From Killing Bacteria to Destroying Cancer Cells: 20 Years of Plasma Medicine

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1. Preamble

With the advent of atmospheric pressure plasma discharges in the early 1990s various industrial and environmental applications that do not require low pressure operating conditions became possible. Among these the biomedical applications of low temperature plasmas took center stage. First, investigations of the efficacy of plasma to inactivate bacteria were conducted in the mid-1990s[1–6] (and references therein). The dielectric barrier discharge (DBD) was the plasma source used during the early studies. Later on, as plasma jets were developed, these were also used with equal success. The inactivation of bacteria on biotic and abiotic surfaces is useful for applications such as sterilization/decontamination[3,4] and wound healing.[5,7] By the early 2000s, investigations on mammalian cells which showed that under some conditions plasma can affect these types of cells without causing damage were conducted.[8,9] Some of the effects include cell detachment and apoptosis. The period between 2006 and 2013 witnessed two major quantum leaps in medical applications of low temperature plasma (LTP): (i) clinical trials on wound healing were conducted by Isbary et al.;[7] (ii) LTP was shown to be able to cause damage or even destroy cancer cells in vitro and, later, in vivo, by several investigators. First, Yonson et al. in 2006 tested a human hepatocellular carcinoma (HepG2),[10] then other adherent and non-adherent cells lines such as melanoma, glioblastoma, and leukemia cells were used by other investigators.[11–23] These crucial advances breathed great confidence and helped cement the idea that LTP could indeed one day revolutionize health care on several fronts.

In this essay, looking back at the last 20 years of efforts, the author's thoughts on the progress of plasma medicine, and especially on the use of LTP to kill cancer cells, are expressed. These thoughts and opinions include personal reflections and assessment of the field and its prospects for the next decade, especially in regards to the use of LTP in cancer therapy.

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2. Historical Perspective: Thoughts and Impressions

It has been about 20 years since the biological and medical applications of low temperature atmospheric pressure plasmas, a field today known as ''Plasma Medicine,'' had its first humble steps. This author's group was fortunate enough to take part and contribute to this exciting multidisciplinary field during its two-decade-long ''formative'' period. Our early work, mid- to late-1990s, focused on investigating the bacterial inactivation efficacy of LTP while in the last few years, 2010 to the present, we have been focusing more on cancer studies. In between these years, various other topics were entertained and experiments were conducted in our laboratory ranging from wound healing, to destruction of pathogenic proteins that cause neurodegenerative diseases, to dental applications. Each one of these lines of research presented its own set of challenges but also offered many rewarding experiences, the collaboration with biologists, biochemists, and dentists being one of these. During these two decades this author witnessed the incredible scientific progress that the field of plasma medicine had undergone as many groups around the world entered the field and achieved new research milestones. Most rewarding is seeing many colleagues who were somewhat skeptical early on (understandably hesitant) become some of the most ardent supporters of the field and many of them become some of the most productive. But regardless of when one enters a research discipline what is important is to positively contribute to the scientific knowledge that is necessary to carry the field forward and many of these colleagues did just that.

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Today, the work on cancer is becoming the most exciting sub-topic of plasma medicine research. This is due to the difficult challenges that this research offers and to the great societal impact it could one day have. A question that a new researcher in the field may ask himself or herself would be: how did our research community arrive to the thought that LTP could possibly be part of a cancer therapy? The answer is that it did not just happen suddenly, but rather gradually. There were many key milestones that various researchers achieved along the way. In the early 2000s, Stoffels and co-workers[8,9] (TU/e, Eindhoven) conducted experiments that showed that low doses of LTP can induce apoptosis in healthy mammalian cells. Then, in the year 2006, a paper from Coulombe's group (McGill University) reported that LTP can detach human hepatocytes from the surface of a Petri dish and in some cases rupture their cell membrane by lipid peroxidation.[10] This work was followed by several seminal experiments that showed that LTP kills various types of cancer cell lines. A non-exhaustive list of these is the following: a report from Fridman's group (Drexel University) on the killing of melanoma by a floating electrode dielectric barrier discharge,[11] Schlegel's group report (University of Munich) on glioblastoma,[12] Keidar's group (George Washington University) reports on papilloma and carcinoma,[13–15] Baek's group (University of Tennessee) report on colorectal cancer,[16] Hori's group (Nagoya University) report on ovarian cancer,[17] and Laroussi's group (Old Dominion University) reports on prostate cancer cells, squamous cell carcinoma, and leukemia, a non-adherent cancer cell line.[18–21] All the above experiments were conducted in vitro and showed the potential of LTP to kill cancer cells effectively (either by apoptosis or necrosis, depending on the applied dose). Successful in vivo works were reported by Pouvesle's group (GREMI),[22] Keidar's group (George Washington University),[15,16] and Kim's group (Clemson University).[23] With encouraging results from in vitro work on several cancer cell lines and in vivo work on a few cancer cell lines, the plasma medicine research community became convinced that LTP could play a role in a future cancer therapy, and therefore adding another weapon to the arsenal of cancer fighting modalities.

3. The Key Questions

Some of the most important questions that the plasma medicine research community is facing today are: (i) can LTP alone be the basis of a new cancer therapy or would LTP be an adjuvant part of a more complex therapy? (ii) When a cancer tumor is destroyed by LTP is the cancer eradicated or will it recur again? (iii) How deep in a tissue can the effects of LTP penetrate in order to treat internal tumors and kill cancerous cells well below the surface? The answers to these questions are not obvious at the present. This author's opinions are the following.

Based on the results published so far in the literature and presented at scientific meetings, LTP does not affect only the cells on the surface but its effects are felt deep within a tissue or a tumor. Two explanations have been put forward on how this happens. Using biological reporter molecules, Hong et al.[24] and Szili et al.[25] reported that oxygen reactive species (ROS) generated by a plasma jet can penetrate not only single cells at the surface but can also penetrate up to 1.5 mm below the surface of a tissue model (gelatin gel). These authors argue that cells deep within a tissue are affected directly by the ex situ ROS generated by the plasma. Other investigators have argued that the effects of plasma deep below the surface could be due to the so-called "Bystander effect." This means that the signaling triggered by LTP is actually transmitted from the cells at the surface to the cells below them (somewhat similar to quorum sensing, requiring intercellular communication pathways). So according to this hypothesis, if apoptosis is induced in a cell after LTP application, the death of that cell causes neighboring cells to undergo the same death pathway. How this happens is still not well understood and remains a matter of debate.

Based on experimental evidence, the answer to the second question seems to be "No," a single dose of LTP does not completely eradicate the cancer: various experiments showed that tumors tend to regrow after a certain time. Therefore, an LTP treatment regimen consisting of several applications for a period of time (maybe weeks) is necessary, but may still not be sufficient.

Based on the above two answers, it appears to this author that LTP may be better suited as an adjuvant therapy (this is an attempt to answer the first key question above). For example, LTP could be used in conjunction with cancer drugs to facilitate and better target the intake of the drugs. Another possibility would be for LTP to be used after surgery to eradicate remnant cancer cells. Other modalities and combinations could certainly be proposed and tested for efficacy. It is also of note at this juncture to mention that some investigators have shown that LTP does not have to be applied directly to a tumor. Instead, plasma activated media (PAM) can be used as a sort of a "drug" to treat tumors or cancer lesions. This line of research is interesting but still needs to be developed further. The study of LTP–liquid interaction is a topic of great interest for these types of experiments.

4. Mechanisms of Action of LTP and Safety Issues

Is it safe to expose biological cells and tissues to LTP? This question is of course relevant to all biomedical applications

of plasma including wound healing, but here we are mainly concerned with cancer treatment application. In order to attempt to answer this question, first it is important to understand the mechanisms of action of LTP against cancer cells. This in itself is an entire research endeavor that is crucial both on the scientific level and on the therapeutic level. What is known at the present time, based on experimental evidence, is that cancer cells appear to be more vulnerable to LTP than healthy cells: several investigators reported that the same plasma exposure kills more cancer cells than their healthy counter parts. Why is this the case? Keidar and co-workers[13] reported that for skin cancer cells LTP causes an increase in the expression of the oxidative stress reporter γH2A.X (pSer 139) and a decrease in DNA replication in the S-phase of the cell cycle. This indicates S-phase damage (S-phase is the DNA replication phase). The effect was much less pronounced in the healthy cells. So, it appears that LTP's is most effective during cellular replication, and more specifically during the DNA replication phase, which makes cancer cells much more susceptible to LTP-induced damage than their healthy counterpart. Other investigators proposed the following hypothesis: because the concentrations of ROS and RNS are much higher in cancer cells than healthy cells, LTP adds more intracellular oxidative stress, up to a level that overcomes (and exceeds) the cancer cells defense, therefore causing their death. However, this argument may hold true for some cancers but not necessarily for all cancer cells (not all cancer cells exhibit elevated levels of ROS).

There are other modes of action of LTP against cancer cells. One such mode is caspase activation. Laroussi and co-workers[26] studied capse-3 activation in the case of squamous cell carcinoma and found higher level of caspase-3 activation in cancer cells treated by LTP than in control samples, indicating that LTP induces apoptosis in these cells. Ishaq et al.[27] suggested that because tumor cells are defective in several regulatory signaling pathways they exhibit metabolic imbalance, which leads to a lack of cell growth regulation. According to these authors, LTP-generated ROS affect the metabolism of the cancer cells by impairing redox balance, which leads to slowing down or arresting the proliferation of the cells. They claim that this is not the case for healthy cells as these are able to adjust/properly regulate their metabolic pathways as the ROS levels change.[27] In a recent paper,[28] the same authors reported that LTP up-regulates intracellular ROS levels and induce apoptosis in melanoma. They identified that LTP exposure causes a differential expression of tumor necrosis factor (TNF) family members in the cancerous cells but not in the normal cells. They found that apoptosis in the cancer cells was induced by apoptosis signal kinase 1 (ASK1), which is activated by TNF signaling.

Although it appears that cancer cells are more sensitive to LTP than healthy cells, some questions do remain to be answered. Two of these are: (i) can cancer cells adapt to LTP treatment? (ii) Could LTP exposure cause mutations in healthy cells that can render them cancerous? Although there are no reports so far that suggest a positive answer to either of these two questions, it may be too early to be sure and more work needs to be done to check these potentially serious safety issues.

5. Quo Vadis?

Where is the field heading and what should we be doing to pave its success for the next decade? With so many in vitro and in vivo experiments done, it appears that time may be near for some clinical trials. The challenges with these are of course the financial costs and obtaining proper approvals. However, clinical trials need to be done at some point, and hopefully soon, if LTP is to be considered as a possible therapy or adjuvant therapy by oncologists. In the meantime, it is important for the plasma medicine community to keep working on the fundamental scientific issues involved in the interaction between LTP and biological systems and for cancer researchers to prove the safety of LTP and that the selectivity of LTP toward cancer cells is indeed true. In addition, scientific meetings such as the International Workshop on Plasma for Cancer Treatment (IWPCT) need to continue being organized on a regular basis so as to offer a forum for investigators from around the world where they can meet, exchange results and ideas, and establish fruitful collaborations. Another very important aspect that the plasma medicine community has not done well so far is to involve medical doctors and healthcare professionals in their scientific meetings. This is crucial as it is the medical doctors who may someday accept LTP as a viable therapeutic modality and use it on their patients. In fact, introducing plasma medicine as a topic in medical conferences and journals should become an urgent priority if the field is to take hold within the health care community.

Last but not least is the funding issue. It is interesting to note here that the funding level for plasma medicine research is the lowest in North America (US and Canada) where the first efforts of using LTP for biological and medical applications (including cancer) were conducted. This is quite a vexing state of affairs for American researchers as European and Asian researchers have fared much better in this regard. Of course, funding priorities of scientific disciplines vary in each country based on various political and economic agendas. Regardless, it is imperative for all of us who are involved in plasma medicine research to be vigilant and expend enough effort to convince the relevant governmental and private funding agencies and foundations to put our field on their budgetary agendas.

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