Review paper

Applications and challenges of low temperature plasma in pharmaceutical field

Lingge Gao [a], Xingmin Shi [a, *], Xili Wu [b, **]

[a] School of Public Health, Medical Science Center, Xi'an Jiaotong University, Xi'an, 710061, China
[b] Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, 710004, China

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ABSTRACT

Low temperature plasma (LTP) technology has shown an outstanding application value in the pharmaceutical filed in recent ten years. This paper reviews the research advances in LTP, including its effects on enhancing or inhibiting drug activity, its combined use with drugs to treat cancers, its effects on the improvement of drug delivery system, its use in preparation of new inactivated virus vaccines, its use with mass spectrometry for rapid detection of drug quality, and the anti-tumor and sterilization effects of plasma-activated liquids. The paper also analyzes the challenges of LTP in the pharmaceutical filed, hoping to promote related research.

1. Introduction

Low temperature plasma (LTP) is a partially ionized gas containing a variety of ions, electrons, active molecules, electric fields and ultraviolet radiation (UV) [1,2]. The non-thermal and non-equilibrium properties of LTP have attracted attention from the biomedical community. At present, LTP has been widely used in blood coagulation, wound disinfection and healing, surgical instruments and medical materials sterilization, and tumor therapy [3,4].

As is known to all, drug therapy is one of the important approaches to treating and preventing diseases. Researchers have long been committed to developing new drugs [5–7] and other auxiliary means [8–10] to overcome the shortcomings of traditional drugs.

As an emerging technology, LTP is gradually favored by the pharmaceutical community. LTP used in the field of pharmaceutical research is generated mainly by two devices: dielectric barrier discharge (DBD) and atmospheric-pressure plasma jet (APPJ). The basic structures of DBD and APPJ are shown in Fig. 1. In general, LTP has two pharmaceutical applications: it is used as an auxiliary means to change the structure and function of drugs, improve drug delivery system, and play a synergistic role with drugs to treat diseases; on the other hand, it is used to treat solution and produce the plasma-activated liquid, which is a "new drug" with anti-infection and anti-cancer effects. In addition, LTP plays an active role in the research and development of inactivated vaccines, and qualitative and quantitative analyses of drugs.

2. LTP's direct effects on drugs

LTP contains rich active components and energy, which act on different compounds to cause a variety of chemical reactions. LTP has two major effects on drugs: producing byproducts with special physiological functions and destructing drug structure or reducing drug activity.

2.1. LTP promotes drug activity

LTP is a safe and environmentally friendly sterilization technology widely used in the food industry, and can extend the shelf life of food [11]. It is found that LTP can improve the activity of the natural ingredients contained in food. Those natural ingredients have many biological effects, such as anti-cancer, anti-oxidation, anti-hypertension and other beneficial properties, and are regarded as important resources for new drug development [12]. Researchers have explored the efficacy and mechanism of LTP on
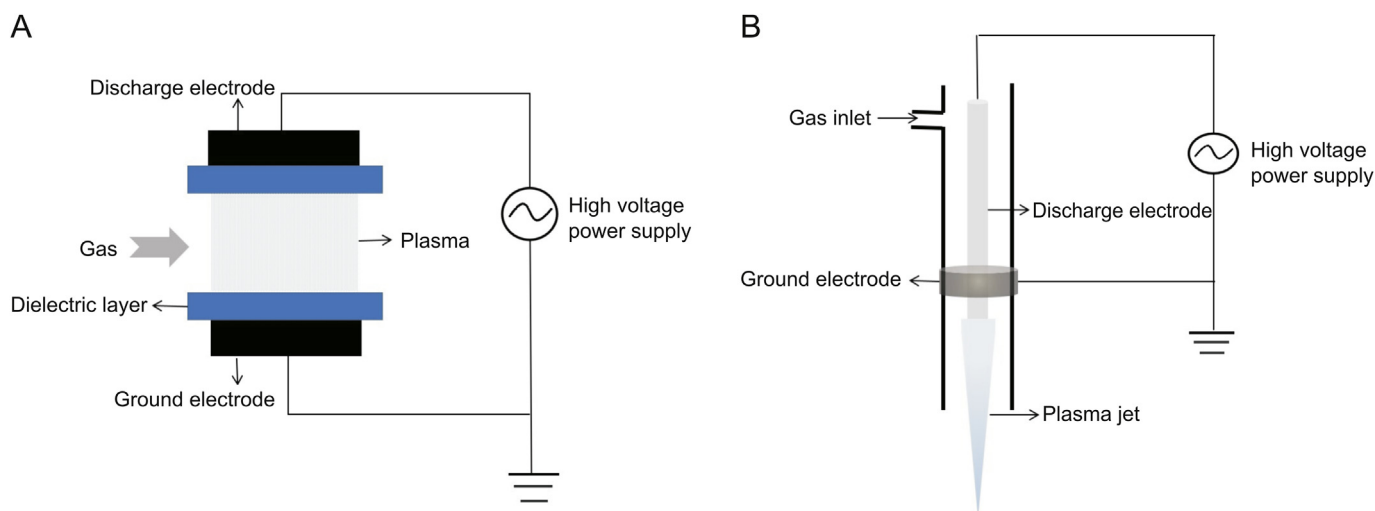
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Fig. 1. Experimental setup of LTP generation. (A) DBD. (B) APPJ. LTP: low temperature plasma; DBD: dielectric barrier discharge; APPJ: atmospheric-pressure plasma jet.

these natural ingredients to develop applications of natural ingredients in the fields of health care, cosmetology and disease treatment.

Phloroglucinol compounds show a vast array of biological activities including anti-inflamation, anti-microbico and anti-oxidation. Choi et al. [13] synthesized phloroglucinol oligomers (dimer, trimer, tetramer and pentamer) by treating phloroglucinol with DBD. Compared with maternal phloroglucinol and other generated oligomers, the phloroglucinol pentamer can inhibit the activity of α-glucosidase more effectively.

Trans-resveratrol (TR) is a stilbenoid found in grapes and low bush berries, and can reduce the risk of aging and heart diseases. Jeong et al. [14] treated TR with DBD and found that after 40 min of treatment, four known products were generated: (−)-ε-viniferin, (+)-ε-viniferin, 3,5-dihydroxybenzaldehyde, and p-hydrox-ybenzaldehyde, in addition to two unusual resveratrol dimers: methylenebisresveratrols A and methylenebisresveratrols B. The two unusual dimers can effectively inhibit the activities of α-glucosidase and α-amylase, to delay the absorption of glucose and reduce the level of postprandial blood glucose. Moreover, LTP acts on quercetin and produces three degraded products, namely, (±)-alphitonin, protocatechuic acid methyl ester, and protocatechuic acid, among which (±)-alphitonin has excellent anti-diabetic and anti-oxidant activities [15].

Compared with classical drug synthesis methods which need harsh reaction conditions, lengthy response time and complex extraction procedures, LTP can promote the biological activities of natural ingredients or generate byproducts with beneficial properties more rapidly and easily. However, there are few researches on the enhancing effects of LTP on commercial drugs. The applications of LTP in increasing drug activity and developing new drugs are still in the initial stage.

2.2. LTP decreases drug activity

Due to abundant reactive oxygen species (ROS) and reactive nitrogen species (RNS), LTP has strong oxidation capacity. Therefore, there are various studies on degradation of drugs by LTP. Amini et al. [16] were the first to study the effects of LTP on saffron compounds. Crocin esters and safranal contents were important indexes to evaluate saffron quality. They found when the working gas was argon, and voltage was 8 kV, LTP could decrease the contents of crocin esters and safranal but increase the contents of isophorone and 4-ketoisophorone. Increasing voltage and adding 5% or 10% oxygen to working gas would cause more crocin esters and safranal to decrease while more isophorone and 4-ketoisophorone to increase.

Lysozyme has been used as a clinical medicine due to its anti-bacterial, anti-inflammatory and antiviral effects [17,18]. Choi [19] compared the effects of lysozymes treated by different working gases (nitrogen and air) and devices (DBD and APPJ), and found that under the same conditions, compared with DBD, APPJ could significantly change the structure of lysozyme and decrease its activity. Compared with air-LTP, nitrogen-LTP could induce more structural changes of lysozyme. Also, the structure of α-chymo-trypsin would be damaged after APPJ treatment for 5 min [20].

LTP can degrade a variety of pharmaceutical components and destroy the activity of drugs, which seems to be detrimental to the development of LTP in the pharmaceutical field. However, from another perspective, this property can effectively destroy the structure and toxicity of drug wastes in sewage, reduce the concentrations of drug pollutants, and finally purify the sewage. Researchers have already used LTP to degrade sulfonamide antibiotics [21,22], β-lactam antibiotics [23] and pentoxifylline [24] in sewage. Therefore, LTP can be a promising technology to destroy non-biodegradable pollutants.

In addition, LTP is an efficient and safe sterilization equipment, which is widely used for sterilization of food and heat sensitive materials [25,26]. If LTP is applied to drug disinfection, it may degrade drug components and reduce drug efficacy. It is believed that researchers will find out the appropriate LTP conditions in the future, which can be used in drug sterilization without damaging drug activity.

3. LTP's synergistic effects with drugs

3.1. LTP's synergistic effects with chemotherapeutic drugs

Chemotherapy has been a major treatment for cancers. However, in recent years, more and more tumor cells have shown drug resistance and the efficiency of chemotherapy is interfered [27]. LTP has been proved to inhibit cell proliferation, induce cell apoptosis, inhibit metastasis and invasion of various cancer cells [28–32]. LTP can be a new treatment for tumors. Many researchers have combined chemotherapeutic drugs with LTP in order to achieve the best efficacy and improve prognosis of tumor patients.

Chang et al. [33] treated oral squamous cancer cells with 1 kV LTP and 10 μg/mL of cetuximab. Combined treatment with LTP and cetuximab could reduce the invasion and migration of oral squamous cancer cells. Sagwal et al. [34] discovered that combining LTP with chemotherapeutic drugs including doxorubicin, epirubicin and oxaliplatin could lead to the significant melanoma cell death. Studies on relevant mechanisms showed that the expression of organic cation importer SLC22A16 in SK-MEL 28 melanoma cells was significantly upregulated after LTP treatment, so melanoma cells could uptake doxorubicin better, and the concentration of doxorubicin in cells was increased.

Chang et al. [35] found if APPJ with helium gas was used to treat BXPC-3 pancreatic cancer cells, followed by low dose (2.5 mg/mL or 5.0 mg/mL) of tegafur, the proliferation capacity of cells could be decreased in a synergistic manner. However, if tegafur was used first, followed by LTP treatment, the tumor inhibition effect was similar to that of using tegafur alone. Further experiments showed that tegafur could react with the short-lived radicals generated by LTP to form 5-fluorouracil (5-FU). 5-FU has a lower anti-tumor capacity than tegafur; thus, the synergistic anti-tumor effect was decreased when using tegafur before LTP to treat cells. Brulle et al. [36] successfully established an orthotopic mouse model of human pancreatic cancer and studied the effects of plasma gun and gemcitabine on pancreatic cancer. The results showed combined treatment with LTP and gemcitabine could synergistically inhibit the growth of pancreatic cancer.

Joint application of radiotherapy and chemotherapy is one of the classical antineoplastic protocols. Damaging normal cells surrounding tumors and inducing radioresistance by repeated radiotherapy are the most important problems that radiotherapy must face. LTP is an interdisciplinary technology of physics, chemistry and biomedicine that has excellent effects in tumor treatment. LTP kills tumor cells selectively without damaging surrounding tissues, and this property overcomes the side effect of radiotherapy. LTP can also improve the sensitivity of tumor cells to drugs, overcome the cell resistance problem and reduce dosage of drugs, thus reducing the side effect of chemotherapy. In conclusion, although researches on the synergistic effects of LTP with drugs are still superficial, the combination of LTP and chemotherapy is a bold attempt in tumor treatment. Combination of LTP and drugs provides a new idea for cancer therapy and may become a new antineoplastic protocol in the future.

3.2. LTP's synergistic effects with nanodrugs

Nanomaterials are widely used as drug carriers due to their ability to adsorb and transport other components [37,38]. In the treatment of solid tumors, nanodrugs can significantly reduce the side effects of chemotherapy drugs, relieve suffering of patients and improve drug efficacy [39,40]. In the past decade, more and more studies have shown that the combination of LTP and nanodrugs can synergistically accelerate the tumor cells death without damaging normal cells [41–43]. Zhu et al. [40] treated breast cancer cells with LTP and core-shell nanoparticles loaded with 5-FU and found they could synergistically inhibit the proliferation of cancer cells. Mechanism studies showed that LTP could promote the cellular uptake of nanoparticles. Yu et al. [44] discovered that combing LTP treatment with paclitaxel-loaded core-shell magnetic nanoparticles could inhibit A549 cells growth.

Gold nanoparticles' surface plasma resonance (SPR) characteristic can cause the scattering and absorption of light, which makes gold nanoparticles widely studied in the field of tumor diagnosis and treatment [45]. At present, in addition to serving as drug delivery carrier, gold nanoparticles can also be used as radiotherapy sensitizers and diagnostic agents in clinical research [46–48]. The

SPR absorption of gold nanoparticles can rapidly transform light energy into local thermal energy, thus damaging tumor cells. In recent years, researches on gold nanoparticles as photothermal therapy have achieved preliminary results [49,50]. Many in vitro experiments showed that LTP and gold nanoparticles could synergistically inhibit tumor cells [42,43,51–54]. For example, Irani et al. [52] investigated the synergy effect of LTP and nanoparticles on HCT-116 colorectal cancer cells. HCT-116 cells were cultured in the presence of gold nanoparticles and then treated with LTP. After combined treatment, the number of viable cells decreased sharply. He et al. [53] treated U373MG glioblastoma multiforme cells with LTP and gold nanoparticles, and drew a conclusion that combination of LTP and gold nanoparticles could induce more cells death. Further studies revealed LTP could enhance gold nanoparticles endocytosis and help intracellular gold nanoparticles transfer to lysosomes.

ROS and RNS are the main active molecules of LTP to exert antitumor effects. In order to overcome the weak permeability of LTP, Kong et al. [55] proposed the chemical non-equilibrium characteristic of LTP could be combined with super penetration potential of nanoparticles to manufacture LTP-coded nano-capsules. The specific structure is shown in Fig. 2, in which the porous nanoparticles captured the reactive oxygen and nitrogen species (RONS) generated by LTP and formed the core of nano-capsules. The nanocapsules are then coated with polymer shells and have surface charges. Finally, the surface of nano-capsules could be connected to a variety of targeting ligands, transferred to sites of tumor growth or infection, and killed tumor cells and pathogens in a specific manner. Due to technical defects, the nano-capsules proposed cannot be successfully manufactured at this stage. This feasible theory may help LTP work deeper in the body.

Nanodrugs have more advantages than traditional drugs. Firstly, nanomaterials can load a great many drugs, delay the degradation of drugs, and reduce the dosage of drugs, so as to decrease the side effects of chemotherapy. Secondly, nanoparticles loaded with a variety of specific ligands on surface will destroy tumors precisely. Thirdly, nanoparticles ranging 1–100 nm in size can reach deep regions which are inaccessible to macromolecules. Furthermore, nanodrugs can be used in the treatment of multidrug-resistant tumors [56]. For decades, LTP and nanodrugs applied separately on tumor treatment have achieved good results. At present, more researches focus on the combination of them in order to gain an optimal treatment efficacy. It is noteworthy that strong permeability of nanodrugs may cause unpredictable damage to the body, also known as nanotoxicity, and the way of LTP working deep in the
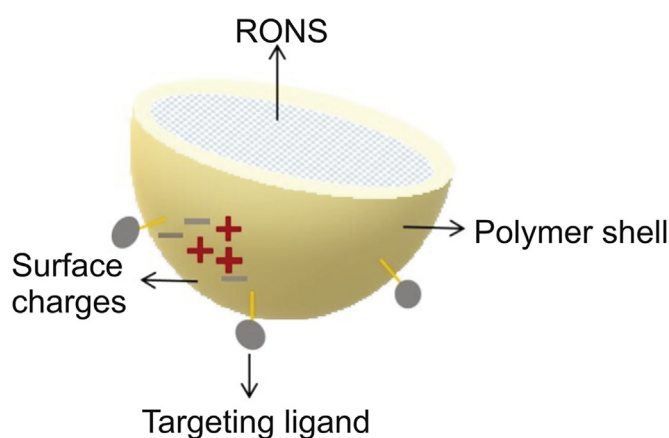

Fig. 2. Structure of LTP-coded nano-capsule. RONS: reactive oxygen and nitrogen species.

body is not yet resolved. All these problems bring difficulties to the clinical application of the combination therapy of nanodrugs and LTP. However, it is undeniable that the combination of nanodrugs and LTP brings a promising future to tumor therapy.

4. LTP's use in drug control and release system

Compared with the traditional material modification techniques, LTP surface modification technique is more rapid and convenient. What's more, LTP only changes the characteristics of nanometer depth of the material surface, while retains most of the properties of the material. As shown in Fig. 3, von Woedtke et al. [2] summarized five basic ways of LTP modification of material surface properties, namely, cleaning, thin film deposition, etching, modification/functionalization, and activation. Cleaning is helpful for the direct contact between LTP and material surface, and can be the first step of thin film deposition [57]. Thin film deposition refers to LTP polymerizing certain monomers on the material surface to form a stable thin film. The functions and properties of film are related to the type of monomers. LTP etching material surface can be used in integrated circuit manufacturing [58]. LTP can promote the production of functional groups on the material surface so as to activate the material surface and improve the hydrophilicity and surface free energy of materials. Activated material surface may graft other components to form graft copolymer with special functions. For example, polyester fabrics after LTP treatment grafting with chitosan oligomers would have a good antibacterial ability and biocompatibility [59].

Biomaterial surface modification is an important application of LTP material modification used in the medical field. LTP is a simple and green modification technology promoting the adhesion of cells on biomaterial surface by increasing biomaterials wettability and functionalization. Hyaluronan (HA) is essential for cell adhesion to extracellular matrix or material surface and is negatively charged by the carboxyl group. So Finke et al. [60] hoped to generate positively charged films by plasma polymerization on titanium surface to attract HA. They used allylamine as the monomer to form an adherent, ultra-thin and stable film. The film on titanium surface was rich in high-density amine groups. Compared with pure titanium, this titanium coated with plasma-polymerized film could promote the formation of osteoblastic focal adhesion and the development of actin cytoskeleton. Egghe et al. [61] used DBD to deposit N,N-dimethylacrylamide-based coatings on glass substrates, and found that coatings treated at a low power were unstable, while coatings treated at higher power were stable and showed a lower hydrophilicity. They found that good cell adhesion and survival were on the coatings obtained at high power. Because the production of plasma-polymerized film requires precise parameter control, Bullett et al. [62] studied different effects of several plasma deposition parameters on the functionality and stability of plasma-polymerized N-isopropyl acrylamide (NIPAAm) coatings. In addition to plasma power, temperature is one of the main factors determining the performance of coatings. The study showed that low substrate temperature and low power produced

unstable plasma-polymerized NIPAAm films. On the contrary, high temperature and high power produced stable films. And the deposition rate of NIPAAm decreased with substrate temperature but increased with plasma power. Appropriate LTP parameters were required to produce plasma-polymerized films with good biocompatibility and stability on biomaterial surface.

In the recent ten years, plasma material modification technique has also been widely applied in drug delivery system, which can regulate drug release rate in vivo and enhance therapeutic effects [63]. LTP regulates drug release rate mainly by promoting thin film deposition, producing crosslinked layers, increasing wettability and free energy of material surface.

Drug burst release refers to the phenomenon that a large dose of drugs is released from the drug delivery system within a short period of time, resulting in a sudden rise in blood drug concentration. Burst release is dangerous to human body and can cause waste of drugs. Slow and lasting drug release is very necessary in tumor therapy, hormone therapy and anti-infection therapy. Therefore, development of new drug release system to delay drug release rate has been a concern of many researchers [63,64]. Canal et al. [65] treated β-tricalcium phosphate ceramics with helium-APPJ. The β-tricalcium phosphate ceramics were loaded with doxycycline hyclate. They found that APPJ did not change the morphological characteristics of the ceramics, but increased the ratio of oxygen atoms to carbon atoms (O/C ratio), and reduced the initial release percentages of doxycycline hyclate. Chen et al. [66] used LTP to treat a chitosan film loaded with ciprofloxacin hydrochloride for 30 s, and then deposited a zein coating on the chitosan/drug film. Results showed that after LTP treatment, encapsulation efficiency, chemical composition and crystal structure of ciprofloxacin hydrochloride remained unchanged, but the hydrophilicity and surface free energy of the chitosan/drug film had a remarkable increase, the sedimentary volume of zein on the surface of the film increased significantly, chitosan was more closely connected with zein, and the 24-h release rate of ciprofloxacin hydrochloride was effectively reduced. Saitaer et al. [67] used LTP to treat polypropylene (PP) meshes, and then LTP-treated PP meshes were coated with polydopamine (PDA). The PDA coated meshes were further loaded with levofloxacin. Compared with LTP untreated PP meshes, LTP-treated meshes could be more effectively in coating with PDA. Plasma treated and PDA coated meshes could load more levofloxacin, delay drug release, and display better antibacterial properties, which might be an effective way to prevent mesh infections in hernia surgery.

In analgesia and sedation, the rapid release of drugs is needed to relieve the pain of patients in a short time. Some studies showed that LTP could act on drug delivery materials and increase drug release. Labay et al. [68] treated polyamide 6.6 (PA 66) fabrics with LTP at different air flow rates (5, 10, 15 L/min). They observed that after LTP treatment, the surface polydimethylsiloxane (PDMS) softeners hydrophobic coatings on PA 66 fabrics were etched, and the release of caffeine from fabrics was increased significantly. The thickness of PDMS decreased and caffeine release increased with the flow rate. When the flow rate was 15 L/min, the 24-h release
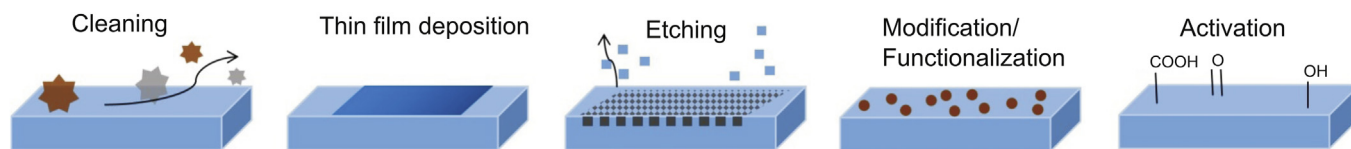

Fig. 3. Five ways of LTP to change material surface properties. Cleaning: LTP can clean the organic pollutants on materials surface. Thin film deposition: LTP can deposit monomers on the material surface to form a thin film. Etching: LTP can cause the loss of material surface particles. Modification/Functionalization: LTP can improve the hydrophilicity of material surface, and promote the generation of crosslinking and graft copolymer. Activation: LTP can promote the production of carbonyl, carboxyl, oxygen atoms and other groups on the material surface.

rate of caffeine could be increased to approximately 90%.

The modification effect of LTP on drug delivery system is related to plasma's parameters, including voltage, treatment time, and working gas. Working gases used in LTP are various, including oxygen, helium, argon, nitrogen, air and a mixture of several gases, and different gases may produce different modification effects on materials. Hagiwara et al. [69] studied LTP effects on curcumin release from poly (ethyl-*co*-vinyl acetate) (EVA) polymer and production of crosslinked layer in EVA using oxygen, argon and nitrogen as working gases. EVA surface became hydrophilic after treated by LTP. The drug release rate of EVA was constant up to 15 s of the oxygen plasma treatment time, and the amount of released drug decreased rapidly after 30 s of the treatment time. However, argon or nitrogen plasma treatment of only 5 s significantly suppressed the drug release. Argon plasma could be the most effective way to establish the crosslinking in EVA, followed by nitrogen plasma, while oxygen plasma would not induce the crosslinked layer in EVA. Ivanova et al. [70] modified poly(ε-caprolactone)(PCL) with LTP to improve the surface wettability and porosity, and promoted the deposition of porous $CaCO_3$ coating on PCL. Different working gases of LTP were used to treat PCL: argon, oxygen and ammonia. All the three LTP devices, especially oxygen plasma could produce strong functional groups on PCL surface and improve the hydrophilicity of PCL. The improvement of hydrophilicity was essential for the deposition of $CaCO_3$. Vaterite $CaCO_3$ was porous, which was conducive to bone tissue adhesion and drug delivery and grew more preferentially on PCL after oxygen plasma treatment than ammonia/argon plasma-treated PCL. And the $CaCO_3$ coating formed on oxygen plasma-treated PCL was smoother and more homogenous than coatings on ammonia/argon plasma-treated PCL. To obtain the best performance of drug delivery materials, scientists need to evaluate LTP's modification effects with different working gases and find the best treatment conditions.

Aging is a common phenomenon in plasma-treated materials. The hydrophilicity of material surface will decrease and the surface will turn into hydrophobic gradually with the aging time. Aging is related to the surrounding environment of materials. The higher the temperature is, the more likely aging is to occur. The higher the humidity is, the more difficult aging is to occur. Murakami et al. [71] used dry nitrogen gas as the hydrophobic aging medium and used distilled water as the hydrophilic aging medium to test changes of the polystyrene (PS), PDMS and phenol-formaldehyde resin (PFR) respectively in two aging media after plasma treatment. The free energy and work of adhesion in all three plasma treated materials decreased when they were aged in nitrogen. It can be explained that polar groups moved from the surface to the depth of materials. When polymers were aged in water, the surface free energy of all materials would turn to be close to the free energy of water. The moving direction of polar groups depended on the level of material surface free energy and water free energy. The surface free energy of plasma-treated PS or PDMS was lower than water energy, so the polar groups moved from the deep to the material surface to improve the surface free energy. The surface free energy of oxygen plasma-treated PFR was higher than water energy, and the polar groups moved from the material surface to the deep layer to reduce the surface free energy of PFR. The results indicate that the movement of groups aims at reducing the free energy gap between the aging medium and material surface, and aging medium is important to aging. Aging impairs the surface properties of materials treated by LTP, and that is an urgent problem to be solved.

Drug control and release systems require sophisticated technical support, so the appropriate LTP treatment condition is crucial for different drug delivery systems. Furthermore, whether the activity of drugs loaded on materials changes after LTP treatment also needs to be further studied. Although the development of LTP in drug delivery system is faced with many difficulties, LTP shows limitless applications in drug controlled release system and is expected to become a new device to control drug release.

5. LTP's use in preparing inactivated vaccines

Several experiments have proved that LTP has the ability to inactivate viruses significantly, such as feline calicivirus [72], adenovirus [73], and herpes simplex virus type 1 [74]. Terrier et al. [75] treated influenza virus type A, respiratory syncytial virus, and human parainfluenza virus type 3 suspensions with cold oxygen plasma generated by subjecting air to high-energy deep-UV light. They found a reduction of all three viruses after cold oxygen plasma treatment. The high efficient inhibition effect of LTP on multiple viruses shows that it has a great potential in the prevention and controlling of foodborne diseases and respiratory infectious diseases.

As LTP has a strong potential to inactivate viruses, Wang et al. [76] further explored whether LTP could only eliminate the pathogenicity of virus while retaining antigenic activity. They treated Newcastle disease virus (NDV) and H9N2 avian influenza virus (AIV) with LTP to explore a new way to prepare inactivated poultry vaccines. In their experiments, the working parameters of LTP were 18 kV voltage, 10 kHz frequency, a mixture of 88% argon, 2% oxygen and 10% nitrogen working gas, and 5 L/min gas flow rate. Both NDV and AIV lost pathogenic activities after LTP treatment for 2 min. And NDV vaccines obtained by 2 min-LTP treatment induced SPF chickens to produce the higher antibody titers compared with traditional vaccines. 2 min-LTP-treated AIV vaccines induced the similar antibody titers compared with traditional AIV vaccines. With overdose LTP treatment, the titers of antibodies induced by the two inactivated viruses were significantly reduced. The inactivated NDV and AIV vaccines produced after LTP treatment for 2 min could effectively prevent chickens from being infected with NDV and AIV strains. Wang's research provided a possibility for the application of LTP in vaccine manufacturing. More studies are needed to find out whether LTP can be used to produce vaccines and how safe and effective these vaccines are.

6. LTP's use in drug quality control

Ambient desorption/ionization mass spectrometry (ADI-MS) is a new type of analytical instrument, and does not require solvents and cumbersome drug pretreatment process. It is fast and accurate, and exhibits multiple advantages in qualitative and quantitative analyses of substances. There are multiple types of ADI-MS, and LTP-MS is one of them [77]. Liu et al. [78] desorbed eleven drugs to be degraded into ions with an LTP probe, and input the ions into MS for analysis. LTP-MS could quickly and accurately show the components of each drug, and determined the authenticity and quality. Jackson et al. [79] analyzed the biological fluid samples (saliva, urine, hair extract) of 14 abused drugs, including opiates, euphoriants, stimulants and sedatives with LTP-MS. Results showed that LTP-MS could directly analyze liquid samples and show good sensitivity. Wiley et al. [80] first introduced a handheld LTP device for material analysis which could be used with a miniature or benchtop mass spectrometer. This LTP device consisted of a circuit system powered by a lithium polymer battery, a glass tube for generating plasma, a miniature helium gas reservoir and a plastic casing, with a total weight of only 910 g. Fig. 4 is a brief schematic diagram of the device. Compared with traditional large LTP device, the handheld LTP source could still analyze experimental drugs accurately. Analogously, Ateacha et al. [81] combined a self-made small LTP probe with a benchtop high-resolution orbital MS, and successfully conducted qualitative analysis of the active ingredients
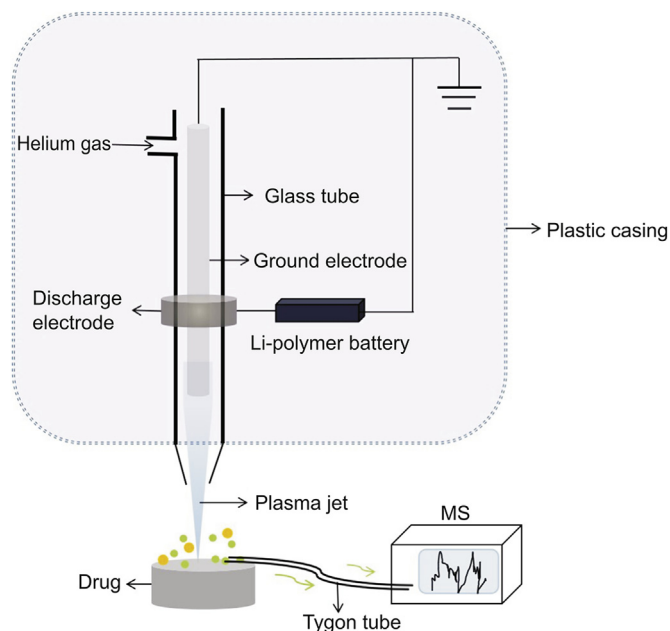
Fig. 4. Brief schematic diagram of LTP-MS.

in Coartem and Malarone tablets, which proved to be a new method for quick and accurate assessment of antimalarial drugs. LTP-MS has shown a broad application prospect in the field of real-time drug quality control.

7. Use of plasma-activated liquids

LTP was used in biomedical research in the following two ways to deal with samples: one is LTP acting directly on the biological samples; the second is LTP pretreating different solutions, and then the solutions are used in the tissues or microbial samples. The water or medium treated by LTP is known as plasma-activated water (PAW) or plasma-activated medium (PAM) [82,83]. Studies showed that plasma-activated liquids had a significant inhibitory effect on a variety of tumor cells and bacteria [82,84–89].

Duan et al. [90] co-cultured hepatocellular carcinoma cells HepG2 with normal liver cells L02 to simulate the tumor growth environment in human body. HepG2-L02 cells co-culture system was treated with PAM. It was found that PAM generated by 10 min LTP treatment could induce the apoptosis of HepG2 cells to the greatest extent without obvious damage to normal liver cells.

Kurake et al. [87] found that PAM induced apoptosis of glioblastoma cells by inhibiting glycolysis. In addition, PAM could kill cancer-initiating cells [91], human lung adenocarcinoma epithelial cells [92], human breast cancer cells [93] and other cancer cells. Chauvin et al. [82] successfully constructed colorectal tumor spheroids to simulate the real structure of tumor growth in human body. After cell culture medium was treated with helium LTP for 120 s, PAM was prepared. When the contact time with PAM reached more than 10 min, the spherules started to disintegrate, and PAM's damage to the spherules became obvious. Further studies showed that PAM could significantly reduce the ATP content in colorectal cancer cells and destroy mitochondrial function. Some in vivo experiments in mice [86,94,95] showed that injecting plasma-activated liquids into mice models could effectively inhibit the growth or metastasis of gastric, ovarian and pancreatic cancers.

Zhang et al. [89] discovered that PAW could inactivate Staphylococcus aureus, and with the longer PAW generation time and treatment time, the sterilization effect was more obvious. PAW

could effectively destroy bacterial membrane, membrane potential, intracellular pH homeostasis and DNA structure. It could be an efficient and environmental friendly sterilizer. Balan et al. [96] tried to explore whether PAW could be successfully used for duodenoscope disinfection and they found after 30 min PAW treatment, the activities of Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae were all decreased significantly. PAW treated duodenoscopes continuously for 30 min daily for 45 days, and did not cause significant damage to the surface structure and material components of duodenoscopes. This indicated that PAW had a strong bactericidal ability and minimal damage to precision medical instruments; therefore, it can be applied in clinical diagnosis and treatment.

Li et al. [97] treated Streptococcus mutans, Porphyromonas gingivalis and Actinomyces viscosus with PAW, and found that PAW could significantly reduce the number of all three bacteria in a short time. As a traditional mouthwash, chlorhexidine will cause side effects such as oral mucosal erosion, tooth staining, taste disturbance and parotid gland swelling. PAW is expected to become a new mouthwash due to its high antibacterial properties, but the issue of PAW biosafety needs further exploration.

In addition, some studies showed that PAW could destroy various viruses. Guo et al. [98] treated bacteriophages T4, Φ174 and MS2 with PAW, and found PAW could effectively inactivate three phages and destroy their proteins and nucleic acids. Su et al. [99] revealed that 30 min PAW treatment could completely inactivate NDV, decrease the protein content and disintegrate RNA into small molecular fragments.

Fig. 5 briefly describes the chemical reactions and RONS generated by liquids after LTP treatment. At present, it is believed that hydrogen peroxide (H_2O_2), nitrite (NO_2^-) and nitrate (NO_3^-) are the main active components of biological functions in plasma-activated liquids [84,100–102]. Plasma-activated liquids can be stored for a long time under suitable conditions. Vlad et al. [103] found that conductivity, pH value, H_2O_2 and NO_3^- concentration of PAW were almost unchanged after PAW was sealed from light for 21 days. Mohades et al. [84] also proposed that PAM produced by 4 min LTP treatment still had some ability to kill cancer cells after being stored at room temperature for 8 h. Shen et al. [104] compared the bactericidal effects of PAW at different storage temperatures ($25°C$, $4°C$, $-20°C$, $-80°C$), and found that PAW could still retain efficient bactericidal efficacy after 30 days of storage at $-80 °C$, and its H_2O_2 and NO_2^- contents were similar to the initial values without obvious attenuation.

Due to the above characteristics, the plasma-activated liquid is considered as a special tumor treatment drug and a green and efficient disinfectant, which has attracted extensive attention from the medical community in recent years. Research on the specific mechanism of active ingredients acting on cancer cells and bacteria is not sufficient, and there is still a long way to go before plasma-activated liquids are commercialized and applied in clinical diagnosis and treatment.

8. Challenges

It is undeniable that LTP has a great application prospect in the field of pharmacy, but it is still in the exploration stage faced with both opportunities and challenges. Due to the variety of drugs and chemical properties of drugs, as well as the multiple factors that can influence the effects of LTP, LTP still faces many challenges.

1) The mechanism of LTP promoting and inhibiting drug activity is unclear. When plasma acts on drugs, the electric fields, charged particles, RONS and UV will react with the drug components and induce the promoting or inhibiting effects. At present, some
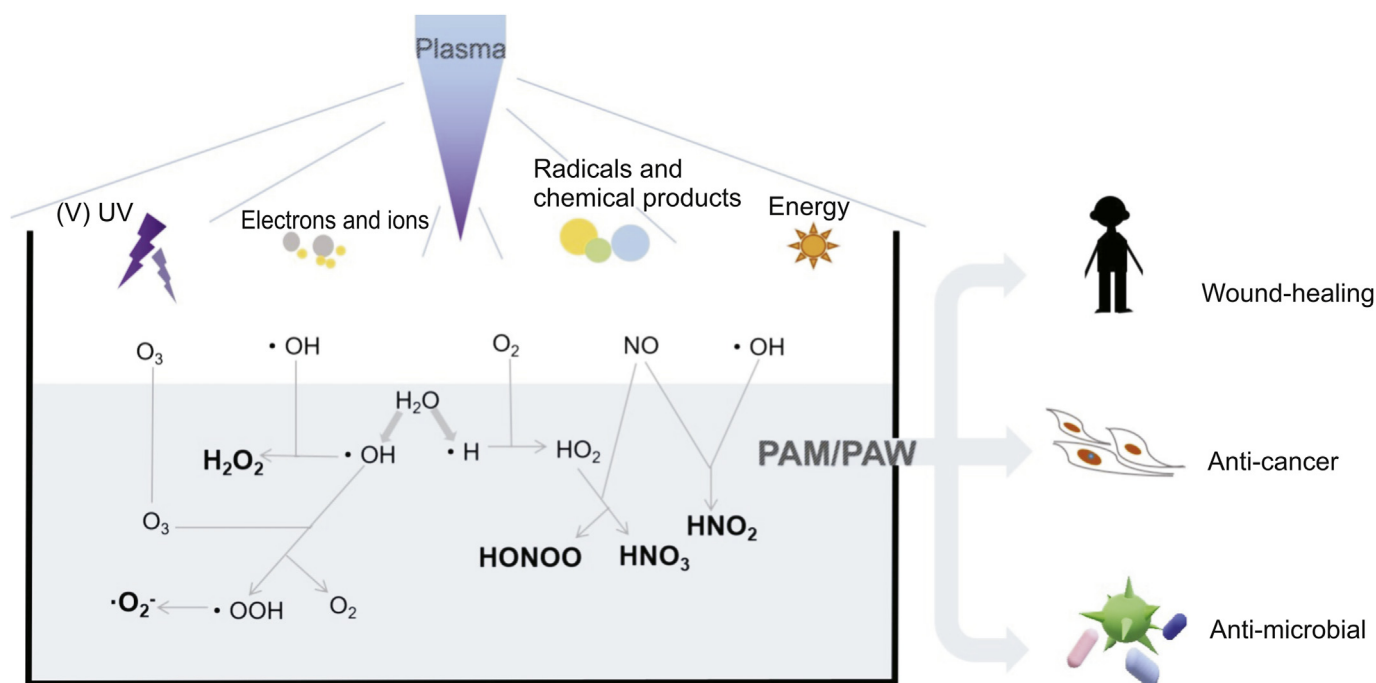
Fig. 5. Schematic diagram of plasma biological effects transmitted through liquid phase. LTP mainly contains hydroxyl ($\cdot$OH), ozone (O_3), nitric oxide (NO), nitrogen dioxide (NO_2) and other RONS. After LTP contacts with liquids, RONS will dissolve in liquids and start various chemical reactions. In addition to containing dissolved RONS, plasma treated-liquids also produce many secondary RONS, including $\cdot$OH, H_2O_2, NO, NO_3^-, NO_2^-, peroxynitrite ($ONOO^-$), etc. Those secondary RONS are considered to be the main factors of PAM/PAW to promote wound healing, kill cancer cells and inactivate microbes.

studies suggest that the degradation of drug components in sewage by LTP is mainly dependent on hydroxylation, while the effect of nitrogen oxides is very small [22,105]. However, it is not clear how deep RONS penetrates into the drugs, and which kind of RONS plays a major role. Therefore, new plasma devices need to be proposed so that they can control the identified types and levels of RONS to reach target drugs.

2) In the process of LTP degrading medical wastes, the toxicity of intermediate products may be comparable to that of the parent drugs, or even higher, so the real-time detection of toxic substances in sewage treatment and selection of suitable plasma treatment conditions to thoroughly purify sewage are important issues [105]. In addition, the effects of LTP degrading active components of drugs limit its application in drug sterilization. How to reduce the inhibitory effects of LTP on drug activity and achieve good bacteriostasis and decontamination effects still need further research [16]. As there are various kinds of drugs but relatively few researches on LTP treatment of drugs, much remains to be done to explore appropriate ways for sewage disposal, drug sterilization and decontamination, and new drug development.

3) The molecular mechanism of synergistic anticancer effect of LTP and drugs remains unclear. So far, there are few studies [33,34], and the molecular mechanism of the co-action of different types of drugs and LTP on tumor cells cannot be fully illustrated. The lack of theoretical basis limits the development of combined use of LTP and drugs in the treatment of tumors.

4) Plasma-activated liquid has been proved to have antibacterial effects, and be able to promote wound healing and inactivate cancer cells [82,106,107]. How does the RONS enter the cancer cells? What is going on inside the cells? How do the transduction of intracellular tumor suppressor signaling pathways and cell to cell communication take place? All these questions remain unanswered.

5) At present, most studies on LTP in the pharmaceutical field are based on in vitro experiments, such as cell experiments, 3D tumor modeling experiments and drug release experiments. There is lack of reliable in vivo experiments and clinical experiments. As a result, it needs strenuous efforts for LTP to become truly a "new drug."

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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