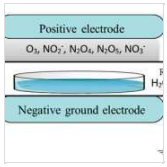

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Di-electric barrier discharge cold atmospheric plasma device protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.br7am9ie

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ABSTRACT

Plasma, the fourth state of matter, is an ionized gas that can directly interact with cells and tissues. A non-thermal plasma with weak collisions between electrons and heavy particles that is applied under atmospheric conditions is known as cold atmospheric plasma (CAP) (Conway et al., 2019). CAP generates short and long-lived reactive oxygen species (ROS), reactive nitrogen species (RNS), neutral particles, molecules, electrons an electromagnetic field, weak ultraviolet radiation and weak heating affect (Yan et al., 2018) that has lead to its' application in wound healing, sterilization and cancer treatment (von Woedtke et al., 2020). Di-electric barrier discharge devices are one of the most common CAP devices that generate short but wide plasma between an anode and a cathode using atmospheric air, and can be used to intensively treat a large sample area (Yan et al., 2016).

The purpose of this protocol is to outline the steps involved in setting-up a di-electric barrier discharge cold atmospheric plasma (DBD-CAP) device for the generation of reactive oxygen species on cell cultures. This protocol provides procedures for direct treatment with and without post treatment storage and also indirect treatment using DBD-CAP on cell cultures.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Conway, G.E., Casey, A., Milosavljevic, V., Liu, Y., Howe, O., Cullen, P.J. & Curtin, J.F. (2016). Non-thermal atmospheric plasma induces ROS-independent cell death in U373MG glioma cells and augments the cytotoxicity temozolomide. British Journal of Cancer. 114, 435-443.

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KEYWORDS

Dielectric barrier discharge, DBD, cold atmospheric plasma, CAP, reactive oxygen species, ROS

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IMAGE ATTRIBUTION

Tsoukou, E., Bourke, P. & Boehm, D. (2020). Temperature Stability and Effectiveness of Plasma-Activated Liquids over an 18 Months Period. Water. 12 (3021), 1-18.

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GUIDELINES

Considerations:
When setting up the device, ensure the electrodes and sample are level and flush to avoid unstable plasma that will arc.

The length of treatment time will effect the outcome of the cells. Longer treatment times will reduce cell viability and eventually kill the cell. Shorter treatment times can enhance gene delivery.

The cell's resistance to CAP will effect the length of treatment time needed on a cell.

MATERIALS TEXT

Materials
- Fresh culture media
- Phosphate buffer saline
- 10% Alamar Blue

Equipment
- Dielectric barrier discharge (DBD-) cold atmospheric plasma (CAP) with maximum output of 120kVRMS at 50Hz, consisting of two aluminium disk electrodes (15cm diameter) and a polypropylene container in the middle (used as a dielectric barrier and sample holder)
- Laminar air flow hood
- Ozone monitor
- Micro-plate reader
- Multi-plate reader
- Cell culture plates (96-well plate, 6-well plate, or petri dish (35, 60 or 100mm))
- 10mm Perspex layer

SAFETY WARNINGS

Reactive oxygen species are a health hazard so ensure the DBD-CAP device is housed in a working laminar air flow hood and an ozone monitor is switched on prior to treatment to protect user safety.

BEFORE STARTING

Turn on the laminar air flow hood that the device will be used in, and ensure it is working.

Turn on the ozone monitor in the room the device will be used in.

Ensure all parts of the device are in working order before setting up.

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Sample preparation

1 Plate cells into desired plate (96 well plate, 6 well plate or petri dish (35, 60 or 100mm)) and allow to adhere overnight.

2 Remove culture media from the sample, leaving a small amount to avoid drying cells.

- For 96 well plate, remove culture media to a minimum of 20µl .
- For 6 well plate, remove culture media to a minimum of 200µl.
- For a 35mm petri dish, remove culture media to a minimum of 200µl.
- For a 60mm petri dish, remove culture media to a minimum of 500µl.
- For a 100mm petri dish, remove culture media to a minimum of 1.2ml.

DBD-CAP device set up

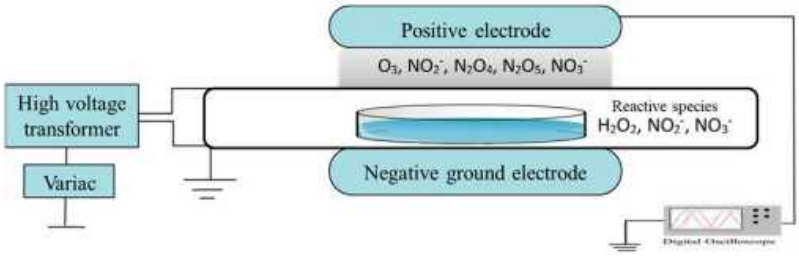
3 Place the sample plate in a sterile polypropylene case.

4 Place a sterile 10mm Perspex layer on top of the polypropylene case.

5 Place the negative ground electrode (i.e. the bottom electrode) underneath the polypropylene case.

6 Place the positive electrode (i.e. the top electrode) on top of the Perspex layer.

7 The set-up of the DBD-CAP device should resemble the following set-up.



Experimental set-up of DBD-CAP device (Tsoukou et al., 2020).

Ensure the electrodes and sample are level and flush to avoid unstable plasma that will arc.

8 Measure the distance between the two electrodes. It should be equal to the height of the container and the Perspex

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layer.

Generation of CAP

9 Follow the appropriate step case depending on the intended treatment and post treatment storage.
Step 9 includes a Step case.
Direct CAP treatment with post-treatment storage
Indirect CAP treatment withpost-treatment storage
Direct CAP treatment

step case

Direct CAP treatment with post-treatment storage

To directly treat sample with CAP and carry out post-treatment storage.

10 Note the relative humidity and temperature of the atmospheric air.

11 Set the transformer to 75kV$_{RMS}$ at 50 Hz.

12 Apply the plasma treatment to the sample for the required time. 30s

13 After the desired treatment time is completed, turn off the voltage.

14 Remove the high barrier propylene bag containing the treated samples from the DBD-CAP set-up.

It is vital for user safety to ensure this is carried out in the laminar air flow hood, as escaping reactive oxygen species are a health hazard.

15 Immediately after CAP treatment, remove any remaining media in the plate and replenish with fresh culture media.

- For 96 well plates, replenish with 100µl fresh culture media.
- For 6 well plates, replenish with 2ml fresh culture media.
- For 35mm petri dish, replenish with 2ml fresh culture media.
- For 60mm petri dish , replenish with 5ml fresh culture media.
- For 100m petri dish, replenish with 12ml fresh culture media.

16 Store the sealed samples in a humidified incubator under 5% (v/v) $C0_2$ at 37°C in for 24-48 hours post-treatment storage time.

Analysis

17 **Determination of ROS generation.**

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17.1 One hour post treatment, measure the fluorescence intensity on a micro-plate reader at 492-495/517-527 nm.

18 Determination of cell viability.

18.1 48 hours post treatment, wash cells once with sterile phosphate buffered saline.

18.2 Incubate cells for 3 hours at 37°C with 10% alamar blue and 90% culture media solution without fetal bovine serum.

18.3 Using a multi-plate reader, measure fluorescence using an excitation wavelength of 520nm and an emission wavelength of 595nm.

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