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## Plasmas meet nanoparticles – where synergies can advance the frontier of medicine

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### Abstract

Nanoparticles and low-temperature plasmas have been developed, independently and often along different routes, to tackle the same set of challenges in biomedicine. There are intriguing similarities and contrasts in their interactions with cells and living tissues, and these are reflected directly in the characteristics and scope of their intended therapeutic solutions, in particular their chemical reactivity, selectivity against pathogens and cancer cells, safety to healthy cells and tissues, and targeted delivery to diseased tissues. Time has come to ask the inevitable question of possible plasma-nanoparticle synergy and the related benefits to the development of effective, selective, and safe therapies for modern medicine. This perspective article offers a detailed review of the strengths and weaknesses of nanomedicine and plasma medicine as a stand-alone technology, and then provides a critical analysis of some of the major opportunities enabled by synergising the nanotechnology and the plasma technology. It is shown that the plasma-nanoparticle synergy is best captured through the plasma nanotechnology and its benefits for medicine are highly promising.

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## 1. Introduction:

In open literature, there have been countless scientific publications, roadmap statements of relevant scientific communities, and government white papers that highlight the immense potentials of the nanotechnology for healthcare. Targeting the same set of major challenges in modern medicine (e.g. infectious diseases, cancers), low-temperature gas plasmas have in more recent years been seen to attract rapidly growing interest in their biological applications. So far, the development of these two technologies for biomedicine appears to be largely independent from each other and each has achieved some notable clinical successes. The scale of their own potential for medicine and the extent of the scientific challenges they face as a stand-alone technology are such that there appears little incentive to explore possible overlaps, in terms of either underpinning science or technological implementation, between these two equally exciting technologies. Yet close examination shows some very intriguing contrasts in their characteristics when considered to meet the same basic requirements for medical applications, for example the efficacy as a therapeutic agent, the selectivity against malignant cells (e.g. pathogens and cancer cells), the toxicity and indeed the safety to healthy cells and tissues, and finally the targeted delivery towards diseased tissues. Nanotechnology has a superior capability in penetrating into a tissue, while its toxicity still represents an area of considerable uncertainties. On the other hand, biological effects of low-temperature plasmas stem from their non-equilibrium reaction chemistry with very high level of chemical dissociation. Their interaction with a living tissue is normally topical with limited penetration, but their cytotoxic effects are better understood through their similarity to free radical biology. Such fundamental contrasts invite the question of the scale of the opportunities brought about by plasma-nanoparticle synergy and call for a detailed analysis of its benefits to the ultimate goal of developing effective, selective, and safe therapies for modern medicine.

This perspective article offers the first critical analysis of whether there is a need for a synergistic combination of the nanotechnology and the plasma technology, what benefits the plasma-nanoparticle synergy may bring about, and how such synergy may be realised in practice. This analysis is built on a concise review of plasma science (Section 2) and nanoparticle-cell interactions (Section 3) as well as a comprehensive review of plasma-cell interactions (Section 4). A number of plasma-nanoparticle synergies are suggested and analysed in Section 5, and the conclusions thus reached are used to highlight in Section 6 the landscape of major opportunities to advance the frontier of therapeutic medicine by capitalising on the synergy between plasmas and nanostructures. It is emphasized that the full potential of synergizing plasmas and nanostructures can only be realized when nanoparticles are fabricated within a low-temperature plasma and delivered by the plasma to a disease target – a technology known as the plasma nanotechnology.

## 2. Plasma Science and Nanotechnology:

Low-temperature plasmas represent a unique thermally non-equilibrium environment where a variety of reactive species as well as different forms of energy (e.g., thermal, electromagnetic, and chemical) can be produced. This ability has been widely used for a large number of applications in nanotechnology. The most common applications are in micro- and nano-scale synthesis and processing of advanced nanomaterials. Representative examples include etching and conformal coating of high-aspect-ratio features in a silicon wafer for nano-electronics, low-temperature synthesis and post-processing of nanopatterns and arrays of nanostructures for sensing, photovoltaics and optoelectronics, highly-selective functionalisation and other post-processing of localised surface areas with nanoscale dimensions, as well as the production of nanoparticles with tailored features such as size, shape, facet expression, and surface reactivity [1]-[5].

The examples of the nanoscale objects produced or post-processed using low-temperature plasmas span all three dimensions and range from zero-dimensional Si-based quantum dots arranged in two- or three-dimensional patterns in a host matrix and arrays of one-dimensional single-walled carbon nanotubes with controlled chirality and metal oxide nano-architectures to two-dimensional free-standing and catalyst-supported graphene sheets and flakes and carbon nanowalls as well as self-

organized patterns of shape-tuned three-dimensional semiconducting nanostructures [6]-[17]. The sizes and other properties of the nanoscale objects can be precisely tailored to meet the requirements of any particular application.

These properties can be very different from the properties of nanoparticles and other nanomaterials produced using other techniques such as thermal chemical vapour deposition (CVD) or wet chemistry. For example, the unique ability of reactive plasmas to dissociate molecular hydrogen has been successfully used to synthesize free-standing silicon and germanium nanoparticles with nearly perfect cubic shape, which is rarely achievable otherwise [18]. Furthermore, carbon nanotubes and other one-dimensional nanostructures show pronounced vertical alignment which is not common to thermal CVD [19][20].

On the other hand, effective control of any particular features of the nanostructure or nanoparticles (NPs) has been intimately related to the ability of the plasma environment to concentrate the building units of the nanoscale matter and the associated energy in designated microscopic and nanoscale surface areas [21]. A balance of plasma-specific forces (e.g., ion drag, electrostatic, thermophoretic) has also been reported as an effective tool for the precise delivery of nanoparticles to the specified microscopic areas on the surface; this approach was also instrumental in almost complete removal of nanoparticles away from the surface areas where their deposition is not warranted [22][23]. For many other unique features and advantages of the plasma-based nanoscale synthesis and processing the reader should be referred to previous publications as well as to other articles in this Special Issue.

In the following we will discuss how the above unique capabilities of low-temperature plasma-based nanotechnology can be used in applications in biology and healthcare.

### **3. Interaction of Nanoparticles with Cells:**

Let us now consider some of the most common effects of nanoparticles on living cells and their applications in nano-medicine and nano-biotechnology. The interactions of plasma-produced reactive species and electromagnetic radiation with living cells will be considered in the next section.

Rapid development of nanotechnology over the last decade made it possible to synthesize different types of nanoparticles whose diameter is of the order of a few nanometers and even less. The surfaces of such nanoparticles can be modified by bioactive molecules or imaging probes that can be adsorbed, coated, conjugated, or linked to them. As such, these nanoparticles were proposed for cell labeling and targeting, tissue engineering, drug delivery, drug targeting, magnetic resonance imaging etc [24]-[28]. Wide-range applications stimulated intensive study of interaction of nanoparticles with a living tissue and in particular the penetration and migration of the nanoparticles inside the tissue. Some authors suggested that the processes governing the penetration of reactive radicals (see extensive discussion of these effects in Section 4) [29] and those governing nanoparticles are not the same [30], making a good case for penetration selectivity argument. In addition, penetration efficacy of nanoparticles inside various tissues is distinctly different. For instance, it was shown that nanoparticles are able to penetrate the hair follicle and stratum corneum (SC), but viable epidermis is reached only occasionally. It was concluded that those nanoparticles **are** unable to permeate the skin. Titanium dioxide nanoparticles of different sizes (from 20 to 100 nm) are not penetrated to SC layers, the human epidermis and dermis, but solely deposited on the outermost surface of the SC [31].

Meanwhile, metallic (e.g., gold) or metal oxide (e.g., iron oxide) nanoparticles have recently demonstrated a reasonable therapeutic efficacy, selectivity, tumor affinity, and concomitant *in vivo* tolerance in cancer therapy [32]-[37]. The treatments are based on targeted delivery of the functionalized (e.g., using glucoproteins) nanoparticles to the tumor-affected areas and using either a brachytherapeutic or hyperthermal/photothermal treatment. In the former case, low-dose electron emission from  $\beta$ -emitting gold-198 ( $^{198}\text{Au}$ ) nanoparticles is used while the latter case relies on localized surface plasmon excitation which generates significant yet localized heat. The nanoparticles

are usually delivered via direct intratumoral injections and penetrate malignant cells through tumor vasculature and pores. Application of nanoparticle treatment makes it possible to significantly reduce the size of the tumor before surgical resection and is an important milestone towards the ultimate ability of complete and irreversible tumor resolution without surgery. This ability has been demonstrated in animal trials and is presently at the stage of human clinical trials [35][36].

In parallel and out of health concerns, an additional research activity stimulated by the rapid developments of nanotechnology is study of human body contamination and health effects of nanomaterials (nano-toxicity) [38]-[42]. This rapidly expanding research field is commonly referred to as nano-safety.

As an example of a combination of plasma treatment considered in Section 4 with the nanoparticle treatment briefly discussed above, let us consider a simple combination of suitably functionalized nanoparticles and a cold plasma jet [43][44]. In such case, the cold atmospheric plasma jet can have two primary purposes. Firstly, the jet will carry nanoparticles and thus will provide their delivery to the desired position and good localization of the treated zone governed by the jet cross-section. Since the intensity of the plasma jet can be limited to below the damaging threshold to living tissues and thus the plasma jet will serve just as the delivering agent. Secondly, it is possible to electrically charge nanoparticles by means of fundamental mechanism of charging of bodies introduced to plasma (due to much higher mobility of electrons in comparison with ions). The value of the charge accumulated on the nanoparticle may be regulated by adjusting the plasma parameters and by the location of the nanoparticle injection point (since the timescale of particle charging in plasma and its flight time through the jet are comparable, both about microseconds [44]). Charging of nanoparticles in plasma enables utilization of electrical field for microscopic level control of nanoparticle characteristics. Symbioses of cold plasmas and nanoparticles can lead to some extremely interesting results. One very promising example of such synergy is cancer therapy. Using antibody-conjugated nanoparticles with cold plasmas led to the five-fold enhancement of melanoma cell death compared to the anticancer efficacy of the plasma treatment alone [45]. The specific mechanisms leading to such a remarkable improvement in the anticancer efficacy are not fully understood.

This paper focuses on direct uses of nanoparticles and nanostructures in the treatment of diseases (i.e. used as active drugs and therapy) so as to explore their synergy with low-temperature plasmas whose biomedical applications are developed primarily as a novel therapy (see Section 4). It is worth noting that most biomedical applications of nanoparticles are related to drug delivery and therapeutic uses of nanoparticles represents only about 2% of nanomedicine publications in 1984 – 2004 and 3% of nanomedicine patent filings worldwide in 1993 – 2003 [46]. So far, 24 nanotechnology-based therapeutic products have been approved for clinical use with total sales exceeding \$5.4 billion [46]. Targeted diseases include leukaemia and cancer, infectious diseases (e.g. fungal and protozoal infections; hepatitis A, B and C), immunodeficiency diseases (e.g., HIV). While cytotoxic effects of metallic materials (e.g. silver) are employed for nanotechnology-based therapies [47], there are increasing interests in encapsulation of nano therapeutic agents in polymers for both treatment of diseases and their preventative vaccination [48]. The reader is referred to recent review papers [48][49].

#### **4. Interaction of Plasmas with Cells:**

For treatment of living tissues, low-temperature plasmas are at present used largely for therapeutic purposes although plasma-assisted imaging is conceivable. Their biological effects are predominately through their non-equilibrium chemistry, highly unique and otherwise difficult to access because of the very high degree of chemical (in particular, electron-impact) dissociation in the plasma. Low-temperature non-equilibrium gas plasmas present a unique environment of reactive oxygen species (ROS) and reactive nitrogen species (RNS), charged particles, photons as well as heat, pressure gradients, and electrostatic and electromagnetic fields, many of which are known to induce biological effects. For example, nitric oxides (NO) are known to promote cell proliferation [50] whereas

hydroxyl radicals ( $\text{OH}^{\bullet}$ ) are very effective against bacteria [51]. The interest in the use of non-equilibrium gas plasmas in healthcare has grown very strongly over the past 10 years [52][53], largely owing to an important technological breakthrough that enables the generation of low-temperature atmospheric pressure plasmas with well controlled thermal stability even in electronegative gases such as oxygen, air, and water vapour [54]-[57]. Such low-temperature plasmas are commonly known as cold atmospheric plasmas (CAPs) and have been shown to be capable of effectively inactivating bacteria, fungi and virus, inducing apoptosis in cancer cells, and stimulating proliferation of mammalian cells, all in a dose-dependent fashion [52][53]. Today, CAPs are increasingly used in an impressive array of healthcare applications such as blood coagulation [58], skin disinfection [58][59] and wound disinfection [60], wound healing [61], sterilisation of surgical instruments and medical devices [62], cancer therapy [63], and food decontamination [64]-[66]. Their potentials for healthcare are profound and their clinical successes so far are exceptionally encouraging [67][68], thus fuelling a rapid expansion of research activities in an emerging but now highly visible field known as plasma medicine. For details of the opportunity, challenge and perspectives of plasma medicine, the reader is referred to several recent reviews [52][53].

#### 4.1. Modes of plasma interaction with biological samples:

With a mean-free path-length as short as a few tens of nanometres in atmospheric pressure plasmas [69], their temporal stability is difficult to maintain over a large gap distance between two electrodes and as such their electrode gap is typically a few millimetres. When a CAP is brought close to a sample to be treated (e.g. an infected human tissue), the sample may become a part of the plasma-containing electric circuit. Here the discharge current flows through the sample and charged particles are likely to play an important role. This mode of plasma treatment is sometimes known as *the direct mode of plasma-sample interactions* [70]. By contrast, the CAP may be used as an afterglow to treat a sample, which is electrically disconnected from the plasma-containing circuit and experiences only little discharge current. The role of charged particles is much reduced and neutral reactive plasma species are likely to play a more dominant role. This mode is sometimes known as *the indirect mode of plasma-sample interactions*. The choice of how a CAP may be configured to interact with a cell-containing sample depends on many inter-dependent factors, many of which are highlighted in Table 1.

| Table 1 Main considerations of CAP design  |
|--|
| Global considerations:   |
| <ul style="list-style-type: none"> <li>• CAP scalability vs localised treatment</li> <li>• Spatially uniform/controlled treatment</li> <li>• Temporally reproducible treatment</li> <li>• Control of gas temperature</li> <li>• Effect of UV, ozone, and <math>\text{NO}_x</math></li> <li>• Presence of liquid</li> </ul> |
| Reaction Chemistry :   |
| <ul style="list-style-type: none"> <li>• Use of charged particles vs neutral species</li> <li>• Role of ROS and RNS and their lifetimes</li> <li>• ROS/RNS transport through liquid and their generation in liquid</li> </ul>  |
| Plasma-cell interactions:  |
| <ul style="list-style-type: none"> <li>• Plasma effects on cellular components</li> <li>• Plasma penetration through cells and tissues</li> <li>• Effect of electric and electromagnetic fields</li> <li>• Safety and toxicity</li> <li>• Selectivity between pathogens and cells</li> </ul>                               |

Figure 1 shows some of the common CAPs currently used in medical applications. Here we consider the CAP jet (see Fig. 1a) [71]-[74] as an example and discuss how some of the current understanding of plasma-cell interaction is used in CAP designs. Formed in a flowing gas, the CAP jet is well suited for precision and localised treatment, such as root canal disinfection [75]-[77] and cell transfection [78]. Some of the current CAP jets rely on the generation of high-intensity plasma in the upstream electrode region (sustained in a radial-directed electric field) and its axial diffusion towards a downstream sample is often unaided. Such devices are known as *the cross-field plasma jet* [79]. A combination of an upstream localised plasma and the short lifespan of many reactive plasma species (typically several tens of microseconds or shorter) means that the biological efficacy of such CAP devices is critically dependent on a very short plasma-sample distance in the region of a few millimetres (often just 1 – 3 mm). Longer plasma-sample distance is desirable for downstream

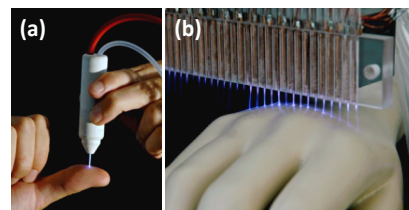


Figure 1: (a) A CAP jet treating a thumb and (b) a CAP jet array treating a hand, both pictures taken at Loughborough.

introduction of gas and liquid precursors, and for controlled treatment of uneven surfaces. To this end, the upstream plasma may be spatially extended by a strong axially directed electric field [79]. With an axially directed flow of the plasma-forming gas, both charged and neutral species can be significantly extended towards downstream to facilitate effective delivery of short-living plasma species to the sample surface. With this strategy, it is possible to extend the plasma jet length to many centimetres and even more than 10 cm [80]. The alignment of the electric and the gas flow fields in such CAP jets (often known as *the linear-field plasma jet* [79]) encourages better penetration of plasma species (see below for further discussion) into a living tissue placed downstream. Importantly, the linear-field plasma jet provides two additional important advantages, when nanoparticles are driven through a plasma region for uptake of reactive plasma species (see further discussion in Section 5.2). Firstly its strong axial electric field can be used to drive the flow of the electrically charged nanoparticles. Secondly, its much longer plasma plume length both enhances the uptake of reactive plasma species by the passing nanoparticles and sustains the ROS/RNS uptake right to the entrance point of the nanoparticles into a tissue. The linear-field plasma jet is therefore ideally placed for synergistic combination with nanoparticles.

Diameter of a single CAP jet is typically a few millimetres [81]. For treatment of large samples having a width of a few centimetres and greater, such as chronic wounds, it is essential to overcome the inherently small size of CAPs through scaling-up [69][81]. This has been shown to be technologically feasible, as shown in several reported up-scaled CAPs such as the 1D and 2D CAP jet arrays (see Fig. 1b) [81][82], the microwave plasma torch [83], and the floating-electrode dielectric-barrier discharges (DBDs) [58]. While the development of these large CAP systems was initially motivated by practical considerations of scaling-up, their interactions with cells and tissues contained in a downstream sample differ from each other. For example, energetic ions with their kinetic energy reaching above 20eV are important in a sample treated by the floating electrode DBDs [84] whereas ions are largely absent in the downstream sample treated using the microwave plasma torch [83]. Depending on the electrode configurations, the CAP jet array can be made to control the involvement of ions and indeed charged particles [85]. Plasma-cell interaction mechanisms are multi-facets and at present are not fully understood. However different applications are likely to be best addressed with different up-scaled CAP sources.

It is important to note that in practice diseased living tissues are either moist or covered by a layer of liquid (e.g. body fluids, blood, and/or wound fluids). When a CAP jet is used to treat a living tissue, its plasma species are delivered to the air-liquid interface and then undergo transportation, and sometimes secondary ROS/RNS generation within the liquid medium, before reaching cells and tissues. Therefore it is important to consider plasma reactions that interact with water molecules [86]-[88].

#### *4.2. Reactive plasma species and their biological targets:*

Reaction chemistry of a CAP device varies considerably with chemical composition of the plasma-forming gas even with the same electrode configuration and the same applied voltage. In a study comparing an atmospheric air plasma with its counterpart in helium/oxygen both sustained in sub-microsecond voltage pulses, it is observed that the air plasma is dominated by  $N_2^*$  species with their emission in the UVC region whereas the helium/oxygen plasma is dominated by oxygen atoms [89]. It is of particular interest to contrast these against bacterial inactivation kinetics data and establish a hierarchical list of bactericidal plasma agents (and their threshold bactericidal doses). However, the distinct difference in reaction chemistry is found to result in a small difference of mere 10 seconds in the timescale over which the two pulsed CAP systems need to achieve 6 log reduction of bacterial spores [90]. In other words, very different combinations of plasma species may yet result in similar efficacy of bacterial inactivation. Different chemical compositions of “lethal plasma dose” seem to exist, each with different synergistic effects among various plasma species, and it appears that the bactericidal effect of a specific plasma species may be compensated by those of others. It is therefore highly probable that there exist many different and viable recipes of bactericidal plasma chemistry, each of which may take different pathways towards bacterial death (e.g. protein, lipid and DNA).

In the gas phase (i.e. before reaching the cell-containing liquid medium), plasma produces UV photons and many bactericidal ROS/RNS, including singlet oxygen ( $O_2^*$ ), atomic oxygen ( $O/O^*$ ), superoxide ( $O_2^{\bullet-}$ ) and nitric oxide (NO). In a moist or liquid environment, hydroxyl radicals ( $OH^*$ ) and hydrogen peroxide ( $H_2O_2$ ) are produced. Complex physicochemical processes in the plasma impose a limit on the maximum achievable concentration of each plasma species and result in a close cross-dependence between concentrations of different plasma species. It is both intriguing and important to note that many of these ROS/RNS are also produced endogenously within the human body itself and are involved in its defence against bacterial infection through, for example, Fenton chemistry [91]. Therefore in a way, plasma disinfection mimics how the body responds to bacterial infection. Having been produced in plasma, ROS/RNS are then delivered to cells and tissues exogenously and their delivery is subject to atmospheric absorption of photons and recombination of short-living species outside the plasma zone. This can filter out the effects of some ROS/RNS, since their concentrations at the cell/tissue location may fall below the threshold of their biological effects. For example, current evidence of bacterial inactivation studies suggests that UV photons play a less important role than ROS [92]-[94]. Figure 2 shows a damaged membrane of *B. subtilis* spores after plasma treatment, typical of a result of oxidation.

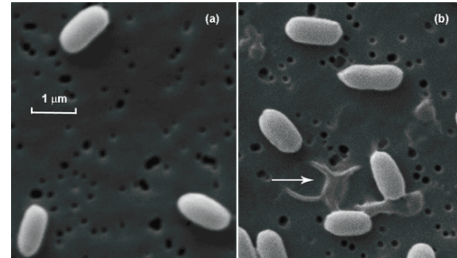


Figure 2: SEM images of *B. subtilis* spores (a) before treatment of a He/O<sub>2</sub> CAP jet and (b) after plasma treatment [100]. Reprinted with permission from IEEE Publishing, 2006.

Upon the arrival of plasma species on the surface of microorganisms, their uptake by pathogens and infected tissues is affected by how plasma species interact with the cell surface structure (e.g. receptors, membrane proteins) and whether they penetrate into the microorganism. These factors mean that the biological effect of each possible plasma agent is likely to be kept within a range of plasma dose and penetration depth. This view also applies to diseases other than bacterial infection such as cancers.

Important insights have been gained from investigations on possible plasma-inflicted damages to key cellular constituents. Studies using isolated protein and plasmid DNA models have shown protein and DNA damages [95]-[99], and more relevant experiments using bacterial cells have provided direct evidence of breaching and rupture of cell membrane [100], reduction and degradation of proteins [101]-[103], lipid damage [104], and (mostly) single-strand breaks (SSB) of DNA [105]. These results have prompted the suggestion of the involvement of ROS, charged particles, and UV photons [92][100][105] -[108], with ROS often linked to oxidation of protein and lipid and with UV photons linked to DNA damages. The view of DNA damages, particularly double-strand breaks (DSB), being largely induced by UV and shorter-wavelength radiation has its root in radiation biology where many hundreds of DSBs per microorganism are not uncommon among irradiated bacteria [109]. The prevailing view of bacterial killing in radiation biology has been “death by DNA damage” for the past 50 years is now challenged and is being replaced by the alternative view of “death by protein damage” for which the role of ROS and RNS is becoming increasingly important [110]. This development in radiation biology may become important in informing and formulating the direction and priority of future studies of plasma-mediated mechanisms against pathogens. It should have a similar impact on studies of plasma-safety for healthy mammalian cells and those of plasma-mediated killing of cancer cells.

The long-standing attempt to understand plasma inactivation mechanisms has very often suffered a common lack of an appreciation that even the same plasma source can have very different plasma chemistries as it transits, often abruptly and rapidly, from one plasma mode to another [89][111]. Similarly under-appreciated is the paucity that the resistance of microorganisms to external stresses is influenced by their micro-environment [112], an obvious example being their interaction with the surface of their supporting substrate [113]. Future studies are likely to include detailed characterisation of both plasma and microbial sample conditions.



Studies of plasma inactivation mechanisms have in the past paid little attention to the effects of the liquid environment that contains bacteria. Energetic electrons, ions and UV photons can react with water molecules and produce additional reactive oxygen species in the liquid medium. Equally importantly, it is possible that plasma treatment may release oxygen, nitrogen, and hydrogen species directly from the chemical structure of cells and tissues and the latter can then be used to alter the local reaction chemistry on the surface of cells. These processes may be referred to as the secondary production or *the on-site production* of ROS/RNS. They introduce both an exciting opportunity for plasma medicine and a complex challenge to the study of plasma inactivation mechanisms. From the standpoint of how ROS and RNS are used to defend against bacterial infection however, the key reactive species include  $O_2^*$ ,  $O_2^{\cdot-}$ ,  $OH^*$ ,  $H_2O_2$ , and  $NO$  and their cellular targets are largely known [114]. These can be considered as the starting group of priority species in mechanistic studies. Similar prioritization of key plasma species may be achieved for treatment of cancers.

#### 4.3. Selectivity and Safety:

When plasmas are used to treat diseased living tissues where pathogens or cancer cells co-exist with healthy mammalian cells and tissues, an important challenge is to achieve plasma selectivity with lethality against pathogens but little damage to healthy mammalian cells and tissues. This is one of the most important questions in plasma medicine but surprisingly has been scarcely addressed in detailed *in-vitro* investigations though histological data from *ex-vivo* and *in-vivo* experiments with animals have so far shown limited damage [52][60]. Mammalian cells have a different structure to bacteria (see Fig. 3), and their responses to external stresses are also likely to be different. In radiation biology, bacteria and pathogens are more resistant to radiation than mammalian cells. Whether the desired reversal exists for plasma produced ROS/RNS remains both critical and intriguing. From the limited *in-vitro* studies reported so far, it has been known that the selectivity window between bacteria and fibroblast cells is significant against *E. coli* [116] but limited against *Staph aureus* [117]. Using an air plasma afterglow, a significant selectivity window is found against bacteria spores [90], suggesting that significant selectivity is attainable even though much more work is needed to examine the presence of possible subtle long-term damages. Similarly there is considerable scope for detailed selectivity studies for plasma-based cancer therapies.

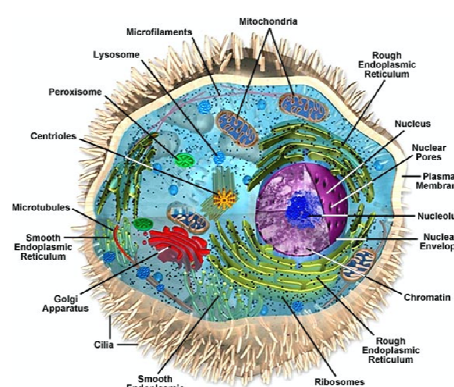


Figure 3: Structure of an animal cell.

Reprinted from ref. [115] by permission from National High Magnetic Field Laboratory, Florida State University, USA.

One practical complication to selectivity is the host-cell interaction where bacterial or cancer cells invade and infect an initially healthy tissue and then may penetrate into the bulk of the tissue thus escaping the impact of the exogenously applied plasma treatment [118]. Bacterial penetration into and co-existence with the host tissues means that the same externally applied plasma dose may be received differently by the pathogens and their host tissues thus complicating the selectivity established from experiments using cell media. While this presents an uncertainty, a useful indicator of its possible consequence is that some non-thermal atmospheric plasma jet systems have already been used in minimal invasive surgery, for example those for removing diseased tissues [67][68][119][120]. These clinical successes provide a critical context of realism and relevance with which to understand the broad nature of the long-term effects of low-temperature atmospheric plasmas [121].

#### 4.4. Plasma Penetration:

The issue of migrating bacteria into the bulk of a living tissue raises the question of plasma penetration. Estimating from the half-lives of the main plasma species, the maximum penetration depth of plasma ROS/RNS into a liquid medium is perhaps at most a few tens of micrometres,

depending on physiological conditions of the liquid medium. For example,  $\text{OH}^-$  radicals are known to propagate for no more than a few angstrom in liquid [110][122] whereas the singlet oxygen has a half-life of only a few microseconds in liquid [123]. On the other hand,  $\text{H}_2\text{O}_2$  are long-living and can diffuse throughout the cell [110][122] even though their reactivity is only modest and, against some pathogens, ineffective. Penetration of ROS/RNS into the skin or a living tissue is even more limited. Therefore the plasma treatment is characteristically topical.

The topical character of plasma treatment is an advantage for localised surgery where spatial control of damages to healthy tissues is critical [119][120]. For living tissues infected exogenously such as open wounds, plasma treatment of the tissue surface may be applied frequently to contain surface infection. Similarly, topical plasma treatment is useful for melanoma tumours. When a diseased tissue is located considerably away from the skin surface, the topical nature of plasma treatment becomes a serious limiting factor. Greater plasma dose may be effective for infected tissues lying immediately below the skin or wound surface, but the issue of plasma toxicity would become a counteracting factor. There is clearly a need for assisted plasma penetration.

It is known that adjustment of pH may considerably increase the half-lives of some ROS [108], such as  $\text{O}_2^{\cdot-}$  which could be made to last up to 10,000 s in liquid [124]. To see whether such extended half-lives of plasma species could enable some ROS to penetrate through the skin and tissues, we note that there are openings and channels in the skin to allow diffusion of small molecules and other externally applied agents such as plasma species. These include the lipidic intracellular routes (5 – 36 nm in diameter) and trans-follicular routes (10 – 210  $\mu\text{m}$  in diameter) [125]. The stratum corneum (SC) and viable epidermis (VE) of the skin (see Fig. 4 and [126]) are about 32  $\mu\text{m}$  and 65  $\mu\text{m}$  respectively, and the length of an aqueous channel through the skin is much greater than the combined depth of SC and VE. We assume that the effective aqueous channel is in the order of 1 mm and the diffusion coefficient of superoxide anions in liquid is in the order of  $D = 8 \times 10^{-5} \text{cm}^2/\text{s}$  [127]. For a lifetime of 10,000 s, the diffusion length of  $\text{O}_2^{\cdot-}$  is  $2(Dt)^{0.5} = 17.9 \text{mm}$ . In fact, the lifetime of  $\text{O}_2^{\cdot-}$  would only need to be more than 32 s for their passage through a 1mm long aqueous channel. Therefore it is possible for reactive plasma species to survive their passage through aqueous channels in living tissues and reach a deep-embedded disease site if the physiological conditions of the aqueous environment could be appropriately adjusted. In addition, it is possible to transiently increase the diameter of lipidic intracellular routes by using, for example, electroporation. This should enable a greater passage efficacy of plasma ROS/RNS through aqueous channels of living tissues. It is worth noting that the delivery of plasma ROS through the membrane of a mammalian cell is also possible as indicated in early *in-vitro* evidence of cell permeation of plasma species [78]. Penetration of plasma species through the membrane of a bacterial cell is possibly more difficult because of much narrow channels through the membrane (~ up to a few tens of nanometres in diameter).

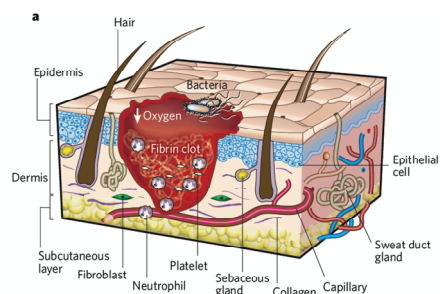


Figure 4: Structure of a skin with an open wound [126]. Reprinted by permission from Macmillan Publishers Ltd, 2008.

The use of such long-living ROS as pre-made antimicrobial or anti-cancer agents implies that liquids after plasma pre-treatment become a drug [128][129]. In this context, research of plasma medicine is becoming the study of how plasma-enabled drugs may be used effectively and safely (i.e. *Plasma Pharmacy*) as well as the study of mechanisms or modes of actions with which plasma-enabled drugs cure their intended diseases (i.e. *Plasma Pharmacology*). It is known that endogenous ROS and antioxidants are often unbalanced in immune-compromised patients and yet their balance is critical in the defence of a patient against bacterial infection and in regulation of many essential biochemical functions. The possibility of pre-prepared long-living plasmas species is exciting, since they can be used as a novel and unique agent to address an imbalance between endogenous ROS and antioxidants thus allowing a patient to fight more effectively against infection and cancer. In addition to directly produced plasma ROS/RNS, it is possible to produce energetic ions at a streamer head [84] and/or energetic electrons in plasmas formed in a micro-scale cavity [130][131] deep inside a living tissue.

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Such energetic charged particles may be extended into a tissue from a parent plasma outside the tissue, and they allow for an in-tissue production of ROS/RNS since their kinetic energy is sufficient to react to water molecules and some chemical bonds of the tissue structure. As a result, they can also be used to address potential imbalance between endogenous ROS/RNS and antioxidants. Such in-tissue production of ROS/RNS may be considered as *long-range effects of plasma species*, and they are almost completely uncharted as an area of investigation but represent a very important route for plasma penetration and indeed plasma pharmacy.

In summary, cold atmospheric plasmas are shown to be capable of inactivating microorganisms and cancer cells through unique non-equilibrium chemistry. They are exceptionally versatile, offering different modes of interacting with a cell-containing sample, and they employ various reaction chemistries to unlock different pathways towards an intended therapeutic objective (e.g. skin disinfection, wound healing, and cancer therapy). With appropriate use of plasma chemistry, selectivity appears to be attainable with controlled safety. CAP treatment is normally topical, ideally for localised cell and tissue manipulation as demonstrated in clinical successes of endoscopic surgery procedures. However there are exciting opportunities to enhance or induce significant penetration of plasma species into a living tissue, with energetic ions and electrons potentially capable of triggering *an in-tissue production* of ROS/RNS in a disease area. In addition, plasma pre-treatment of a physiologically-optimised liquid medium (e.g. using pH adjustment) may allow the in-liquid production of ROS/RNS that last sufficiently long to survive their passage through tissue. The delivery of both energetic charges (for in-tissue ROS production) and pre-prepared plasma ROS (for penetrating to a remote site) offers a great opportunity to work with the chemistry of endogenous ROS/RNS, by addressing the potential imbalance of endogenous ROS/RNS and antioxidants. The role of CAPs as a complementary regulator of free radical biology within the body may open up a whole new host of opportunities to treat human diseases.

## 5. Synergies between plasma and nanoparticles:

Both the nanoparticle technology and the cold plasma technology offer exceptional opportunities to biomedicine. There are growing momentums that drive forward each technology towards a full realisation of their immense promise to healthcare, and there are significant evidences that each technology is likely to influence very strongly how modern medicine may be practiced in future. While the exceptional scale of these opportunities tends to encourage a focus on one technology alone, there may be even greater opportunities in their synergy. Given that CAPs have so far been used largely for therapeutic purposes, our discussion of synergy is confined to the treatment of diseases.

### 5.1. Comparison of CAPs and nanoparticles:

For treating diseases, medical applications of CAPs and nanoparticles often require at least four considerations, namely their reactivity, their selectivity against pathogens or cancer cells, their toxicity to healthy mammalian cells and tissues, and their penetration to the contaminated regions. Table 2 is a summary of the properties of CAPs and nanoparticles when compared against the above four considerations. CAPs provide non-equilibrium reaction chemistry with many of their main reactive species also present in the body with known effects on bacterial and mammalian cells [114]. The biological implication of plasma chemistry is not dissimilar to those related to free radical biology. Provided that future studies result in appropriate understanding and control of plasma dosage, it is highly probable to achieve selective kill of pathogens or cancer cells with little damage to healthy mammalian cells and tissues. There have been clear successes of some CAP-based clinical procedures [119]-[121].

|             | CAPs                          | Nanoparticles   |
|-------------|-------------------------------|---|
| Reactivity  | ROS/RNS, UV Charged particles | Intracellular ROS, metal, nanomaterials                                   |
| Selectivity | Potentially large             | Can be very high subject to appropriate functionalization/ Bioconjugation |
| Toxicity    | Possibly controllable         | Varied, depending on material, structure, surface chemistry, etc.         |
| Penetration | Topical                       | Penetrating   |

On the other hand, nanoparticle-based therapies are yet to enjoy a comparably similar level of clinical acceptance despite of far greater extent of scientific advances [132] and this is partly related to the current anxiety over their toxicity. Many nanoparticles generate ROS when incubated with different cells or when inoculated *in vivo* [133], and nanotoxicity represents both a key area of research and a significant uncertainty [134]. Notwithstanding future progress of nanotoxicity research, it is highly desirable to reduce the minimum amount of nanoparticles necessary for a targeted therapeutic effect. This is one of many areas where plasma-nanoparticles synergy is particularly beneficial.

## 5.2. Synergy in reaction chemistry:

Consider the *in-vitro* study discussed in Section 3 in which antibody-conjugated nanoparticles were used to enhance plasma-mediated killing of melanoma cells [45]. It may have benefited from a synergetic combination of the anticancer properties of both CAPs and antibody-conjugated nanoparticles. This synergy can and should be taken advantage of to reduce the minimum plasma dose against cancer cells and as a result reduce plasma toxicity. In reversal of roles, plasmas could be used as a supplementary agent to assist nanoparticle-based therapies against cancer and/or infection. It has already been shown that plasma species induce apoptosis among cancer cells (*in-vitro* device) [52] as well as suppress the growth of melanoma and lung cancers (*in-vivo* evidence) [63]. So plasma pre-treatment of a tumour can reduce the defence of its cancer cells and as a result reduce the minimum inhibition concentration of intratumorally injected nanoparticles when used alone. This is yet to be reported in open literature but is anticipated from the current understanding of anti-cancer effects of both plasmas and nanoparticles. The obvious patient benefit of reduced risk of nanotoxicity could be potentially profound for nanoparticles-based therapies, as it is likely to alter the parametric space and indeed the paradigm of nano-safety. Furthermore, the benefit extends also to other major diseases such as skin and wound infection, infectious diseases, and wound healing.

One exciting area of plasma-nanoparticle synergy is to combine the unique non-equilibrium plasma chemistry with the superior penetration of nanoparticles. Such synergy can be realized at the stage of nanoparticle synthesis or post-synthesis processing. This is illustrated in Figure 5. Suppose nanoparticles are produced first with an iron core, which can be made to acquire a high affinity to oxygen atoms and hence trap oxygen atoms, for example if the core has a porous structure of appropriate pore size, shape, and surface-volume ratio [135]. The iron nanoparticles are then coated with biocompatible silica (with reduced toxicity and optimized wettability) and finally bio-conjugated by attaching specific molecules that are designed to bind with specific proteins in a targeted cell. Such nano-particles can be delivered to a diseased area by, for example injection via bloodstream, and by minimising dissolution during their passage to the diseased site. The magnetic core of the nanoparticles can then release heat and oxygen atoms to the proteins of the targeted cell to which the nanoparticles are attached, by means of redox reactions. The release of oxygen atoms onto a buried site of a tumour tissue or an infected tissue induces a site-specific effect against either cancer cells or pathogens. While fabrication of such multifunctional nanoparticles is a major challenge, the use of plasmas in the fabrication of the nanoparticles allows the nanoparticles to be immersed in oxygen atoms of very high concentrations thus achieving high uptake rate. The bonding of oxygen atoms onto the nanoparticles needs to be not too strong so as to be released readily on the target site, yet not too loose to avoid being lost on their way to the targeted site. Nanoparticles of different surface-to-volume ratio release oxygen atoms at different locations down a tumour body or an infected tissue. If the diseased tissue is subject to a concomitant plasma treatment so that pores and other aqueous channels

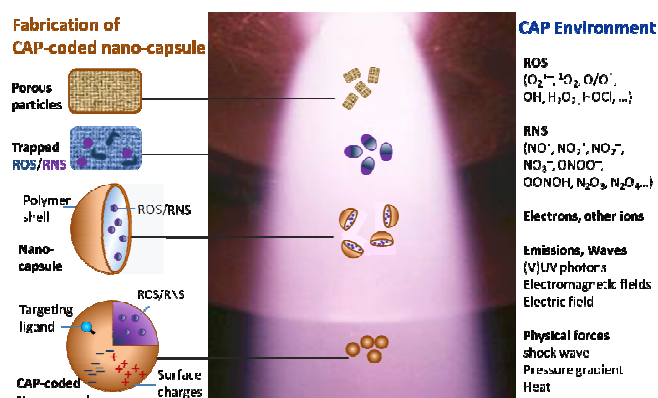


Figure 5: Fabrication of nano-capsule with enclosed plasma ROS and/or RNS, a polymer coat, and conjugated surface-borne, protein-binding molecules, fully completed in a cold atmospheric plasma (jet) environment.

are temporally opened by plasma (see Section 5.3), the delivery of the nanoparticles carrying oxygen atoms will be made more efficient.

Discussion in Section 4.4 indicates that some ROS (e.g.  $O_2^{\bullet-}$ ) may be made to last longer and could also be carried by nanoparticles as in the case of oxygen atoms, though further work is needed to substantiate its practicality. The above discussion suggests a new concept of plasma-seeded nanotechnology in which nanoparticles are prepared with encapsulated plasma reaction chemistry by immersing their fabrication in a non-equilibrium plasma environment where very high concentrations of ROS and RNS can be up-taken by nanoparticles. Material properties and surface functionalities of nanoparticles are hardly exploited for trapping and sustaining ROS/RNS during the production of nanoparticles and before them being coated and bio-conjugated.

The above synergy takes advantage of the superior capability of nanoparticles to penetrate through the skin barrier [30], thus liberating plasma from its limitation as an intrinsically topical technique. The potential specificity of nanoparticles to attach to diseased cells as against healthy cells [136] offers selectivity, and the use of ROS or RNS carried by nanoparticles as the main agent against either cancer cells or pathogens reduces the reliance on toxic materials (e.g. silver). These anti-cancer or disinfecting effects are realised selectively on a nano-scale and their study may be considered as *nanoscale plasma pharmacy*. While there are clearly significant engineering and scientific challenges, the benefits and implications of nano-scale plasma pharmacy are very exciting.

### 5.3. Synergy in cell permeation and cellular manipulation:

There have been some reports of plasma-mediated cell permeation [78], and possible mechanisms may include surface deposition of charges, electroporation by global and local electric field, exothermic recombination of excited species and radicals, and locally released heat. Energy and/or heat can be released by either plasmas or nanoparticles, though via different routes. Once above a certain threshold, the global effects of the released energy can contribute to cell permeation. However localised energy release is of greater interest. Plasma ROS/RNS may act on specific sites on the cell surface and exothermic radical recombination is likely to be surface roughness-selective [137], both capable of localised deformation to the cell surface and hence induction of enhanced cell permeation. Furthermore, plasma ROS are known to degrade adhesion proteins [138] and integrin [139]. These alter the cell-substrate and cell-cell attachment, thus deforming the cell and affecting permeation of its membrane channels. Equally, it is possible to achieve a spatially preferential pre-deposition of nanoparticles at specific cell surface sites (e.g. surface proteins and integrin) so that localised plasma degradation and hence cell deformation are enhanced, leading to more significant cell permeation and intracellular uptake of plasma species.

Electrically assisted or mediated cell permeation is of particular interest, as electroporation [140] can be induced at the level of electric field in atmospheric plasmas. For example, the electric field is at the order of 0.5 kV/cm at the tip of a CAP jet [74][141] and higher at that of a plasma streamer [84]. In RF microplasmas, the sheath electric field is of the order of 80 kV/cm [130]. The temporal opening of membrane pores, usually over a microsecond timescale, allows increased intracellular uptake of plasma species and/or nanoparticles. It is worth noting that the electric charging time of the membrane of a mammalian cell is about a few hundreds of nanoseconds for mammalian cells [140]. When the temporal scale of the electric field in the plasma is shorter than the electric charging time of the cell membrane, strong intracellular electric field can be established and this would lead to forced intracellular penetration of plasma species and nanoparticles. Nanosecond pulsed CAPs are known to be possible [142][143] and can be used to set up intracellular electric field inside a mammalian cell. This is useful in treating cancer cells. Equally, nanoparticles carrying charges could be rapidly deposited on the cell membrane over a nanosecond scale, for example through a CAP jet, and this can set up a permeating electric field into the intracellular space. Strong intracellular electric fields not only enhance the uptake of plasma species and/or nanoparticles, but also induce other important biological effects such as intracellular calcium release and enhanced gene expression [144]. Therefore

the benefits of electrically assisted cell permeation could go beyond enhanced uptake of plasma species and/or nanoparticles.

Interestingly, plasma-borne nanoparticles can be intentionally used to create a clear difference in morphological features on the surfaces of targeted and non-targeted cells. These properly labelled and functionalized nanoparticles can be delivered and distributed around the cellular surface thus acting as externally delivered “etching masks” which are commonly used in nanoscale plasma etching to enable selective treatment of the unmasked areas. Similarly, the ion focusing effect discussed above may lead to an effective and selective heating of the nanoparticles rather than the open surface areas. In this case one could expect ROS/RNS radicals to interact with the unmasked (open) areas of the surface only. On the other hand, selective heating of nanoparticles may lead to the controlled heating of the cell membrane underneath the nanoparticles. This may in turn induce heat-enhanced inter-diffusion of radicals between the nanoparticles and the cellular membrane. For comparison, these effects cannot be achieved using focused laser beams since the typical sizes of laser beam spots are in the micrometre range (which is comparable with the sizes of small cells) and cannot resolve sub-micrometer features on the cellular surfaces. By tailoring the nanoparticle sizes one can mask and treat surface areas of virtually any dimensions, which offers exciting opportunities for unprecedented increase in selective treatment at the cellular level or even the organelle level. The many important benefits discussed above stem from the opportunity to induce spatially differentiating effects, on a nanometre scale, by exploiting the synergy between plasma species and nanoparticles. For future reference, this may be referred to as *plasma-nano cellular manipulation*.

#### 5.4. Synergy for enhanced penetration and selectivity:

The above discussion on permeation through cellular pores can be extended to permeation through aqueous channels through a living tissue, though the timescale is likely to be larger because of the larger length scale of a tissue. Surface deposition of energy by plasmas and/or nanoparticles can be made spatially selective, and electrically induced temporal opening of pores and channels within a tissue is equally possible. It has recently been shown that an impinging plasma streamer incepting a tissue moves preferentially to a nearby channel in the tissue and establish a strong in-channel electric field there [84][145]. Consistent with the discussion in Section 5.3, the spatially extended electric field can trigger an in-tissue production of plasma ROS/RNS well away from the streamer-tissue inception point. This offers an interesting route for plasma penetration into a living tissue, particularly in tissue cavities and openings in a wound by putrescence or other disease-caused routes. Again, ROS/RNS can be delivered into a tissue by nanoparticles encapsulated with plasma ROS/RNS (see Section 5.2).

Synergistic combination of plasmas and nanoparticles could even facilitate *selective penetration*. It is known that nanoparticles may preferentially deposit near cancer cells rather than healthy cells [136]. Therefore a combined use of plasmas and nanoparticles is likely to enhance the directionality of the propagation of both plasma species and nanoparticles towards diseased sites within a tissue. There are many different implementation routes, some of which are realized in plasma-based nanotechnology, such as mutual orientation of ion fluxes and nanoarrays [146]. This orientation is very different for different surface morphologies. If the surface features some micro/nanostructures with reasonably large aspect ratios (e.g. >3-5), the effects of incoming ion fluxes and the resulting feature electric field enhancement effects may be very different. For example, the outer layers of many eukaryotic cells feature hierarchical brush-like structures. Different elements of this structure would thus draw different ion fluxes and would then be charged differently. More importantly, normal and cancerous cells have very different membrane external structure, so-called brush [136]. Therefore, the direct effect of the electric field (electric field enhancement) and charging is expected to be different for the normal and cancerous cells thereby providing an excellent opportunity to achieve the as yet elusive selective treatment of the normal and cancerous cells by plasma ion species.

It is possible to facilitate the charging and discharging of different locations in a tissue surface, using plasmas, nanoparticles or their combination, in order to establish possible ion-focusing channels for enhanced penetration of plasma species or nanoparticles. Many of the above-discussed enhancements

of plasma and/or nanoparticle penetration are enabled by charged particles and their electric field, which can be used to charge nanometre and micrometre scale structures and as such enable plasma chemistry on nano- and micro-features of a diseased area (either cells or tissue sites). The importance of charges and micro-scale tissue charging provides a unifying point for plasmas and nanoparticles – nanoparticles are most conveniently charged in plasma and much less useful by using other means. In addition, it is possible to control the charge polarity of nanoparticles by tuning plasma conditions [147] and, thus, to enrich the therapeutic potential of nanoparticles.

The enhanced penetration by synergising plasmas and nanoparticles offers a spatial selectivity and indeed a way to enhance their safe use as a therapeutic strategy. It offers an unexpected aid for up-scaling, since the selective move of a plasma streamer to a nearby channel means that a CAP jet array may preferentially establish strong electric fields in many natural or putrefied channels regardless their relative locations to the jet-tissue inception points. Plasma jet-specific feedback control helps maintain similar plasma-tissue interaction [81], and the design of the CAP jet array could become reasonably independent of the specific properties of the diseased tissue that it would be used to treat.

As one final example of synergistic approach for applications other than therapies, hydroxyapatite [HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] has been widely used to promote biological functions of various biomedical and dental implant/filling materials [148]. This bioceramic material is the main mineral constituent of bone and tooth tissues, and HA coatings have revealed inspiring clinical advantages in promoting efficient implant fixation and implant-to-bone adhesion shortly after the implantation, as well as faster bone remodeling due to enhanced bidirectional growth and formation of a bonding interlayer between bone and implant [149]-[151]. Recently, nanoparticle HA dental enamel paste has been used for rapid repair of early caries lesions without the need of removing of healthy tooth material, commonly practiced in dentistry to ensure the filling stick [152]. However, the issues of appropriate sterilization of the damaged tooth areas still remain. This is where CAPs and HA nanoparticles can be used simultaneously to implement both effects – the CAPs to be used not only as the carrier of the HA nanoparticles to the targeted tooth but also as simultaneous sterilization of the tooth surfaces. The HA nanoparticles, precisely delivered to the targeted growth sites, will then be used for dental tissue remodelling. This exciting synergistic opportunity awaits its realization.

## 6. Concluding Remarks:

Against a backdrop of truly immense progress in the presently parallel fields of the nano-technology and the cold atmospheric plasma technology for biomedicine [46]-[49],[52]-[53], this perspective article was motivated primarily by the intriguing contrast between these two well-established technologies in terms of their reactivity, selectivity, toxicity, and targeted penetration to diseased tissues. Inevitably the latter invites the question of their possible synergy and the related benefits to the ultimate goal of developing effective, selective, and safe therapies for modern medicine.

In terms of their underpinning science, nanomedicine is more advanced than plasma medicine. To this end, the paper started with a brief review of nanoparticle-cell interactions and a comprehensive review of plasma-cell interactions. A key advance of cold atmospheric plasmas is their non-equilibrium reaction chemistry with very high levels of chemical dissociation that is otherwise difficult to access and that strikes an intriguing similarity to the chemistry of endogenous ROS/RNS produced by cells themselves. Evidences of key plasma ROS/RNS and their cellular targets were presented, and, when appropriate, linked to those known in free radical biology and radiation biology. While normally used as a topical treatment, the cold atmospheric plasma technology has already found important clinical success in endoscopic surgery with good indication of their safety to healthy cells and tissues. For treatment of a diseased area buried inside a living tissue, technological viabilities of several plasma penetration techniques were suggested and assessed. These included the in-tissue production of ROS/RNS by microscale plasmas penetrating into aqueous channels in a tissue whilst reacting with water molecules and chemicals bound to the tissue structure. Another technique was direct penetration of physiologically adjusted liquids with their antimicrobial or anticancer effects induced by plasma

pre-treatment. Such plasma-treated liquids thus form a novel form of drugs, and their studies were referred to as *plasma pharmacy* and *plasma pharmacology*, an important avenue of future investigations of biomedical applications of plasmas. Plasma-mediated cell permeation was also discussed.

When plasmas and nanoparticles are combined, a number of important benefits were shown to emerge, such as reduced toxicity through combinatorial antimicrobial or anticancer effects of plasmas and nanoparticles. Given the current focus on safe use of nanoparticles, this synergy was indicated as particularly beneficial in reducing the minimum inhibition concentration of nanoparticles for cancer cells or pathogens. To advance one step further, one exciting opportunity of synergy was shown to be related to the fact that porous nanostructures may be used to trap reactive plasma species (e.g. oxygen atoms) before the nanostructure was encapsulated with a bio-polymer coat and then bio-conjugated. Coupled with the possibility that some plasma ROS may be made to extend their half-lives and so survive their passage to a diseased tissue, nanoparticles could become a carrier of pre-prepared plasma ROS/RNS. This was shown to be a powerful strategy, partly because plasma ROS/RNS could be delivered deep into the diseased tissue or even into an infected mammalian cell (thus overcoming the topical character of plasma treatment) and partly because less chemically reactive materials may be used for manufacturing nanoparticles thus mitigating the issue of nanotoxicity. While there remain considerable fabrication challenges, one implementation route was thought to involve nanoparticles being prepared in an atmospheric plasma jet where nanoparticles are immersed in very high concentrations of plasma ROS/RNS for efficient uptake and are charged simultaneously for subsequent directional penetration into tissues towards cancer or infected cells. These suggest the possibility of *nano-scale plasma pharmacy*.

Upon the arrival on the region of malignant cells, it was shown possible for nanoparticles to target specific cellular sites, for example, by difference in sub-microscale features on the cell surface and by mutual orientation of plasma ion fluxes and nanoarrays. Also shown possible was a pre-deposition of nanoparticles onto specific surface sites of cell membrane to form a spatially selective cell-surface mask for spatially selective treatment of subsequently applied plasmas. These opportunities could lead to an unprecedented selectivity of plasma/nanoparticle treatment at the cellular level or even the organelle level. This suggests a highly desirable capability of *nanoscale plasma manipulation of cell*, and it would mostly likely be materialized when the plasma technology and the nanotechnology are synergistically combined. As an illustration to the power of the plasma-nanoparticle synergy beyond direct treatment of major diseases, the use of nanoparticles for enhancing bone and tissue remodelling was considered. The benefits of this important nanotechnology would be enhanced significantly if a simultaneous step of plasma sterilization was used.

In essence, the synergy of low-temperature plasmas and nanoparticles aims to take advantage of distinct and different strengths of each of the two technologies in terms of reactivity, selectivity, safety, and diseases targeting. Its benefits go well beyond simple combinations of the two technologies and indeed can only be fully exploited when nanoparticles are synthesized and then post-processed/functionalized within the plasma environment, because of the need for an *in-situ* access to high density plasma ROS/RNS and because of the benefits of charged nanoparticles for preferential cell targeting. The plasma-nanoparticle synergy not only enables a step change in their performance matrix for biomedical applications, but also unlocks unexpected doors to many presently unknown opportunities. These combine to offer an unprecedented grand opportunity with potentially enormous impact on modern medicine.

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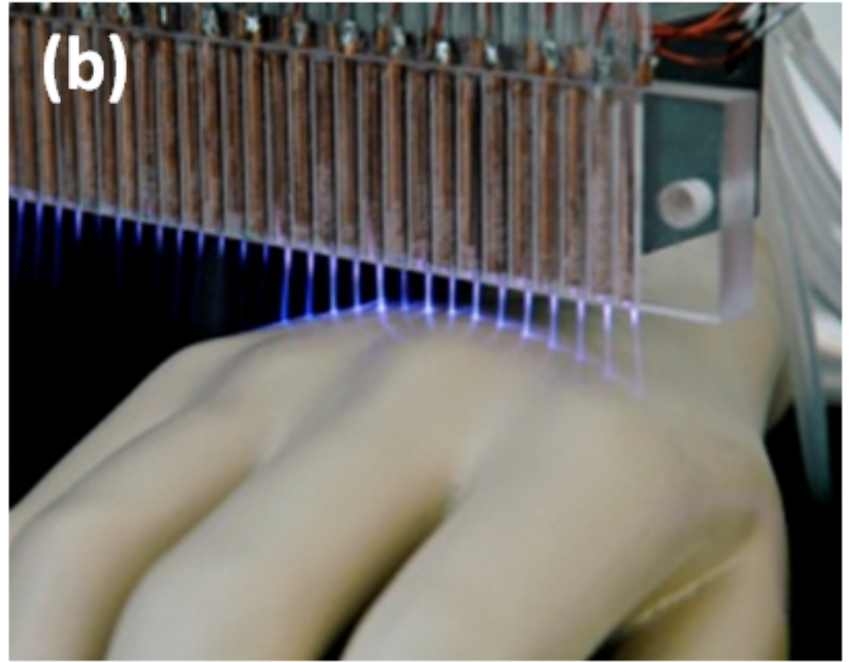


Figure 1 (Fig-1.tif)

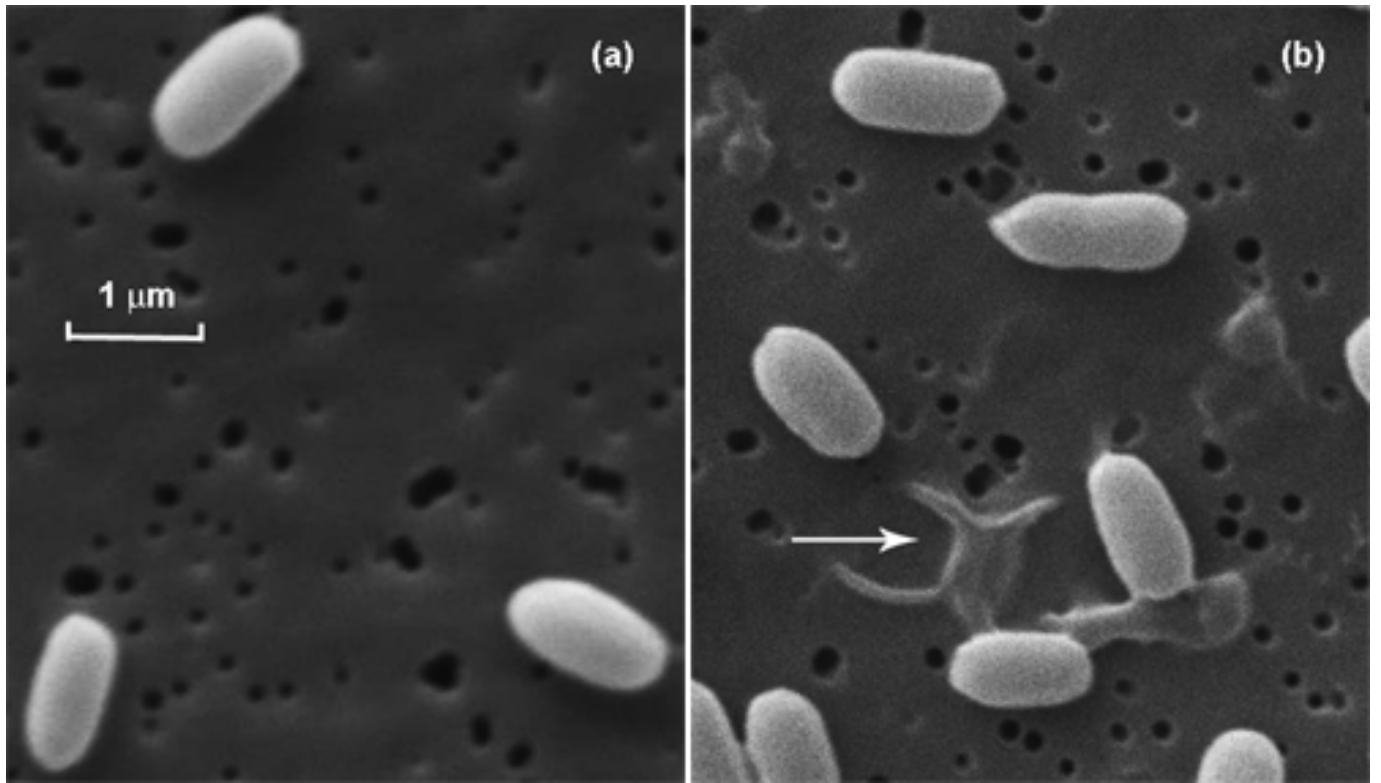


Figure 2 (Fig-2.tif)

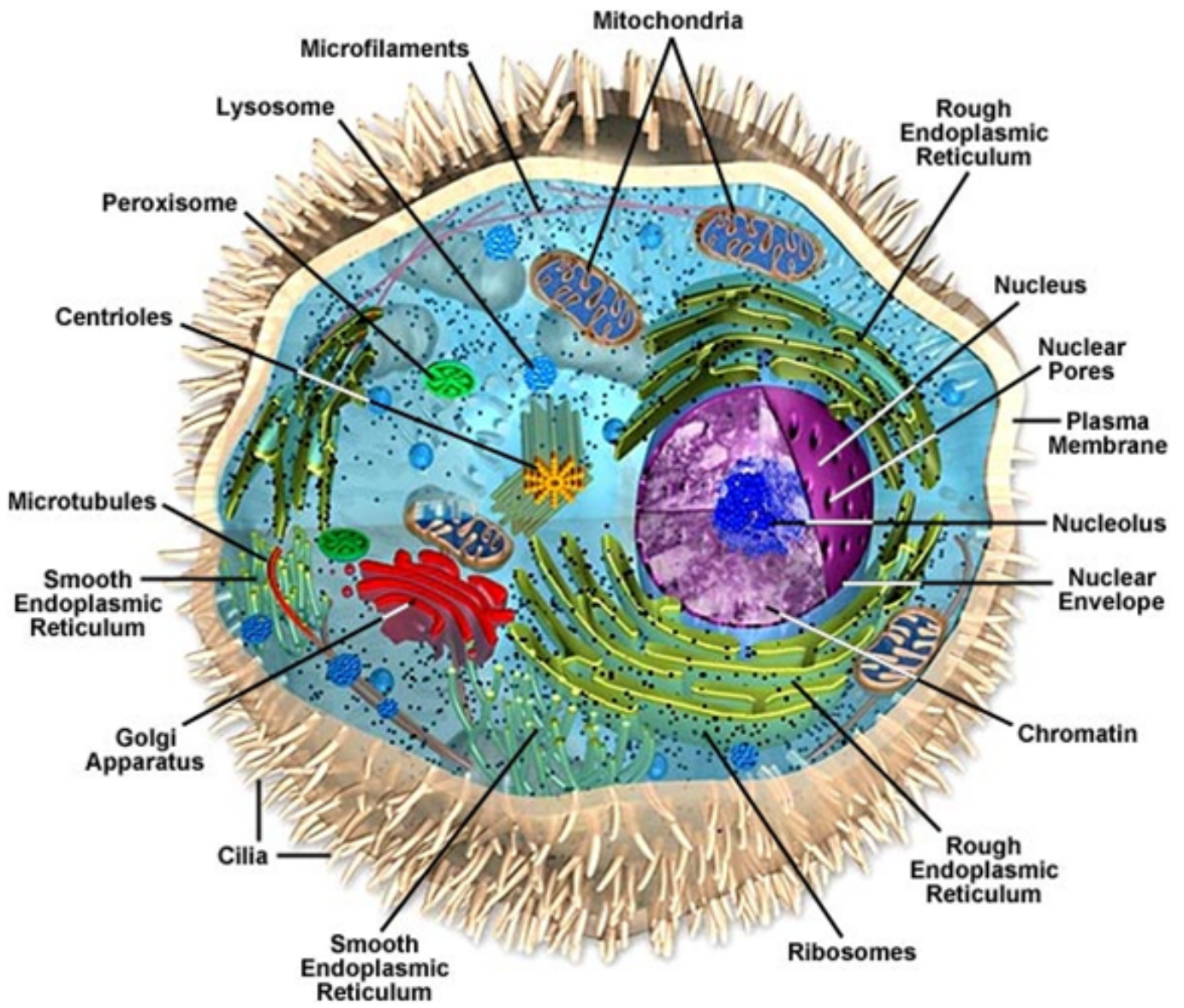


Figure 3 (Fig-3.tif)

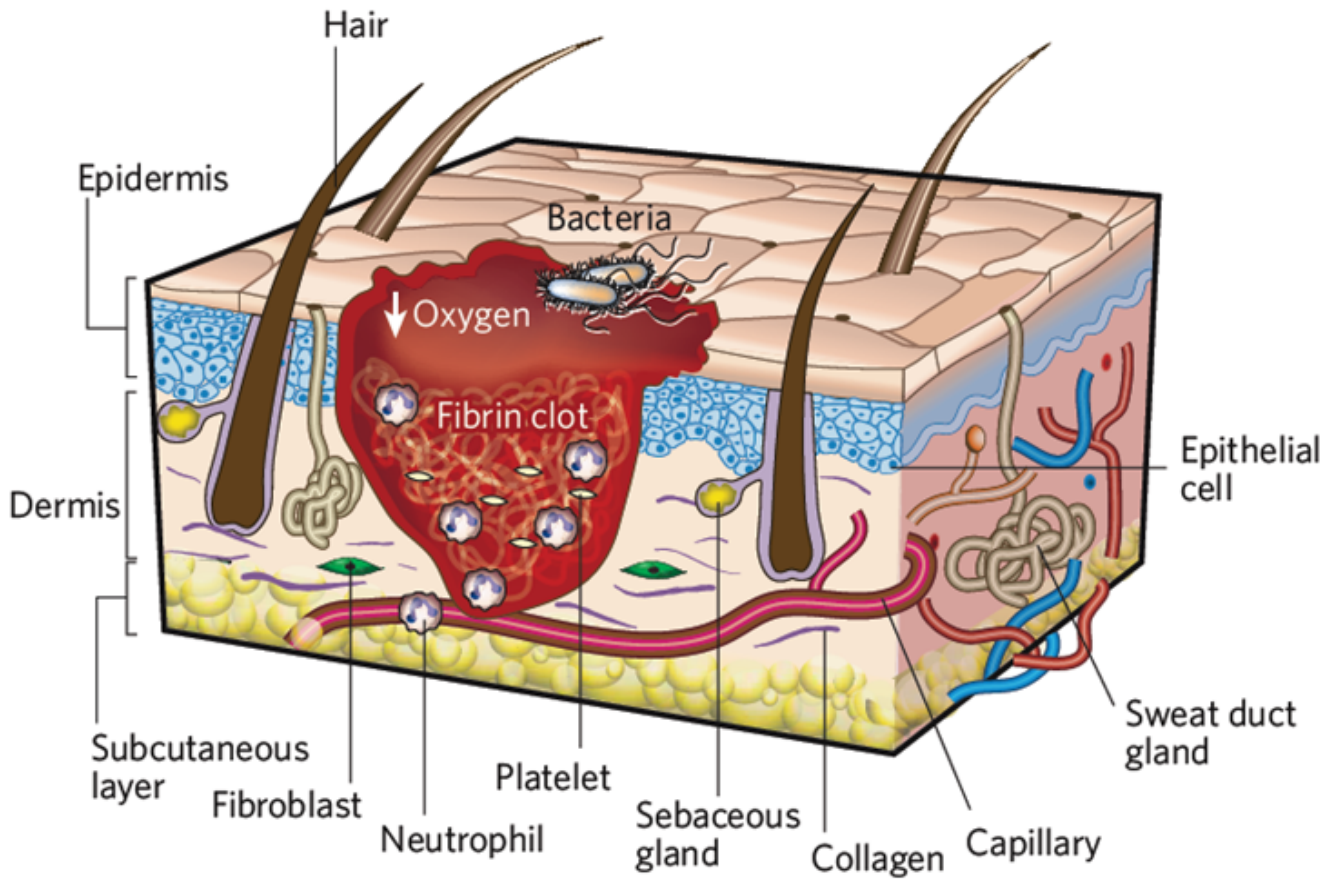
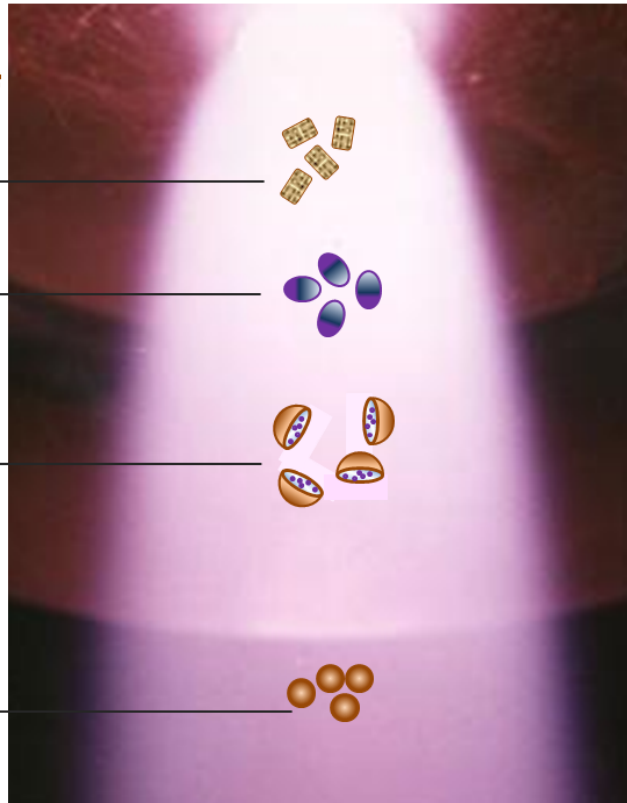
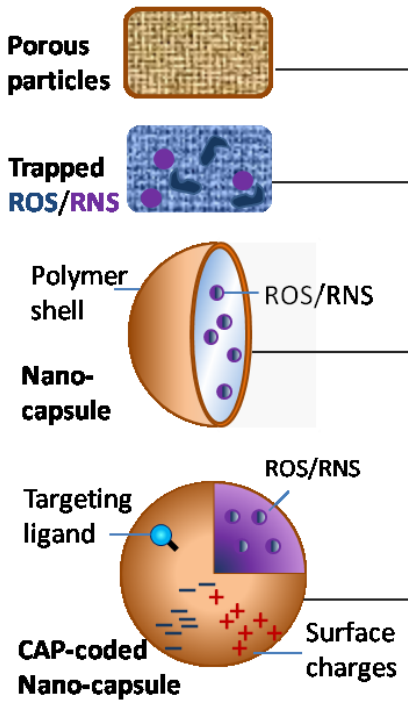


Figure 4 (Fig-4.tif)



## Fabrication of CAP-coded nano-capsule



## CAP Environment

**ROS**  
( $O_2^{\cdot-}$ ,  $^1O_2$ ,  $O/O^{\cdot}$ ,  
OH,  $H_2O_2$ , HOCl, ...)

**RNS**  
( $NO^{\cdot}$ ,  $NO_2^{\cdot}$ ,  $NO_2^-$ ,  
 $NO_3^-$ ,  $ONOO^-$ ,  
 $OONOH$ ,  $N_2O_3$ ,  $N_2O_4$ ...)

**Electrons, other ions**

**Emissions, Waves**  
(V)UV photons  
Electromagnetic fields  
Electric field

**Physical forces**  
shock wave  
Pressure gradient  
Heat

Figure 5 (Fig-5.tif)