Low Temperature Plasma Causes Double-Strand Break DNA Damage in Primary Epithelial Cells Cultured From a Human Prostate Tumor

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***Abstract*— Research in the new field of plasma medicine continues to demonstrate the efficacy of low temperature plasmas (LTPs) for numerous biomedical applications. Responses such as reduction in cell viability and cell death for cancer therapy, cell proliferation for wound healing, and bacterial inactivation have been observed as a result of plasma treatment. In this paper, we applied LTP to prostate cancer primary cells derived from patient tumour tissue to inflict irreparable DNA damage.**

***Index Terms*— Atmospheric pressure plasma, cells (biology), DNA, oncology.**

IN RECENT years, the prognosis for prostate cancer (PCa) patients has improved as a result of early detection and improved treatments. Advances in radiotherapy such as the CyberKnife linear accelerator, and the advent of focal therapies, including photodynamic and cryotherapy, have improved treatment options for patients. However, cancer recurrence and adverse side effects are still common [1]. Low temperature plasmas (LTPs), operated at atmospheric pressure and room temperature, could present a new treatment option for localized PCa, with fewer side effects than current treatment options. The transfer of reactive oxygen and nitrogen species from the core plasma to the cell media, such as O_3, NO, $^1O_2$, O_2^-, OH, and H_2O_2, can lead to adverse cellular effects and ultimately cell death [2]. Here, we present the first steps in treatment development for primary PCa cells with LTP, where we have demonstrated potentially lethal DNA double strand breaks (DSBs) within the cell nucleus.

The LTP used in this paper was a dielectric barrier discharge (DBD) device, operated at 6 kV, 30 KHz. Helium was used as a carrier gas at 2 slm, with 0.3% O_2 admixture. The distance from the nozzle to the sample surface was maintained at 10 mm. Treated samples received 5 min exposure to the plasma. Exposure times of up to 10 min did not raise the media temperature beyond 36.5 °C.

The PCa tissue was obtained with patient consent from targeted needle core biopsies following radical prostatectomy. Primary cells were cultured from cancer tissue and plated onto a chamber slide at a density of 10 000 cells per well in 200 μL of stem cell media [3]. Cells were fixed in paraformaldehyde 30 min after treatment before staining. The treated sample was previously identified as Gleason grade 7 cancer through tissue histopathology.

Immunofluorescence staining clearly demonstrated DNA damage in the plasma-treated primary prostate epithelial cells cultured from a patient tumor. Fig. 1(b) and (c) shows a plasma-treated primary cell and an untreated control cell, respectively. The presence of red γ-H2AX foci in Fig. 1(b) is indicative of DSB DNA damage in the nucleus of the treated sample. The blue 4', 6-diamidino-2-phenylindole (DAPI) and green phalloidin markers were used to indicate cell nuclei and cell cytoskeleton (cell structure), respectively.

This result is a first demonstration that LTP is capable of causing potentially lethal DSB DNA damage in primary cells cultured from patient cancer tissue. The DSBs are particularly difficult for the cell to repair, and in turn lead to apoptosis (regulated cell suicide). It is anticipated that normal tumor-neighboring prostate cells would also sustain DNA damage; however, this would be a localised effect and would not negatively affect the prostate as a whole. Further, study now needs to be conducted to ascertain, comprehend, and manipulate the exact interaction mechanisms between the plasma and cancer cells; however, this result clearly shows the potential of LTP as a therapeutic for cancer patients.

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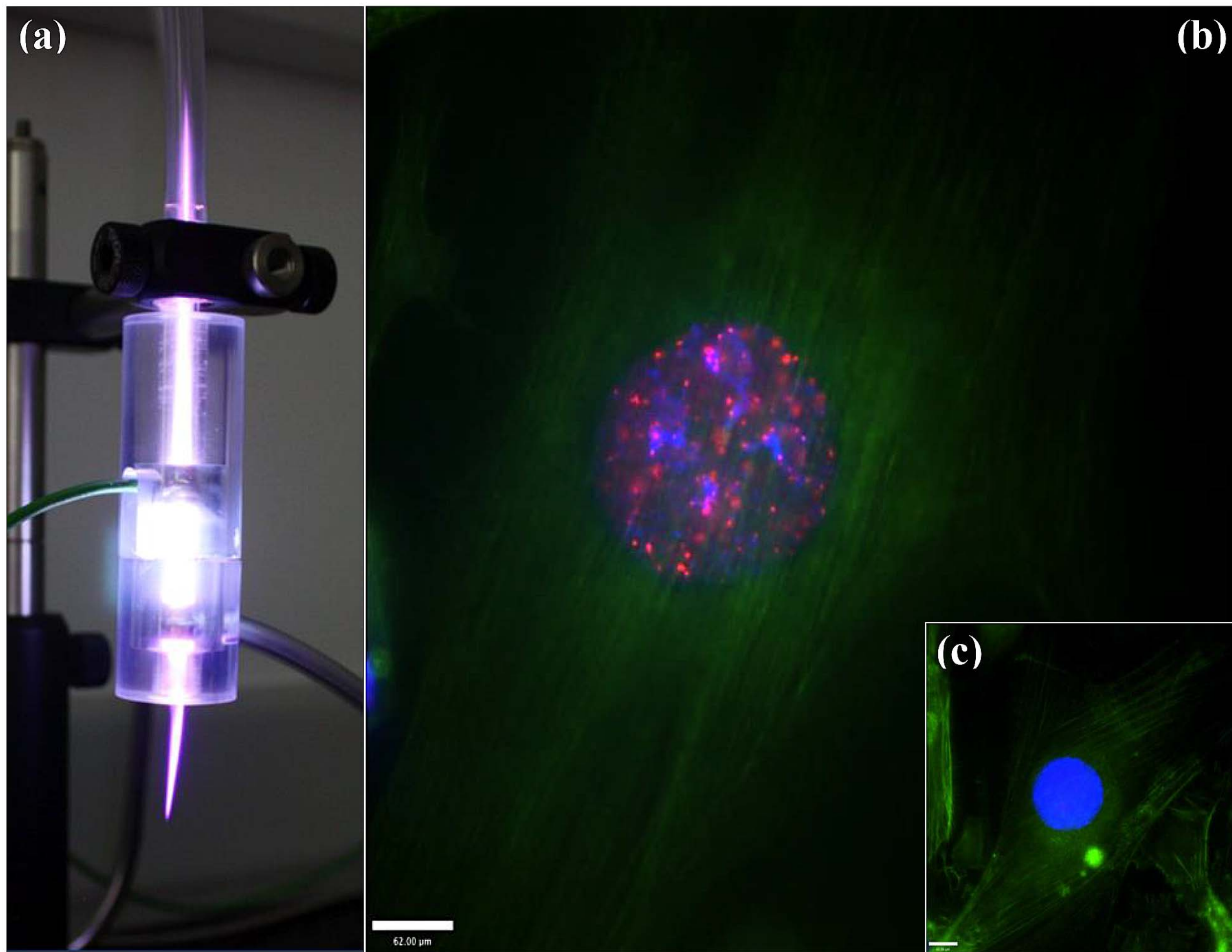

Fig. 1. (a) Primary prostate epithelial cells were treated with low temperature DBD plasma, operated at 6 kV. Helium was used as a carrier gas at 2 slm, with 0.3% O_2 admixture. Core discharge is created between the grounded (top) and powered (bottom) electrodes, with the resulting effluent directed onto the cell culture media surface. (b) Cells were cultured from a Gleason grade 7 patient tumor, plated at low density and exposed to 5 min low temperature plasma treatment, or (c) left untreated. Cells were fixed at 30-min posttreatment and stained for γ-H2AX (red, DNA damage foci), DAPI (blue, cell nucleus), and phalloidin (green, cell actin filament structure). Note the multiple red dots within the cancer cell nucleus after plasma treatment, indicating widespread and potentially lethal damage to the DNA. Scale bars = 62 μm in both (b) and (c).