma Discharge

16 Tune the Plasma Discharge

16.1 If plasma discharge is weak, increase the Voltage parameter to its maximum. If the discharge is still weak, decrease the voltage to around 70% and increase the Duty parameter by one value and again increase Voltage.

16.2 If the plasma discharge has reached an acceptable level, increase the Frequency Discharge parameter to increase the discharge (Pulses) frequency.

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

16.3 If the plasma discharge is too strong and there is a strong local discharge, deactivate the output and PSU and wait again for initialization.

Sample Treatment

17 Sample Treatment

17.1 Seed cells in accordance with the analysis that is intended after treatment. For assays using a 96-well seed plate, seed cells in 100 µl of the appropriate media. Use the 24 central wells of the 96 well-plate as indicated in *Figure 6* below to ensure symmetry in plate loading to stabilise the plasma field during treatment.

It was found that there were significant IC_{50} differences in DMEM used with and without the supplementation of 0.11g/L of sodium pyruvate, suggesting sodium pyruvate had a protective effect on cells. Media without sodium pyruvate should be used when evaluating the full effect of pin-to-plate discharge.

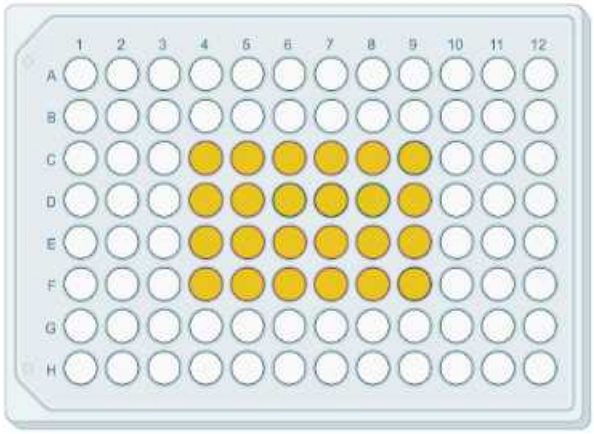

Figure 6: A 96 well-plate indicating in yellow the correct wells to seed to ensure symmetry in plate loading to stabilise the plasma field during treatment

17.2 Leave cells to adhere overnight at 37 °C in a humidified atmosphere at a density of 2 x 10^3 cells/well or 1 x 10^4 cells/well for 96 hours incubation time post-treatment and 24 hours incubation time post-treatment, respectively. If using Petri dishes, leave cells to adhere in the same conditions as the well plates but at a density between 0.2 x 10^5 and 1.2 x 10^5 cells/dish for a 35mm dish. For a 60mm dish, seed cells at a density between 2.5 x 10^6 and 3 x 10^6 cells/dish.

17.3 Ensure that there is 20 µl of media in each well before treatment. In some assays, the media may need to be fresh. In this case, remove all media and add 20 µl of fresh media into each well.

17.4 Place the sample (plate without lid) in the centre of the system, at the air gap between the pin electrode

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

and the ground plate as shown below (*Figure 7*).

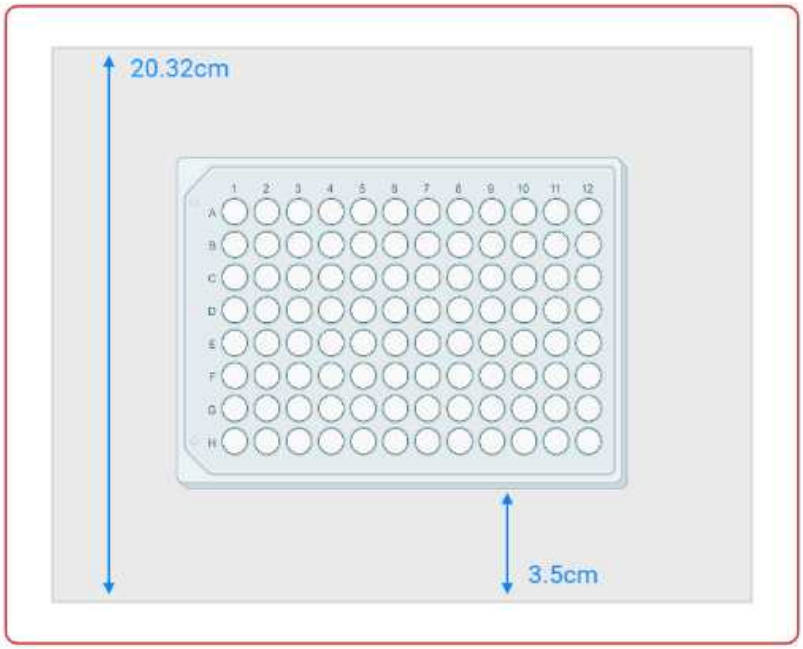

Figure 7: Schematic of the bottom of the device including a 96-well plate. The ground plate is in grey, the sample plate in the centre of the ground plate, and the container outline in red

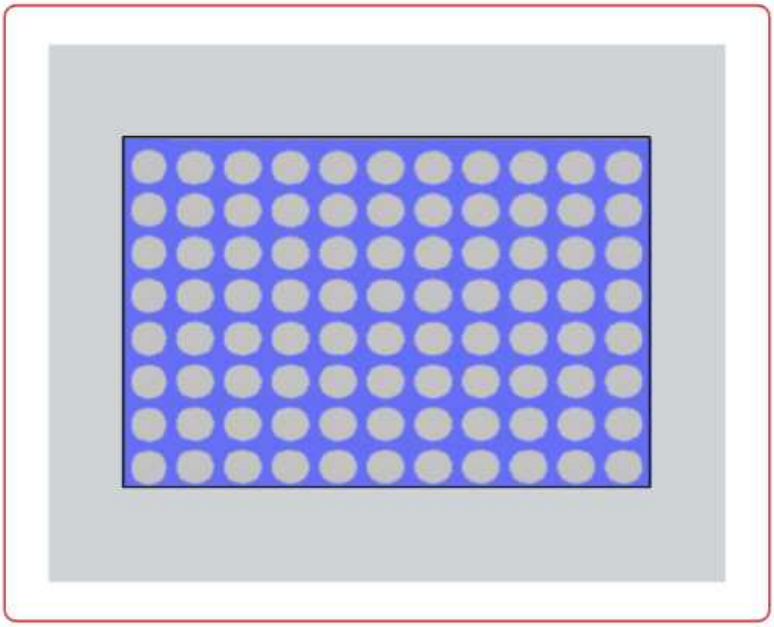

Figure 8: The underside of the top of the device showing the 88 pin electrodes and plasma discharge area in blue, and container outline in red

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

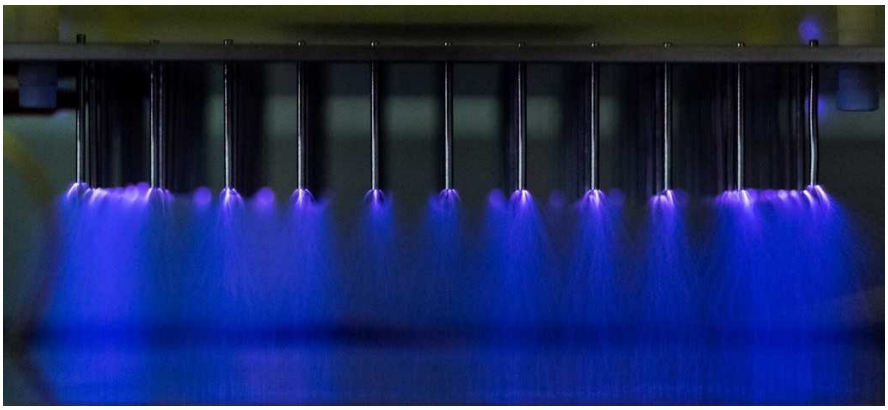

Figure 9: Plasma discharge emanating from the pin electrodes

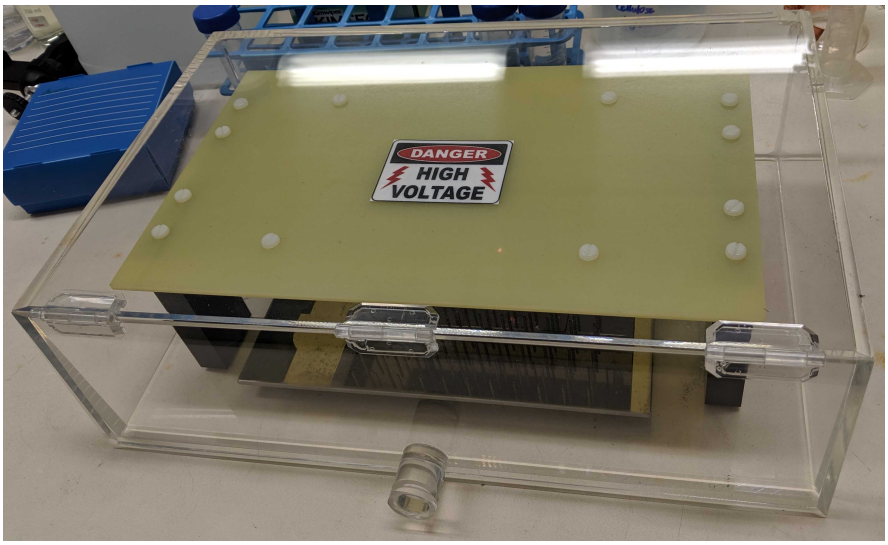

Figure 10: The clear container surrounding the device

N.B ENSURE CONTAINMENT BOX IS PLACED FIRMLY ON DEVICE. Cell viability is dependent on the containment of the plasma during treatment. In the absence of containment, no cytotoxicity will be observed.

17.5 Turn on the device by pressing the main switch.

17.6 Start initialization by pressing the INIT Itb LCD touch button and pressing the START button. The STATUS LED will stop flashing and turn green indicating that the device is ready to use.

17.7 Press the RUN button on the touch LCD.

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6

17.8 Set the parameters using the encoder as described in the SETTING PARAMETERS section above. A parameter is changed by touching the LCD screen in the position of that value and then turning the encoder left or right to change the active value.

> 1000Hz was found to be the optimum frequency in previous studies as this produced the greatest combination of Reactive Nitrogen Species and Reactive Oxygen Species within the plasma. The device has been used to treat cells for up to 320 seconds and was found to be stable, safe and had a cytotoxic effect on cells at a voltage of 240V. No significant differences were found between the use of 52 µs or 73 µs duty cycles.

17.9 Treat the samples with the plasma using the parameters that were set.

17.10 Following treatment, remove sample plates from the containment box and add 80 µl of fresh media to the wells and incubate at 37 ℃ using 5% CO_2 for 96 hours. 96 hours was the optimum incubation time in this study, however, optimum incubation varies between assays.

17.11 Use dimethyl sulfoxide (DMSO) (20%) or 1mM Hydrogen Peroxide (H_2O_2) as a positive control.

17.12 Cells are now ready for analysis.

Citation: Almha Gilheany, Sebnem Gunes, Laurence Scally, Patrick J Cullen, Brijesh Tiwari, James F Curtin, Andressa Maria Aguiar de Carvalho (05/04/2021). LEAP100 Pin-to-Plate Device Protocol for Treating Cancer Cells. https://dx.doi.org/10.17504/protocols.io.buqznvx6