5. Two Biomedical Applications of LTP

To illustrate the effects of LTP on biological targets, two applications are shown here. The first concerns the bactericidal property of LTP and the second shows the effects of direct plasma exposure as well as plasma activated media on cancerous and healthy epithelial cells. The results presented below are based on the use of the plasma pencil described earlier. The results shown were obtained by the application of the LTP plume generated by the plasma pencil on a bacterial lawn seeded on the surface of a Petri dish (see Figure 4). The bacterium used was *Acinetobacter calcoaceticus*, a gram-negative soil bacterium also found in the tiger mosquito, which is known to be a transmission vector of yellow and dengue fevers. Figure 5 shows zones of inactivation (dark circular areas) around the center of the dish where the plasma plume was applied. The photo to the left is for an initial bacteria concentration of 10^9/mL, while that on the right is for an initial concentration of 10^7/mL. It is clear that the killing effects are more extended and pronounced for the lower initial concentration. For more information on the dependence of inactivation on the plasma exposure time and on the type of bacteria, the reader is referred to [93].

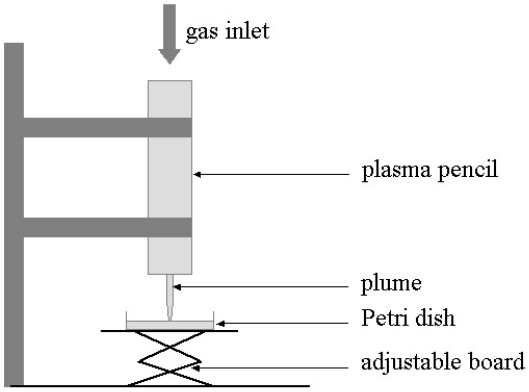

Figure 4. Experimental setup for the bacterial inactivation experiments.

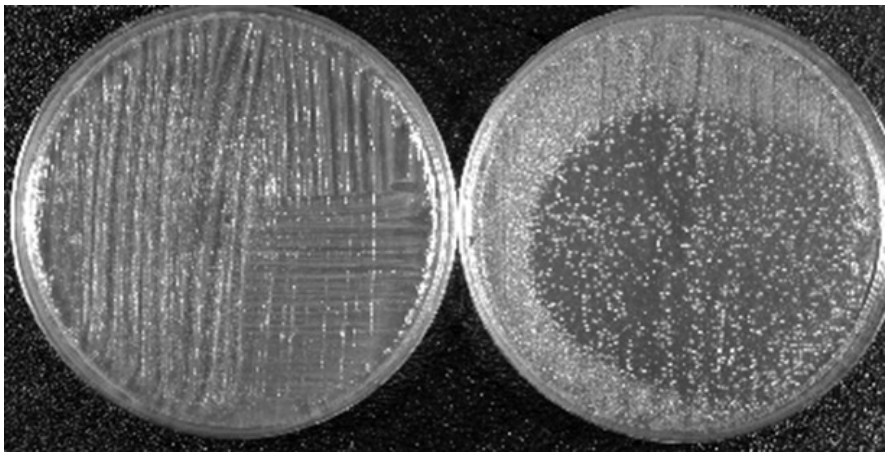

Figure 5. Killing property of LTP: Dependence of the killing efficacy on the initial bacteria concentration. Left picture is for 10^9/mL and right picture is for 10^7/mL. Bacterium is *A. calcoaceticus*. LTP source is the plasma pencil operated with helium as a carrier gas [93].

Figure 6 shows the effects of direct application of LTP on suspensions of cancerous cells. The cancer cell line used was a squamous cell carcinoma of the bladder (SCaBER, ATCC HTB-3^{TM}) originally obtained from a human bladder. After LTP exposure and proper incubation process (37 °C under 5% CO_2 atmosphere), Trypan-blue exclusion assay was used to count the number of live and dead

cells. For details of the experimental protocol please refer to [40]. The counts immediately after LTP treatment (at 0 h) revealed no dead cells, which suggested there were no immediate physical effects. However, the viability of cells reduced to around 50% at 24 h after a 2-min LTP treatment. As seen in Figure 6, higher plasma exposure times result in more cells killed (5-min plasma treatment results in 75% of loss of viability at 24 h post-treatment) [40]. These results indicate that LTP does not apply immediate brute physical force on the cells, but its effects require longer biological times to show. This is an indication that plasma agents, such as reactive species and electric fields, interact with the cells and induce reactions and/or trigger biochemical pathways that ultimately result in the death of the cancer cells hours later.

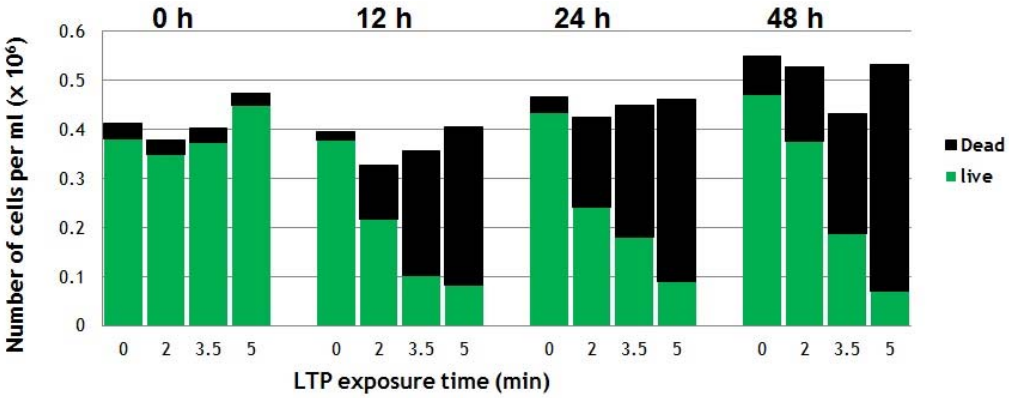

Figure 6. Viability of SCaBER cells in media treated directly by the LTP plume of the plasma pencil reveal dead (black bars on top) and live (green bars) cells. The viability was monitored at 0, 12, 24 and 48h post-LTP treatment [40].

Figure 7 shows the selective effect of LTP when it comes to destroying cancer cells versus healthy cells in vitro. The viability results shown in the figure below were obtained using plasma activated media (PAM), which was created by exposing biological liquid media to the plasma pencil for certain lengths of time. The cancerous cell line used was SCaBER and the healthy/normal cells were MDCK (Madin-Darby canine kidney) cells from normal epithelial tissue of a dog kidney. The media used to make PAM were MEM (minimum essential media) for SCaBER and Eagle Minimum Essential Media (EMEM) for MDCK. Figure 7 shows the results [57].

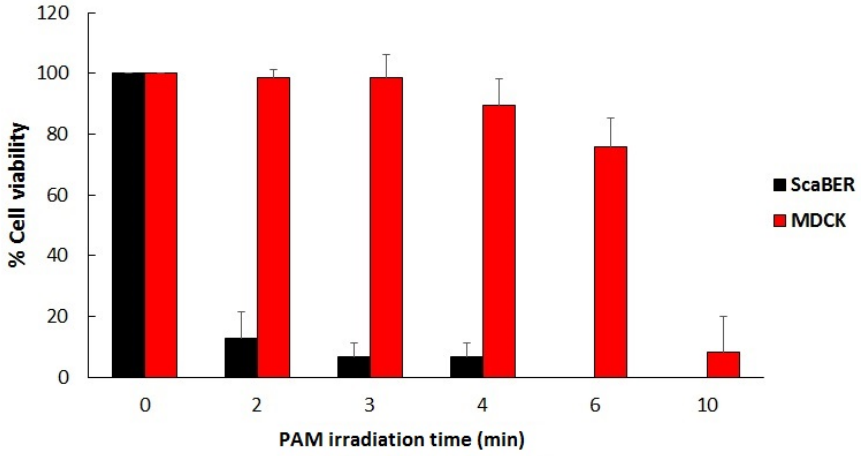

Figure 7. Viability in percent of SCaBER (cancerous) and MDCK cells (noncancerous) treated by PAM for various lengths of time. Viability was assessed after 12 hours incubation with PAM using MTS assay and Trypan-blue exclusion assay [57].

Figure 7 shows that PAM created using longer exposures to LTP has increasing killing effects on SCaBER cancer cells, reducing their viability to below 10% for irradiation times longer than 2 min. However, normal MDCK cells were able to withstand exposure to PAM for 3 min. This illustrates the selectivity of PAM in killing cancer cells while sparing healthy cells. But for PAM created with longer exposures to LTP (6 minutes and more) extensive killing of MDCK cells was obtained. This illustrates that the plasma dose is an important factor to take into consideration for optimal outcomes.

6. Penetration of RONS in Tissues

One of the key questions in plasma medicine is the following: Do the RONS generated by LTP only interact and affect cells on the surface of a tissue (or tumor) or do they penetrate the tissue and affect cells in deeper layers? Experimental evidence has shown that LTP does indeed affect cells underneath the tissue surface but what remains unclear is how. One possible explanation is what is referred to as the "bystander effect," which implies that there are chemical signals sent by the cells on the surface (in contact with plasma) to cells in the layer below [41]. These signals would trigger reactions similar to those occurring at the cells on the surface, including the onset of apoptosis. However, and to the best of this author's knowledge, there has been no experimental proof this occurs when LTP interacts with tissues. So, the possibility is there, but reliable data that can be replicated needs to emerge first. Therefore, in this section, only experiments that reported qualitatively and/or quantitatively on the penetration of RONS are presented.

In order to qualitatively and quantitatively elucidate RONS penetration into tissues, investigators used various in vitro models. Oh et al. investigated the penetration of RONS using a model made of an agarose film covering a volume of deionized water contained in a quartz cuvette [67]. They found that RONS kept being delivered from the agarose film to deionized water underneath it for up to 25 min after the plasma was removed. To study the delivery of reactive oxygen species (ROS) into cells, Hong et al. used a model comprising phospholipids vesicles encapsulated within a gelatin matrix and equipped with reactive oxygen species (ROS) reporter [68]. They found that ROS were delivered to the cells without rupturing the membranes of the vesicles. To simulate biological tissue, Szili et al. used gelatin gel, a derivative of collagen, and reported on the penetration behavior of H_2O_2 through a 1.5 mm thickness gelatin film [69]. The same authors also investigated the effects on DNA in synthetic tissue fluids, tissue, and cells [94].

Tissue models are useful and provide preliminary data regarding the penetration of RONS through biological targets. However, to simulate more realistic conditions, Duan et al. used slices of pig muscle tissue of different thicknesses placed on top of a PBS solution [72]. Figure 8 shows the experimental setup. A plasma jet operated with a helium/oxygen mixture was used. To ignite the plasma sinusoidal high voltages at a frequency of 1 kHz were employed. The plasma treatment times were 0, 5, 10, and 15 min.