


Review

Cold Argon Atmospheric Plasma for Biomedicine: Biological Effects, Applications and Possibilities

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Abstract: Currently, plasma medicine is a synthetic direction that unites the efforts of specialists of various profiles. For the successful formation of plasma medicine, it is necessary to solve a large complex of problems, including creating equipment for generating cold plasma, revealing the biological effects of this effect, as well as identifying and justifying the most promising areas of its application. It is known that these biological effects include antibacterial and antiviral activity, the ability to stimulate hemocoagulation, pro-regenerative properties, etc. The possibility of using the factor in tissue engineering and implantology is also shown. Based on this, the purpose of this review was to form a unified understanding of the biological effects and biomedical applications of argon cold plasma. The review shows that cold plasma, like any other physical and chemical factors, has dose dependence, and the variable parameter in this case is the exposure of its application. One of the significant characteristics determining the specificity of the cold plasma effect is the carrier gas selection. This gas carrier is not just an ionized medium but modulates the response of biosystems to it. Finally, the perception of cold plasma by cellular structures can be carried out by activating a special molecular biosensor, the functioning of which significantly depends on the parameters of the medium (in the field of plasma generation and the cell itself). Further research in this area can open up new prospects for the effective use of cold plasma.

Keywords: cold plasma; argon; molecular mechanisms of action; biological effects



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1. Introduction

Plasma medicine is a relatively young scientific field, which is the result of successful interdisciplinary interaction of physicists, biologists, and doctors [1–3]. Over the past decade, the efforts of researchers from various regions of the world have gradually revealed broad horizons for the use of cold plasma in various fields of medicine. These studies historically originate from technical disciplines within the framework of which the ability of cold plasma to disinfect various surfaces was discovered and investigated. This fact made it possible to extend the scope of application of the physical factor in question to its antibacterial and viricidal activity and, in the future, to other biomedical applications [2–5]. At the same time, despite the large number of experimental works, their systematization and integration have been carried out partially and only for special areas of application. Insufficient knowledge of the biological effects of cold plasma, as well as their molecular mechanisms, does not allow us to fully test its sanogenetic activity in *in vivo* models. This determines the fact that most studies are based on the results of *in vitro* experiments.

Based on this, the purpose of this review was to form a unified understanding of the biological effects and biomedical applications of argon cold plasma.

2. Cold Plasma as a Physicochemical Concept

According to the traditional ideas of physicists, plasma is a special, fourth state of matter, along with solids, liquids and gases. Plasma is a partially ionized gas, and the degree of its ionization is one of its key characteristics that determine temperature and other basic parameters and properties. It was the temperature factor that made it possible to divide the plasma into standard (“thermal”), having an average temperature of about 4000–5000 K, and low-temperature (“cold”) with a characteristic temperature of 30–50 °C [1,2,4]. Given the peculiarities of the functioning of living systems, high-temperature plasma cannot be used for medical purposes. On the contrary, low-temperature plasma, causing the generation of reactive oxygen and nitrogen species in solutions and biological objects, can have both positive and negative effects on them [4,6–9].

In connection with this circumstance, a wide range of generator equipment has been developed using various principles of gas flow ionization. In particular, for this purpose, the authors used spark, arc, barrier, corona discharges, and pulse current, but all these effects provided single process-controlled ionization of the carrier gas [1,4,6]. At the same time, one of the significant difficulties in using these devices is the lack of a configurable variable parameter. On this basis, the gradation of the severity of the action of cold plasma must be carried out only by changing the exposure of the treatment.

3. Cold Plasma as a Means of Disinfection

Traditionally, the first biomedical application of cold plasma was the consideration of this physical solution as a method of disinfection and detoxification and decontamination of surfaces. For more than 10 years, there have been reports in the literature about the antibacterial activity of argon cold plasma. One of the most extensive should be considered the study by R. Matthes et al. (2016), in which the resistance of 78 genetically different strains of *Staphylococcus aureus* to the action of cold plasma was studied in detail [10]. It has been shown that the surface properties (in particular, the characteristics of capsule lipopolysaccharide) and antibiotic resistance are associated with resistance to cold plasma treatment. At the same time, a number of pathogens of chronic wound infections (for example, methicillin-resistant strains of *S. aureus* and *Pseudomonas aeruginosa*) were inactivated by exposure to cold plasma [11]. Also of interest are the data of R. Matthes et. al. (2016) on the unequal antibacterial activity of plasma formed from different carrier gases [12]. It was found that argon plasma with 1% oxygen admixture had the greatest effectiveness against staphylococci and pseudomonads. It is important that the presence of oxygen in the medium is more significant than the type of carrier gas [12]. The mechanism of this antibacterial effect is considered to be a toxic effect on the cell wall of microorganisms [13] as well as on the inactivation of key oxygenases of bacteria [14].

Of particular importance is the possibility of eliminating pathogens in biofilms. It is known that such bacterial symbioses significantly complicate the possibility of their destruction by standard antibiotics, whereas the use of cold argon plasma allows for layer-by-layer destruction of biofilms formed by *S. aureus* [15]. Additional ways of antibacterial action of argon cold plasma are the triggering of oxidative stress, DNA damage and the formation of phosphate starvation [16]. This information has been confirmed by other studies involving a wider range of indicators (in particular, the secondary product of lipid peroxidation—malonic dialdehyde) [17].

In addition to the antibacterial activity, the antiviral and fungicidal activity of argon cold plasma flow was later identified and proved. Thus, on a number of bacteriophages (T4, F174 and MS2) it was demonstrated that the considered effect, both in the form of a gas stream and plasma-treated water, successfully inactivates these viral particles [18].

The mechanism of this effect is also based on the oxidative destruction of nucleic acids and proteins. At the same time, a significant increase in the antiviral effect is observed with

the additional introduction of 1% of air into the system. These results are fully consistent with the study by H.A. Aboubakr et al. (2018), who evaluated the ways of inactivation of feline calicivirus with a mixture of 99% argon with 1% oxygen [19]. It was revealed that short-term treatment (15 s) causes only moderate changes in the structure of the virus capsid but complicates its interaction with the infected cell in vitro. Prolonged exposure (2 min) leads to the disintegration of the main capsid proteins, in particular, the main structural domain of the VP1 protein, as well as oxidative damage to viral RNA.

The generation of a massive amount of reactive oxygen species (ROS) also provides a fungicidal effect of argon cold plasma [20,21]. At the same time, this process can be controlled. Thus, according to M.H. Kang et al. (2014), the addition of sodium chloride to the reaction medium significantly reduces the toxic effect of cold plasma on the reproduction, structure and genomic DNA of *Neurospora crassa* [20]. At the same time, the effect of sodium chloride turned out to be slightly more significant than the change in pH, osmolarity, and even the concentration of ROS in the solution under consideration. It can be assumed that such quenching properties of sodium chloride are due to the expenditure of the formed radicals on the generation of hypochlorite (ClO^-).

On the contrary, the introduction of ROS generation inductors (FeCl_2 and FeSO_4) into the system, triggering the Fenton reaction, significantly increases the sterilizing properties of argon plasma against *Aureobasidium pullulans* [21]. This fungus produces the black pigment melanin, which ensures its resistance to most damaging effects (up to radiation). Under standard conditions, *A. pullulans* is resistant to cold plasma, but the use of iron salts eliminates this effect, which can be explained by the potentiation of ROS generation in the Fenton reaction [21].

Thus, studies of the reaction of microorganisms to cold plasma treatment, on the one hand, are of interest in terms of using the factor as a means of disinfection and antibacterial action. Furthermore, on the other hand, it helps to understand the mechanisms of action of cold plasma on various biological systems.

4. Cold Plasma in the Treatment of Biologically Significant Surfaces

An important task of transplantology, dentistry, traumatology and regenerative medicine is the optimal preparation of the implant surface. It is necessary to ensure maximum biocompatibility of these implants, as well as their most complete and successful colonization by cellular elements. It has been shown that pretreatment of the surface of titanium implants with argon cold plasma provides an improvement in the adsorption of bovine serum albumin on them, comparable to the effect of ultraviolet radiation for 2 h [22]. In addition, a series of publications by L. Canullo et al. (2017, 2018) confirmed an improvement in the colonization of scaffolds by osteoblasts after their preconditioning with argon cold plasma due to increased adhesiveness [23,24]. An additional positive characteristic of the effect is the decontamination of the implant surface, and these properties are realized for materials of various compositions [24]. At the same time, stimulation of cell proliferation and cell adhesion to the implant surface is realized in a wide range of exposures (from 5 s to 1.5 min) with equal success [25].

5. Non-Thermally Operated Electrosurgical Plasma Sources

Along with the antibacterial activity of cold plasma, procoagulation properties serve as the second earliest discovered property of the ionized gas flow. Thus, the works of M. Zenker (2008) demonstrated the possibility of using thermal plasma flow as an electro-surgery technology that provides hemostasis [26]. The optimal modes of action of the factor were selected, additionally realizing the devitalization of tissues due to thermal exposure. These developments were improved by I. Justin et al. (2010), who described the design, operation and efficiency of the Cesar 136 brand “plasma knife” operating at a frequency of 13.56 MHz [10]. At the same time, the inner diameter of the blade was about 0.4 mm, which made it possible to achieve high accuracy of the surgical procedure.

The design characteristics of the impact made it possible to successfully apply the “plasma knife” in endoscopic surgery. In particular, within the framework of a randomized pilot clinical trial conducted on patients with Barrett’s esophagus (BRIDGE), the comparability of the results with the effect of radiofrequency ablation (in terms of the effectiveness of removing areas of dysplasia and the frequency of side effects) was demonstrated with a multiple reduction in the cost of the procedure [27].

Currently, the development of cold plasma technologies in surgery has allowed the creation of a number of devices for electrosurgical treatment. In particular, some researchers have shown the possibility of successful application of non-thermal argon plasma in oncogynecology [28,29] and urology [30] based on changes in the state and parameters of the mucosa [29].

6. Cold Plasma in Regenerative Medicine and Wound Treatment

The use of ionized inert gas flows in surgery and regenerative medicine is based on two main factors: stimulation of regeneration processes, and antibacterial effects [31–33]. The second mechanism is the most studied, as already reported in the relevant section of this review. It should also be noted that both factors are clearly interrelated: the removal of bacterial contamination of the wound creates favorable conditions for adequate restoration of the structure of damaged tissues [11,31,33]. In addition, this is further facilitated by the oxidative removal of necrotic and non-viable tissues under the influence of oxidative stress induced by exposure to cold plasma [31,33]. In this case, the effect of cold plasma therapy will be determined by the exposure of the biological object and the parameters of the plasma itself, primarily by the degree of ionization of the gas stream, which determines the intensity of generation of oxygen, nitrogen radicals and, secondarily, lipids, proteins and nucleic acids. Thus, when using a short exposure (up to 3 min), the bioregulatory effect of the factor is realized, which, in relation to regenerative medicine, manifests itself in stimulating cell proliferation and accelerating the rate of wound healing. This has been demonstrated by various research groups on a wide range of biomodels: from simple (regeneration of the damaged caudal part of the body in Danio fish) [34], to more complex (reproduction of streptozoin-induced diabetic wound [35] and experimental damage to the ear area in rats [36]). All authors note the synergetic, complex effect of cold argon plasma on the regeneration processes and decontamination of pathogenic microorganisms from the wound surface.

Currently, there are some small clinical trials that also prove the effectiveness of the use of the factor in question in the treatment of wounds of various etiologies [32,33].

An important additional pathogenetic mechanism of action of cold plasma is a positive effect on the microcirculatory bed. Thus, in our previous studies on the model of contact thermal burn, a pronounced stimulating effect on the intensity of blood flow through small-diameter vessels was established. It should be emphasized that such an effect is provided by physiological regulatory factors—an increase in the release of nitrogen monoxide by the vascular wall, as evidenced by a significant increase in the amplitude of the endothelial component.

In addition, in our experimental studies using the same model, it was demonstrated that exposure to cold plasma induces the generation of reactive oxygen and nitrogen forms, not only in the wound itself and the near-wound zone, but also in the blood of animals. This indicates the presence of a systemic effect of the physical factor in question, which was first established by us both by metabolic (the level of oxidative processes in the blood and various tissues) and functional (the state of heart rate variability and microcirculatory bed in the area remote from the wound) criteria.

Along with the effectiveness, an important issue determining the possibility of clinical application of cold plasma therapy technology is the safety of the latter. To clarify this, an assessment of the mutagenic effect of argon cold plasma [37] was carried out in modes an order of magnitude higher than the intensity of the standard ones used. The absence of genotoxic effects of the studied factor was shown. A study of the long-term effects of

argon cold plasma treatment of the body was also performed. Based on the analysis of the cytokine profile (IL-1, TNF, fetoprotein, calcitonin), as well as the level of cancer-embryonic antigens, there was no increase in the risk of neoplasms one year after the course of cold plasma therapy.

All of the above makes it possible to talk about the relative safety of argon cold plasma; however, additional research is required in this regard.

Finally, the use of prolonged plasma treatment and high-intensity ionization of the gas stream ensure the destructiveness of this factor in relation to biological tissues (“plasma knife technology” [38]) and pathogenic microorganisms.

7. Application of Cold Argon Plasma in Oncology

Cold argon plasma can be effectively used for the treatment of tumors of various nature, while its effect can be either direct (directly on tumor structures), or indirect, for example, through plasma-treated aqueous solutions [39–41]. It has been shown that the maximum destruction of cancer cells is achieved precisely by using a direct method of exposure [42–44]. However, it is worth noting that the location of the tumor is not always available for cold plasma treatment. In these cases, they resort to an indirect method of influence, which has a relatively lower efficiency [40].

The pro-apoptotic effect of cold argon plasma on cancer cells is achieved by generating an excessive amount of long-lived and short-lived reactive oxygen species (ROS) and reactive nitrogen forms (RNS) [43–46]. In the case of drinking water treatment, stable forms— H_2O_2 and NO_2 —have the primary damaging effects [46]. Special attention is paid to the role of glutathione in shifts in the redox status of cells induced by cold argon plasma and resulting from the treatment of prostate cancer [30].

The cellular mechanisms of the proapoptotic effect of argon cold plasma have been studied in detail in experiments *in vitro* on various tumor cell lines. It has been shown that the treatment of tumor cells causes the launch of apoptotic changes in them. In particular, activation of the caspase system (3, 7, etc.) was detected on osteosarcoma cells, leading to a significant inhibition of the growth of the neoplasm focus as a whole [42]. At the same time, it was found that this effect does not depend on the type of cold plasma generator [42].

Additionally, modulation of the expression of anti-apoptotic genes and stimulation of the production of chaperones—heat shock proteins—are involved in the implementation of programmed tumor cell death induced by the action of cold plasma [43]. Similar results were obtained using the human lymphoma cell line U937, and the effect of cold plasma was enhanced by saturation of the matrix carrier gas with molecular nitrogen, which made it possible to switch the generation of bioradicals from ROS to RNS [43].

In the experiments of another research group (R. Moniruzzaman et al., 2018) performed on Molt-4 lymphoma cells, the fundamental role of hydrogen peroxide formation in ensuring the pro-apoptotic effect of cold plasma on them was established [44]. Hypersynthesis of this molecular messenger determines the activation of the caspase cascade and increases the expression and sensitivity of the FAS receptor to the corresponding ligands [44]. In addition, a valuable result of the work is the proof of the presence of effective modifiers of the damaging effects of the factor in question, in particular, sulfasalazine, which inhibits the cysteine–glutamate antiporter, which provides reduction of the intracellular level of reduced glutathione [44]. This makes it possible to assume the prospects of using this compound as a sensitizer for potentiating the pro-apoptotic effect of cold plasma.

The central role of hydrogen peroxide in the realization of the destructive effects of cold plasma was also confirmed on the A549 lung cancer cell model [39]. Interestingly, during the treatment of cell culture with plasma, a progressive increase in intracellular H_2O_2 concentration was recorded, while NO-dependent mechanisms were practically not involved, which was verified by maintaining the level of nitrite ions at the initial values [39]. This highlights the key importance of hydrogen peroxide in the induction of plasma-associated apoptosis of tumor cells.

The involvement of NO-dependent mechanisms in the treatment of tumor cells was demonstrated by the example of the glioblastoma cell line U87MG [40]. It has been shown that in this case, the formation of RNS occurs, which can lead to the development of both apoptosis and necrosis of tumor cells [40,47,48]. It should be noted that the generation of this type of reactive molecules is not necessarily associated with an increase in the concentration of nitrogen in the matrix carrier gas [40]; however, it requires prolonged treatment of a biological object with cold plasma to effectively inhibit cell proliferation and activate cell death [49].

Currently, there are data on the possibility of using cold argon plasma in clinical oncology. Thus, Marzi J. et al. (2022) showed the pilot data of an in-human clinical study using plasma to successfully treat cervical cancer precursors [50]. This allows us to consider oncology as one of the most significant areas of application of cold plasma therapy.

8. The Authors' Own Research in the Field of Studying Local and Systemic Effects of Cold Plasma

Our research attempts to sequentially decipher the biological effects of cold plasma under *in vitro* and *in vivo* (on Wistar rats) conditions. In the first stage of our work, ideas about the peculiarities of the action of cold plasma on biosystems of various levels of organization were integrated, and unresolved issues of plasma biomedicine were shown, including deciphering the mechanisms of the biological effect of the factor in question, the effect of the carrier gas on them, the dose dependence of the response of the biological object, etc. [51]. Human whole blood samples served as the main test biosystem, convenient and indicative in terms of evaluating the effects of cold plasma in experiments *in vitro*. It was found that cold helium plasma modifies various components of blood plasma metabolism [52,53] and erythrocytes [54]. In particular, in blood plasma, the studied factor induced moderate stimulation of free radical processes with a short exposure (1–2 min) against the background of more pronounced activation of the antioxidant system [52]. Similar metabolic shifts have been recorded not only in animals and humans, but also in plants treated with argon cold plasma [55].

It should be emphasized that there was a clear dose dependence of the identified effect on oxidative metabolism. In further studies, it was shown that with an increase in exposure time (in the range of 3 min and more) the degree of activation of free radical oxidation increased progressively [56], which was associated with an avalanche-like increase in the amount of reactive oxygen and nitrogen species, which was confirmed by the data of other authors [4,44,45,48]. These shifts in oxidative metabolism were accompanied by a sharp depletion of the antioxidant reserves of the biological fluid [56].

A similar trend was registered with respect to energy metabolism. According to a number of researchers, the restructuring of this component of metabolism, manifested in shifts in the level of coenzymes (in particular, concentrations of NAD and NADH) and products of these processes (for example, lactate and pyruvate), is due to the regulation of the expression of the corresponding genes (ATP a1, ATP a2, ATP b1, ATP b2, ATP b3, TOR, GRF 1-6) [57].

Interesting features of the response to the action of helium cold plasma were discovered by us when assessing the state of red blood cells. It was found that blood treatment with the studied factor contributed to a pronounced increase in the level of malonic dialdehyde, a standard marker of the intensity of free radical processes, in erythrocytes [56]. Further disclosure of the mechanism of these shifts made it possible to find out that exposure to cold plasma did not lead to stimulation of lipid peroxidation in the membranes of these blood cells, and the detected effect was due to inhibition of aldehyde dehydrogenase utilizing malonic dialdehyde [56]. Subsequently, this pattern was confirmed in experiments *in vivo* [58].

In addition, the subject of our research was the disclosure of the systemic effects of cold plasma. They were analyzed when exposed to a cold plasma stream on a section of pre-epilated skin of a laboratory animal (Wistar rats). It was found that the treatment under

consideration caused nonspecific (adaptive) shifts in heart rate variability [59], as well as stimulation of the intensity of blood flow through small-diameter vessels, realized mainly due to the release of NO by the vascular wall [60]. The conjugacy of the action of cold argon and helium plasma with the modulation of nitric oxide production was previously shown in experimental studies on the model of melanoma [61] and other pathological processes [62], as well as in the mechanisms of the pro-regenerative effect of the studied effect [63]. The systemic nature of the response to the studied physical factor included metabolic rearrangements, which, in particular, were expressed in the activation of energy metabolism enzymes (stimulation of the catalytic properties of lactate dehydrogenase in a direct reaction, accompanied by an increase in pyruvate production) and moderate inhibition of aldehyde dehydrogenase activity [58].

A separate issue is the clarification of the role of the carrier gas in the implementation and features of the action of cold plasma on biological objects. To solve this problem, we compared the modification of the physicochemical properties of blood plasma during its treatment with helium and argon plasma [64]. It was found that the use of helium as a carrier gas provides a more physiological response of the biosystem, contributing to the optimization of the crystallographic picture of the biological fluid. On the contrary, the use of argon plasma causes more destructive shifts in the crystallogenesis of blood plasma, but this effect cannot be unequivocally regarded as negative. To a greater extent, it makes it possible to distinguish the areas of practical application of helium and argon cold plasma. We assume that helium plasma mainly has a pro-regenerative effect, while argon plasma has an antibacterial and pro-apoptotic effect. The absence of a negative effect of argon plasma on metabolism was confirmed based on an assessment of the dynamics of the parameters of free radical oxidation and components of the antioxidant system of the blood of rats who received a course of external cold plasma treatment with this carrier gas [65].

In general, the results of our previous studies create prerequisites for further study of the sanogenetic potential of monocomponent cold plasma, primarily antibacterial and regenerative effects, in the treatment of wounds and burns.

9. Analysis of Molecular Mechanisms of Cold Plasma Action on Biological Objects

Numerous studies in the field of plasma biomedicine postulate that the cellular, tissue and organismal effects of cold plasma on biosystems are mediated by the formation of reactive oxygen species and nitrogen. Among them, hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet\text{OH}$), ozone (O_3), singlet oxygen ($^1\text{O}_2$), superoxide-anion radical ($\text{O}_2^{\bullet-}$), atomic oxygen (O), nitrogen monoxide (NO), peroxyxynitrite ($\bullet\text{ONOO}^-$), nitrite (NO_2^-) and others are mentioned [40,66,67]. These compounds are usually classified into short- and long-lived, while the pool of the latter also includes oxidized forms of proteins, DNA and lipoproteins [45,48,66,68,69]. However, for a long time the question of the specific participation of each of these ions has remained open.

According to the literature, the central link of the metabolic sensing system–biosensor for cold plasma is hydrogen peroxide, which mediates many other external effects on the cell [39,66,70]. On the other hand, other researchers have reported on the significant role of atomic oxygen [70], singlet oxygen [71], ozone [72], hypochlorite [66] and other reactive molecules in the realization of the effects of cold plasma. Such a diversity of opinions is due, in our opinion, to the emergence of all possible reactive oxygen and nitrogen forms during the processing of biological objects with cold plasma and their integration into a single molecular cascade. The analysis of the physico-chemical foundations of this multifaceted process allowed us to form an integrated circuit of the perception of plasma radiation by the cell and the transduction of this signal to various intracellular structures, including DNA molecules and proteins (Figure 1).

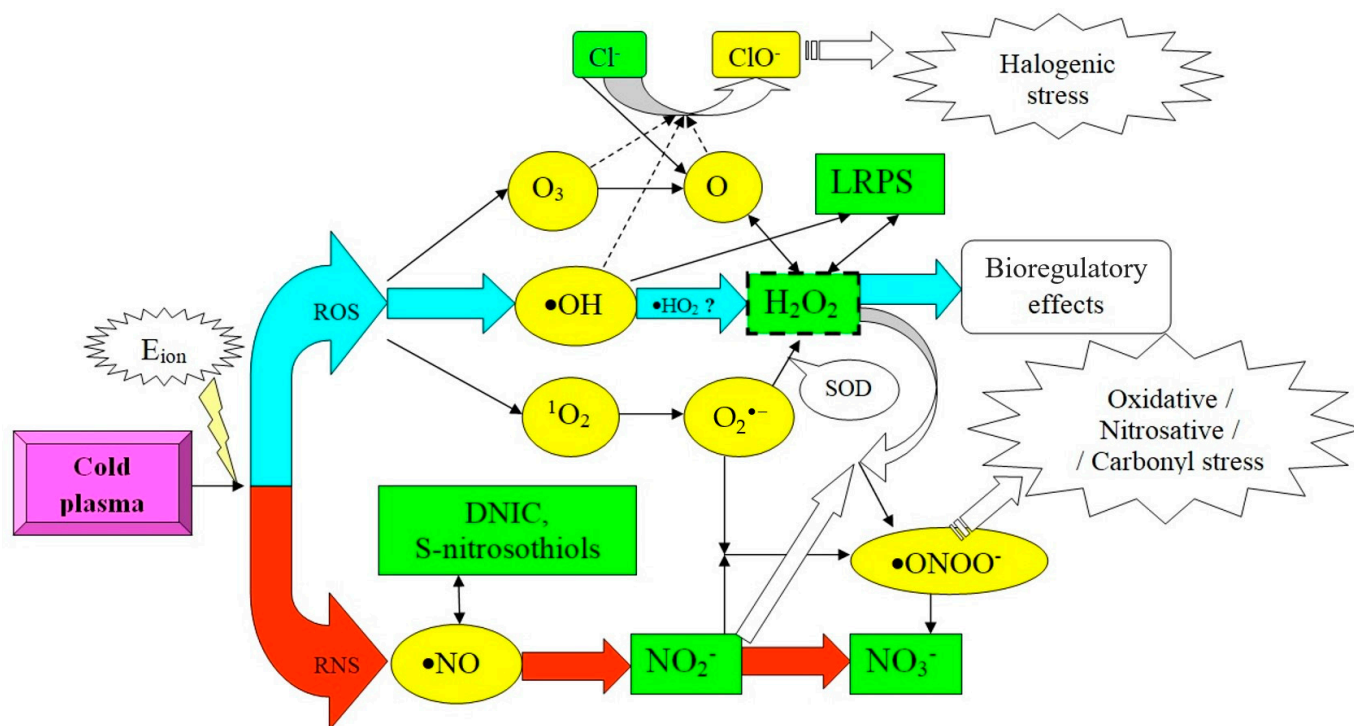


Figure 1. Molecular cascades, induced by the action of cold atmospheric plasma (ROS—reactive oxygen species, RNS—reactive nitrogen species, LRPS—long-living reactive protein species, DNIC—dinitrosyl iron complexes, E_{ion} —ionization energy, SOD—superoxide dismutase).

From the presented Figure 1, it can be seen that the effect of cold plasma, which contributes energy to the biosystem for the ionization of endogenous molecules, in a heterogeneous medium leads to the formation of a wide range of reactive oxygen species (the upper branch of the scheme), the main representatives of which in this case are hydroxyl radical, ozone and singlet oxygen. All these compounds are short-lived and are immediately transformed. Thus, ozone decomposes to form atomic oxygen, the hydroxyl radical dimerizes into hydrogen peroxide (as an intermediate stage of this process, the formation of hydroxonium- $\bullet\text{NO}_2$ can occur), and singlet oxygen forms a superoxide radical. Further, atomic oxygen is capable of oxidizing halides (in particular, Cl^-) to their oxygen-containing derivatives (ClO^- , respectively, which can realize halogenating stress) or interact with water to form hydrogen peroxide. Interestingly, the second process can be reversible. It is known that ozone also has the ability to oxygenate halides. In turn, the superoxide anion radical under the action of superoxide dismutase is also transformed into H_2O_2 . Thus, the entire ROS-dependent cascade of reactions is integrated into a single point—relatively stable hydrogen peroxide. At the same time, long-lived active forms of proteins can act as a kind of depot for this compound. In our opinion, it is hydrogen peroxide that ensures the realization of the bioregulatory effect of cold plasma.

The second (lower section in Figure 1) branch of the molecular sensor for cold plasma is associated with the formation of nitrogen reactive species and is triggered by the generation of the most universal of them—nitric oxide—which can be stored in the form of endogenous depositing compounds (S-nitrosothiols, dinitrosyl iron complexes with various ligands—glutathione, serine or protein [73,74]). The second, more preferable, scenario is the formation of nitrite from NO, which, interacting with a superoxide-anion radical or hydrogen peroxide, provides the synthesis of peroxynitrite, which has an extremely high oxidative potential and probably implements the toxic effects of cold plasma. This creates the conditions for the development of plasma-induced oxidative, nitrous or carbonylating stress (occurs during prolonged exposures of the factor). Finally, both nitrite and peroxyni-

trite can be transformed into stable and low-reactive nitrate ions, which can be considered as the end point of the process.

It should be noted that the given paths and their preferences depend significantly on the parameters of the environment. In particular, when processing cells in a nitrogen medium, only the NO-dependent branch of the molecular biosensor is involved [43,71]. In the case of additional saturation of the plasma jet with oxygen, its toxic effect is significantly potentiated [19]. A similar effect was achieved when Fe^{2+} ions were introduced into the biosystem, reproducing the Fenton reaction and progressively increasing the concentration of free radicals [21].

On the contrary, the use of various antioxidants or traps of reactive oxygen species (for example, singlet oxygen) partially or completely neutralizes the effect of cold plasma [71]. Additionally, saturation of the system with increased concentrations of sodium chloride reduces the toxic effects of cold plasma even in the case of large exposures [20], which may be due to the “quenching” of the formed reactive oxygen species by chloride ions with the formation of the less reactive hypochlorite. All of the above clearly indicates the controllability of the action of the factor in question through the modulation of the components of the cellular biosensor.

The considered molecular cascades are successfully integrated into the cellular mechanisms of the action of cold plasma on biological objects (Figure 2). Forming reactive oxygen species in the perimembrane space (Figure 3 [75]), cold plasma intensifies their intracellular generation, triggering a metabolic response to the action of this factor [76–79]. In particular, it is implemented in the oxidative modification of proteins and nucleic acids, as well as in the regulation of the functioning of cell organelles. In addition, changes in the cell cycle and the processes of proliferation and differentiation can occur [57,80,81]. The mechanism of these effects may be due to the effect on plasma proteins (hemoglobin, myoglobin, lysozyme, etc.) [82], as well as with the involvement of p53 protein modulators [83].

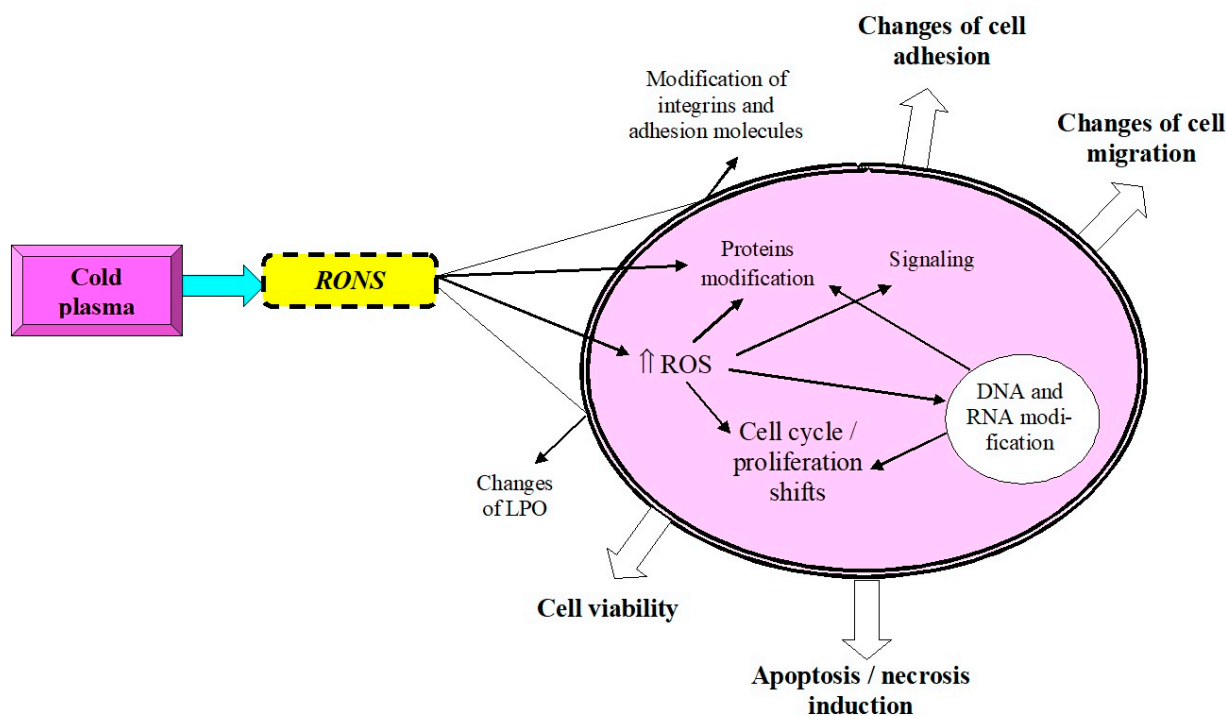


Figure 2. Cellular response to exposure to cold atmospheric plasma (RONS—reactive oxygen and nitrogen species, ROS—reactive oxygen species, LPO—lipid peroxidation).

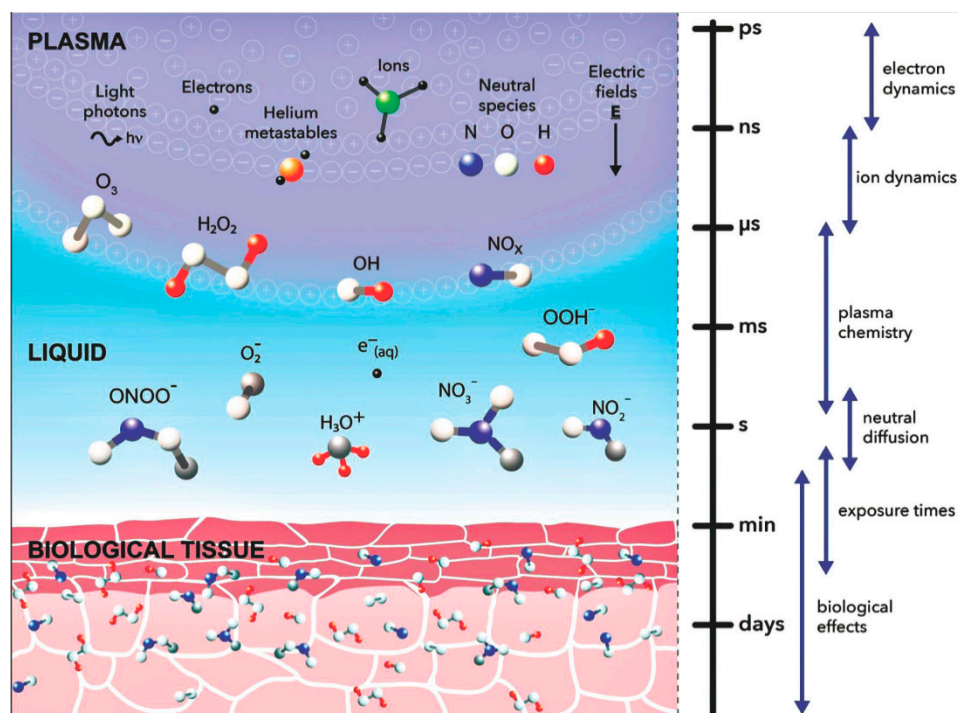


Figure 3. Plasma-induced production of reactive oxygen and nitrogen species in perimembrane space (some data from [9]).

A study of plasma–3D–tissue interactions showed tissue penetration depth of physical plasma in human mucosa [84]. The authors used marker-independent Raman spectroscopy to determine the functional tissue penetration depth of direct plasma treatment. A working group at the NMI (Natural and Medical Science Institute) published data comparing the analysis of protein expression using Digi-West technology following plasma treatment of 2D single cells and 3D tissue [85]. This study shows the importance of investigating plasma effects on 3D tissue due to significantly differing results compared to single cell studies, which so far represent the (incomplete) data basis of our understanding of plasma effects. Holl M. et al. (2022) showed that in the culture of peritoneal cells, such effects can be caused not only by direct irradiation with cold plasma, but also by plasma-activated water [86]. Interestingly, this liquid form of exposure to cold plasma can be used as a drink. In particular, it has been shown that oral administration of plasma-activated water for 28 days can inhibit proliferation and angiogenesis, as well as stimulate apoptosis in a model of non-small cell lung cancer in mice [87].

The rearrangement of the cell membrane caused by the modification of the peroxidation of its structural lipids and proteins in combination with conformational and oxidative shifts of integrins and adhesion molecules can lead either to stabilization of the plasmalemma or to its desiccation [88,89]. The latter serves as a mechanism for the implementation of plasma-induced apoptosis (in some cases, with prolonged exposure to the action of the necrosis factor). Additionally, these shifts in the membrane state can significantly change the adhesive properties of the cell and affect its migration [90–92].

Yan et al. suggested that an increased density of aquaporins in the membrane of tumor cells facilitates the entry of plasma-derived ROS and RNS into tumor cells and thus causes selective apoptosis induction [93]. The model by Van der Paal et al. is focusing on the role of cholesterol for the control of ROS influx into cells [94]. These two models are based on the concept that plasma-derived ROS and RNS are sufficient to induce apoptosis. This is contrasted by a model presented by Bauer and Graves that is based on an initial signaling effect of singlet oxygen, either derived from plasma directly or formed through the interaction between long-lived ROS and RNS with the extracellular redox system on

the surface of tumor cells. This may trigger singlet oxygen formation by tumor cells and induction of NADPH oxidase 1 (NOX1)-driven and ROS- and RNS-mediated apoptosis [95].

Thus, the treatment of biological objects with cold plasma, activating specific molecular mechanisms, ensures the transformation of the functioning of cells and tissues.

10. Conclusions

In general, plasma biomedicine currently appears to be a multifaceted interdisciplinary field that has already demonstrated broad prospects in various fields of medicine. Like any other physico-chemical factors, cold plasma is dose-dependent, and the variable parameter is the exposure of its application. One of the significant parameters determining the specificity of the cold plasma effect is the carrier gas used, which is not just an ionized medium, but modulated the response of biosystems to it. Finally, the perception of cold plasma by cellular structures can be carried out by activating a special molecular biosensor, the functioning of which significantly depends on the parameters of the medium (in the field of plasma generation and the cell itself). Further research in this area may open up new prospects for the effective use of cold plasma.

In our opinion, an in-depth understanding of the biological effects of argon cold plasma, as well as the molecular mechanisms of their implementation, makes it possible to extend the scope of this method. In particular, it becomes possible to use cold plasma more widely as a means of stimulating regenerative processes, as well as a modulator of the state of the microcirculatory bed. In addition, the disclosure of the systemic effects of cold plasma makes it possible to correct the functional and metabolic status of the body with a wide range of pathology. Thus, cold plasma therapy can be considered not only as a method of local treatment, but also as a systemic medical technology.

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