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Plasma pharmacy – physical plasma in pharmaceutical applications

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Dedicated to Professor Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

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During the last years the use of physical plasma for medical applications has grown rapidly. A multitude of findings about plasma-cell and plasma-tissue interactions and its possible use in therapy have been provided. One of the key findings of plasma medical basic research is that several biological effects do not result from direct plasma-cell or plasma-tissue interaction but are mediated by liquids. Above all, it was demonstrated that simple liquids like water or physiological saline, are antimicrobially active after treatment by atmospheric pressure plasma and that these effects are attributable to the generation of different low-molecular reactive species. Besides, it could be shown that plasma treatment leads to the stimulation of specific aspects of cell metabolism and to a transient and reversible increase of diffusion properties of biological barriers. All these results gave rise to think about another new and innovative field of medical plasma application. In contrast to plasma medicine, which means the direct use of plasmas on or in the living organism for direct therapeutic purposes, this field – as a specific field of medical plasma application – is called plasma pharmacy. Based on the present state of knowledge, most promising application fields of plasma pharmacy might be: plasma-based generation of biologically active liquids; plasma-based preparation, optimization, or stabilization of – mainly liquid - pharmaceutical preparations; support of drug transport across biological barriers; plasma-based stimulation of biotechnological processes.

1. Introduction

Currently, a new independent field of medical research is emerging worldwide combining plasma physics, life science and clinical medicine to use physical plasma for mainly therapeutic applications – meanwhile known as plasma medicine (Fridman et al. 2008; Kong et al. 2009; Weltmann et al. 2010). A specific field within plasma medicine is plasma pharmacy which means the use of physical plasma for pharmaceutical purposes. It is directed on the use of physical plasma to generate, modify and stabilize pharmaceutical preparations or to support its application.

1.1. Physical plasma

Plasma which is also called the "fourth state of matter" is an ionized gas produced by supply of energy. The high reactivity of plasma is a result of different plasma components: electromagnetic radiation (UV/VUV, VIS, IR, high-frequency electromagnetic fields, etc.) on the one hand and ions, electrons and reactive chemical species, primarily radicals, on the other, which are generated by supplying of – mostly electrical – energy to not directly effective gases (e.g. argon, helium, oxygen, nitrogen, air, or mixtures thereof, respectively) resulting in ionization and subsequent reactions in the gas/plasma phase (Fig. 1). Quantity and composition of plasma components are strongly dependent on parameters like energy input and nature as well as composition of the gas or gas mixture. A high concentration of fast reactive ions, atoms or small molecules determines the plasma to be hot (local thermal equilibrium – LTE) whereas cold or non-thermal plasmas (non-LTE) comprise of a lesser number of fast electrons embedded in a hull of normal non-excited atoms and molecules. Whereas thermal plasmas are naturally occurring phenomena e.g. the sun, which are tried to simulate technically for power generation in field of fusion research, non-thermal plasmas are pure technical ones whose composition and temperature are adjustable in a wide range by parameters like type of energy input, input power, type of gas, gas pressure and composition and others. In most cases, cold plasmas for technical applications are generated by applying electrical energy to a neutral gas or gas mixture, respectively (Braithwaite 2000; Conrads and Schmidt 2000).

1.2. Technological plasma applications

At the end of the 20th century, low-temperature plasmas were established in a wide field of technological applications – in several cases as enabling technology which is not primarily in the knowledge of the user or consumer. Most important fields are energy-saving lamps, pretreatment of polymer materials as for painting and bonding, improvement of packaging materials, several surface finishing technologies, waste and air pollution management, microelectronics, flat panel displays, and many more (Suchentrunk et al. 1997; Bonizzoni and Vassallo 2002;

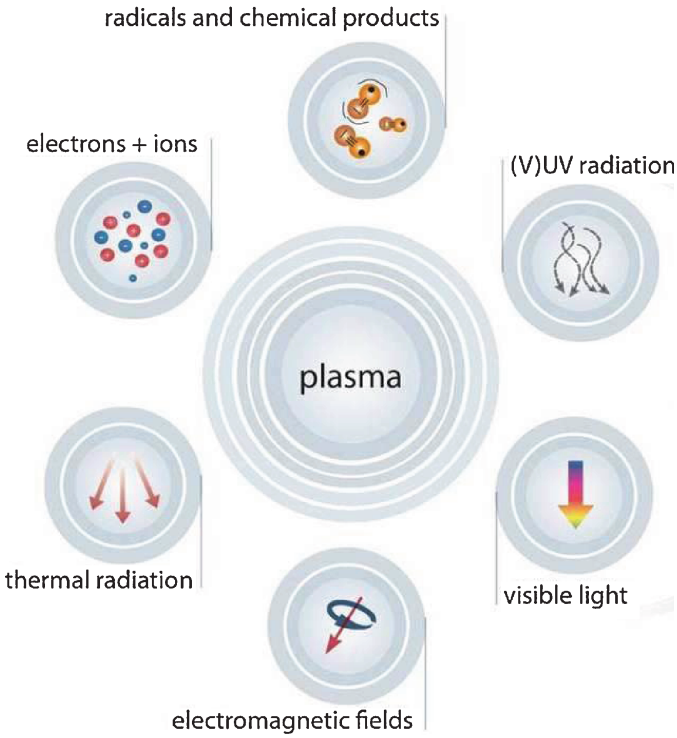

Fig. 1: Active components of physical plasma.

d'Agostino et al. 2005; Hess and Weltmann 2007; Kaiser et al. 2008; Brandenburg et al. 2010).

1.3. Plasma in medicine

Even if the medical use of plasma technology has been established since several years in so-called electrosurgical applications for tissue removal as well as cauterization (Stalder et al. 2005; Raiser and Zenker 2006), the new and rapidly growing research field of plasma medicine which is opened up for no longer than 10 years represents a new quality of plasma application in medicine.
In a more general perspective, medical application of physical plasma can be subdivided into two principal approaches:

- use of plasma-based or plasma-supplemented techniques to treat surfaces, materials or devices to realize specific qualities for subsequent special medical applications, and
- direct application of physical plasma on or in the human (or animal) body to realize therapeutic effects based on direct interaction of plasma with living tissue – the core area of plasma medicine.

The field of plasma application for the treatment of medical materials or devices is intensively researched and partially well established for several years. Plasma-assisted modification of bio-relevant materials is an established technique to optimize the biofunctionality or biocompatibility of implants or to qualify polymer surfaces for cell culturing and tissue engineering (Favia and d'Agostino 1998; d'Agostino et al. 2005). Modifications of biomaterial surfaces by plasma treatment range from changes of surface morphology and texture up to special physically and/or chemically based surface properties. These include increase of surface wettability or specific functionalization of surfaces to increase and optimize adhesion of living cells on the one side as well as realization of non-fouling surface conditions to inhibit adhesion of organic matter like proteins, bacteria or cells on the other (Favia and d'Agostino 1998; Ohl and Schröder 1999; Chu et al. 2002; Siow et al. 2006; Förch et al. 2007; Ohl and Schröder 2008; Cheruthazhekatt et al. 2010; Bazaka et al. 2011; Morent et al. 2011).
Another important field of plasma application closely related with plasma treatment of surfaces is its use for sterilization or bio-decontamination of materials or devices for medical purposes (Moisan et al. 2001; Laroussi 2005; Moreau et al. 2008; von Woedtke et al. 2008; Ehlbeck et al. 2011; Morfill and Zimmermann 2012; Weltmann et al. 2012a, b).
The direct use of plasma in medicine is intended in fields like dermatology, plastic surgery or dentistry, and is up to now focused on tissue regeneration, infected and/or chronic wounds as well as infective and inflamed skin diseases (Fridman et al. 2008; Heinlin et al. 2010; Lloyd et al. 2010). Another field of big interest is oncology (Keidar et al. 2011; Vandamme et al. 2012).

1.4. Basic research of biological plasma effects

However, to realize such medical plasma applications, the basic understanding of mechanisms of plasma effects on different components of living systems is a main challenge. Therefore, a comprehensive, systematic, and careful physical characterization of plasma sources and a comprehensive characterization of biological effects is essential (Weltmann and von Woedtke 2011).
During the last years, a multitude of findings about plasma-cell and plasma-tissue interactions have been provided and published.
There are several key insights that are useful not only for a better understanding of mechanisms of plasma effects on living systems but also to think about new and promising applications in the pharmaceutical field. Current considerations to use plasma for pharmaceutical purposes are mainly based on the following three key facts:

1. It was demonstrated that several biological effects do not result from direct plasma-cell or plasma-tissue interaction but are mediated by liquids.
2. It could be shown that plasma application results not only in lethal effects like inactivation/killing of microorganisms or local destruction and ablation of tissue in electrosurgery, but can also induce "soft" effects on cells, i.e. much more differentiated interaction of specific plasma components with special structural elements as well as functionalities of living cells which can possibly lead also to stimulation of cellular function.
3. It was demonstrated, that plasma treatment of cultured cells as well as tissue like skin may result in a short-term and reversible increase of permeability of biological barriers.

1.5. New field: plasma pharmacy

Apart from the fact that plasma use for decontamination/sterilization of pharmaceuticals and pharmaceutical packaging materials can be considered as a field of pharmacy, too, innovative application fields of plasma might be:

- preparation of antimicrobially active liquids for disinfection and antiseptics,
- modification of complex liquid components to influence cell and tissue behavior, e.g. stimulation of cell proliferation,
- preparation of specific drug containing carriers as micelles or nanoparticles, activation of drugs before application,
- support of drug transport across biological barriers,
- promotion of DNA transfer into host cells in gene technological processes,

- stimulation of growth and productivity of microbial and/or mammalian cells in biotechnological processes.

This knowledge focuses attention on a new and innovative field of pharmaceutical plasma application where the plasma could be used

- to generate, optimize and/or stabilize products which contain active agents, above all liquids,
- to support application as well as increase therapeutic efficiency of pharmaceutical preparations, and
- to optimize biotechnological processes for the production of pharmaceutically or otherwise useful compounds.

2. Possibilities of plasma application in pharmacy

2.1. Plasma-based generation of biologically active liquids

One of the recent key findings of basic research in plasma medicine is that several biological effects do not result from direct plasma-cell or plasma-tissue interaction but are mediated by liquids. It was demonstrated that simple liquids like water or physiological saline, are antimicrobially active after treatment with atmospheric pressure plasma and that these effects are attributable to the transient on-site generation of different low-molecular reactive species. The persistence of antiseptic activity in liquids and consequently the long-term stability of antimicrobially active substances generated in the liquid need further detailed investigation. According to the actual state of knowledge such plasma-treated water-based antiseptic liquids are stable over more or less long periods, possibly dependent on the special plasma treatment conditions (Naitali et al. 2010; Oehmigen et al. 2011a; Traylor et al. 2011; von Woedtke et al. 2012; Julák et al. 2012; Naitali et al. 2012).

Plasma treatment of more complex liquids like cell culture media results in changes of organic components which could induce various effects on living cells and their components (Kalghatgi et al. 2010, 2011, Vandamme et al. 2012; Höntsch et al. 2012; Haertel et al. 2012).

These results are important not only to underline the key role of the vital liquid environment of cells in the transmission of biological effects from atmospheric pressure plasma to living cells or microorganisms, respectively, but opens up the door for taking into consideration the plasma-supported generation and/or optimization of active substance containing liquids for medical applications.

Because of the assumed complex and multiple action mechanism of different plasma or plasma-generated components against microorganisms, according to the present state of knowledge it is not only possible to inactivate a broad spectrum including multiresistant microorganisms but it is considered to be most improbable that microorganisms develop resistance against plasma treatments (Daeschlein et al. 2012; Klämpfl et al. 2012). The same should be true for plasma-treated liquids even if this remains to be proved. In general the use of antiseptically effective liquids generated by plasma treatment might be a chance to overcome the increasing clinical problems of bacterial resistance against antibiotics and antiseptics. Based on primarily non-active liquids like water or physiological saline, antiseptically acting preparations can be generated on-site by plasma treatment.

However, it could be demonstrated that the generation of active species by atmospheric-pressure plasma treatment of aqueous liquids is a result of reactions at the plasma/gas-liquid interface. Consequently, any volume effects of plasma in liquids are based on diffusion and convection processes from the liquid surface into the bulk (Oehmigen et al. 2011b). Therefore, the activation of bigger liquid volumes is a practical challenge. It could be overcome by special technical solutions like electro-spraying technologies (Machala et al. 2010, 2012).

Apart from this practical restriction the use of plasma-activated liquids could be a promising alternative whenever limited amounts of broad-spectrum antiseptics are needed and the use of antibiotics has to be avoided.

2.2. Plasma-based preparation, optimization, or stabilization of pharmaceutical preparations

Another very promising field which might be considered as potential part of plasma pharmacy is the use of atmospheric pressure plasmas to induce or catalyze chemical reactions in liquids.

Some years ago a method was published to use a low-temperature plasma discharge reactor for polymerization of unsaturated compounds. Using amphiphilic monomers (tensides) with unsaturated molecule parts, plasma treatment was used to stabilize shape and size of micelles in aqueous media below the critical micelle formation concentration (CMC) (Dwars et al. 2003). This opens up the possibility to solubilize and/or stabilize poorly soluble or non-soluble substances in aqueous media using micelles without the detrimental side effect of high surfactant concentrations of the respective liquids.

Moreover, the variety of chemistry which is induced by plasma treatment can also be used in the innovative and growing field of nanoscience. In principle, several synergies between plasma technology and nanotechnology could be imagined. Synthesis, functionalization and processing of nanomaterials and nanoparticles could be used for the fabrication and optimization of innovative nanoparticulate drug-delivery systems (Graham and Stalder 2011; Kong et al. 2011).

Another conceivable possibility is the plasma-based activation of drugs in liquids. Several substances which are not stable in liquids have to be applied as prodrugs which are needed to be activated by first-pass metabolic processes inside the body after application and absorption. By special tailoring of such substances, similar drug activation might be realizable outside the body immediately before application of liquid pharmaceutical preparations utilizing the possibilities of specific in-liquid physical plasma reactivity.

However, such processes are not limited to liquid preparations. As mentioned already, the potential of surface modification by plasma treatment is broadly used to obtain specific characteristics of biomaterials (Fig. 2) (Chu et al. 2002; Siow et al. 2006; Bazaka et al. 2011).

Kuzuya et al. (2001) described the possibility of using plasma to prepare or modify controlled drug release systems as multilayered tablets or functionalized composite powder for matrix systems by specific plasma-surface interactions. In these and related studies low-pressure plasma techniques are used. In practice, vacuum systems are needed for these techniques resulting in a limitation to batch production processes. But similar surface-modifying effects have been demonstrated for atmospheric pressure plasmas, too (Foest et al. 2005; Schäfer et al. 2008, 2009; Vogelsang et al. 2010, 2011; Fricke et al. 2011, 2012). This opens up the possibility to integrate plasma-based surface treatment steps into continuous production processes of solid pharmaceutical preparations for controlled drug release.

2.3. Support of drug transport across biological barriers

It was hypothesized by some authors that non-lethal plasma effects on cells could be related to sub-lethal influences on diffusion characteristics of cell membranes (Stoffels et al. 2006;

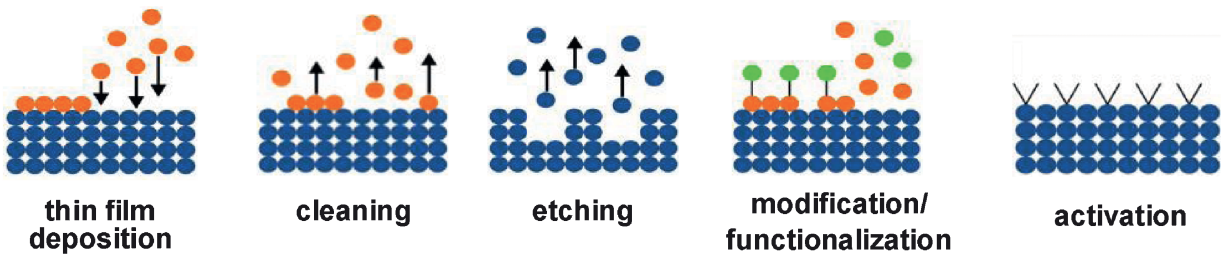

Fig. 2: Basic categories of plasma-chemical interaction with material surfaces.

Kalghatgi et al. 2010). Such kind of plasma induced non-lethal but reversible cell permeabilization was described by Leduc et al. (2009) who demonstrated by *in vitro* experiments that dextrane molecules which are not freely diffusible through cell membranes could be transferred into HeLa cells dependent on molecular size by plasma treatment. Other authors used similar plasma treatments for transfection of DNA into cells without DNA degradation (Ogawa et al. 2005; Sakai et al. 2006).

As an example of plasma-nanoparticle synergy it was shown that killing of cancer cells *in vitro* by antibody-conjugated gold nanoparticles could be significantly stimulated by nonthermal air plasma treatment (Kim et al. 2009, 2011; Lee et al. 2011).

Moreover, by *ex-vivo* studies using pig ear skin it could be shown that the penetration of both chemical substances and nanoparticles into the skin was enhanced by atmospheric-pressure plasma treatment (Lademann et al. 2011a, b).

Such reversible manipulation of transfer characteristics of cell membranes as well as biological barriers like skin is called "plasma poration" (similar to well-known electroporation or sonoporation effects). Consequently, in the future plasma treatments could be used to support intracellular effectivity of pharmacological substances e.g. in cancer therapy or to permit or enhance transcutaneous drug delivery (Weltmann et al. 2011).

2.4. Plasma-based stimulation of biotechnological processes

High-yielding microbial and/or mammalian production strains are the condition for economic production of metabolites by biotechnological processes. These high-yielding strains are generated by a combination of mutagenesis and selection. Besides genetic engineering, X-ray, gamma-ray, UV ray or radical forming chemicals are often used to induce desired changes of the DNA. The plasma glow contains components like UV radiation and highly reactive radicals which could possibly work synergistically in generation and improvement of production strains.

There are few investigations demonstrating the efficiency of atmospheric-pressure plasma for breeding high-yielding bacterial strains (Chen et al. 2010; Dong et al. 2008; Wang et al. 2010).

Dong et al. used dielectric barrier discharge (DBD) plasma to generate a stable strain of *Klebsiella pneumoniae* with improved 1,3-propanediol production (Dong et al. 2009, 2010). They showed an increase of the specific activities of producing enzymes (glycerol dehydrogenase, glycerol dehydratase and 1,3-propanediol oxidoreductase) and an increase in product formation. The bacteria were treated by direct exposing an agar plate containing *Klebsiella* to DBD plasma. The highest percentage of positive isolates (31%) after treatment of several wild strains was obtained from a plasma treatment time of 120 s. The survival rate of DBD plasma-treated cells decreased with increases in treatment time, but a slight recovery occurred. Further cultivation was done under fed batch conditions. The concentration of 1,3-propanediol in the medium after fed batch cultivation for 36 h in the best plasma treated strain (Kp-M2) was 76.7 g/L and 49.2 g/L in the wild type strain. The selected strain Kp-M2 grew faster and showed more rapid glycerol consumption in batch fermentation than the wild strain.

The aim of the work of Chen et al. (2010) was to improve the antagonistic activity of *Bacillus subtilis* JA towards *Fusarium graminearum*, a crop pathogen, by generation high-yielding mutants using the atmospheric-pressure plasma jet (APPJ) with helium as operating gas. The antifungal activity of *B. subtilis* is exhibited by lipopeptide antibiotics. The bacteria were loaded on a sterilized filter paper and this was put on a Petri dish. Then, the filter paper was exposed to the plasma jet below the nozzle. After plate culture the bacteria were transferred into suspension cultures. The successfully screened out strain B06 showed larger inhibition zones against *F. graminearum* (+23%) than the original strain. The lipopeptide production of the plasma-treated strain was the 2.3-fold of the original strain. Besides, faster growth of the treated strain was observed.

Streptomyces avermitilis produces the antiparasitic avermectins which are commercially used in animal health and agriculture. The entire genome sequence of *S. avermitilis*, including that of the gene cluster for the biosynthesis of avermectins, is already known (Ikeda et al. 2003). Wang et al. (2010) employed an atmospheric pressure glow discharge (APGD) plasma jet, driven by a radio frequency power supply with water-cooled and bare-metallic electrodes, to treat the spores of *S. avermitilis* and to improve the fermentation efficiency for the production of avermectins. The plasma jet yielded high total (over 30%) and positive (about 21%) mutation rates on the bacteria. A mutated strain (G1-1) with high productivity of avermectin B1a, the main component of the avermectins, and genetic stability could be obtained.

In a recent study, the influence of atmospheric pressure low temperature plasma jet (Weltmann et al. 2009) on growth and metabolite production of cultured cyanobacteria and higher fungi was investigated. Short time plasma jet treatment of a *Nostoc* strain as example for cyanobacteria resulted in increased production of cytostatic and antimicrobial cyclophanes (Neidhard 2012). Treatment of the mycelial cultures of a *Ganoderma* species with plasma resulted in positive effects on growth, extract yield, content and spectrum of bioactive components of some extracts. The effects were dependent on time, plasma equipment, plasma producing gas and treatment conditions (Wucherer 2011).

The existing examples demonstrate the potential of physical plasma for an optimization of biotechnological production processes. Until now, only growth and yield of biomass and some products have been investigated. It can be expected that also the product spectrum, the release of metabolites from cells

into nutrient medium and other parameters, will be influenced. Generally, for a better understanding and evaluation of the plasma induced processes in the cells an exact knowledge of the genes responsible for the biosynthesis of the desired products is necessary. All published results have been obtained with prokaryotic cells. Our observations with mycelial cultures of a basidiomycete show that also eukaryotic cells can be influenced. Further investigations to estimate the possible application of physical plasma for the stimulation of biotechnological processes in eukaryotic cells are in progress. One idea is the above described modification of membrane permeability of cells by plasma. Such a "plasma poration" could improve the production of recombinant biopharmaceuticals by allowing the effective transfer of DNA without damages into suitable expression systems.

Another pharmacy and biotechnology related field of plasma application could be the plasma stimulated degradation of drug metabolites in sewage. The removal of pharmaceutical compounds, e.g. antibiotics (Magureanu et al. 2011), from water samples in laboratory scale could be shown.

3. Conclusion and outlook

Plasma pharmacy is a special field in medical application of physical plasma. In contrast to plasma medicine, what means the direct use of plasmas on or in the living organism for therapeutic purposes, in this field plasma is used to support manufacturing or application of pharmaceutical preparations. Especially the recent key insight of plasma medical basic research that plasma effects on microorganisms and mammalian cells are mainly caused by changes of the surrounding liquids has accelerated some research on biological effects of plasma-treated liquids and its potential use for medical applications. One of the advantages of plasma-treated liquids is that direct contact of plasma with living tissue is avoided and, consequently, some possible side effects (e.g. caused by UV radiation) can be excluded. Another advantage is the possibility of generating such plasma-activating liquid from non-active basic liquids like physiological saline on-site immediately before its application. However, it has to be taken into consideration that such plasma-pharmaceutical products have the character of drugs. Together with the innovative technique of generation of such plasma-activated liquids, the licensing of such products according to regulatory requirements demands further attention.

In biotechnology plasma could become an important tool to breed high yielding strains for production of drugs or other compounds and to promote the transfer of DNA into suitable expression systems e.g. for the generation of biologicals. This field of plasma application is just scarcely examined. Its future success depends on a better understanding of the genes responsible for the synthesis of the desired products and the effects of the different plasma components on it.

Besides the generation of active agents-containing liquids or stimulation of metabolite-producing cells or microorganisms, respectively, because of its specific reactivity plasma can be used to stabilize pharmaceutical preparations or to generate specific characteristics e.g. of drug delivery. Here, plasma technique is not limited to liquid preparations, but the well-established surface-modifying potential of plasma can be used for specific drug preparations too.

Last but not least, physical plasma can be used to support drug delivery processes across biological barriers. Accompanying application of plasma devices could be helpful to bring active agents into living cells or to enhance transdermal drug permeation. Above all the latter field should be tested as an alternative to several technical systems for transdermal drug delivery as laser microporation, sonoporation, iontophoresis or micro-needles being under investigation or in use already.

In general, plasma application in pharmacy is in a very early stage of research, yet. However, because of the world-wide rapidly increasing field of plasma medicine, several options of pharmaceutical plasma application become apparent. Pharmacists should use this chance to launch an innovative tool like physical plasma into their field of research.

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