Chapter 11. Drug delivery using cold plasma

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Chapter Abstract

Cold Plasma (CP) is an ionised gas generated in atmospheric pressure and at room temperature. CP technology has already shown great promise for a wide range of biomedical applications. This chapter provided insights into the various applications and types of CP devices/configurations used to promote drug delivery. CP is a promising method to enhance the treatment of diverse skin diseases and some types of cancers by improving drug delivery and reducing the required dose. CP has also been utilised to modify the structure of a range of biocompatible materials to enhance drug delivery in different tissues. This technology has showed remarkable growth in recent years; however, future studies should be focused on *in vivo* and clinical studies to facilitate the adoption of CP in clinical settings for drug delivery.

11.1 Introduction: Fundamentals of CP and drug delivery

Over the past few decades, the trend of replacing traditional techniques in drug delivery has been intensively investigated. Since 1879 when plasma was first discovered as the fourth state of matter by Sir William Crookes and named in 1927 by Irving Langmuir (Gates, 2018), it has been significantly optimised to substitute traditional treatments within various fields with remarkable efficacy. Plasma treatment can be divided into thermal and cold/non-thermal atmospheric plasma. CP or cold atmospheric pressure plasma (CAPP) can be conveniently generated in an atmospheric environment with a gas temperature as low as room temperature, while higher power and pressures need to obtain thermal plasma. CPs can be generated using a wide range of adjustable temperatures, energy and power input, type, pressure, and gas composition.

In most cases, CPs are generated by using a range of single gases or gas mixtures, such as oxygen (O_2), nitrogen (N_2), carbon dioxide (CO_2), helium (He), and argon (Ar). From the physical and chemical reactions that are taking place, a non-equilibrium state between electrons and neutral ions and free radicals is achieved (Ma et al., 2022). Additionally, these reactions may lead to the production of numerous stable reactive atomic and molecular molecules and atoms, e.g., reactive oxygen species (ROS), reactive nitrogen species (RNS) and finally, the generation of highly energetic UV photons.

In the past, CP has been used for sterilisation and decontamination of various surfaces and, more recently, as a novel processing technology in the food industry. The application of CPs has been gaining increasing importance in inactivating many types of common pathogenic bacteria on different substrates (Asimakopoulou et al., 2022; Ekonomou and Boziaris, 2021; González-González et al., 2021) as well as inactivating endogenous

enzymes (Sonawane et al., 2020). Moreover, the broad field of technological applications of CPs and the continuous research for innovative cold plasma applications in the new century resulted in the use of CPs in medical technology, biotechnology, and pharmacy. This ground-breaking and emerging field is called plasma medicine, and the medical applications of CPs can be branched into i) "Indirect" and ii) "Direct" cold plasma-based techniques. In this way, indirect plasma techniques can be applied to treat various materials, coatings and surfaces, while the direct techniques are focused on the direct application of CPs in the human or animal body and living tissue (von Woedtke et al., 2013). CPs can be generated by a range of devices, with dielectric barrier discharge (DBD) being the most widely used indirect plasma source (Figure 11.1a). Indirect plasmas are produced between two electrodes and then transported to the desired area via the gas flow. Indirect plasmas are preferable for the treatment of living cells and tissues. However, the more versatile direct jet plasma (Figure 11.1b) can be applied to perform *in vitro* and *in vivo* treatments using plasma needle or torch devices (O'Connor et al., 2014). Hybrid plasmas are less commonly used and combine the plasma production technique of DBD with the properties of jet plasmas (Heinlin et al., 2011). A broad spectrum of direct, indirect, and hybrid plasma sources offered for biomedical applications has been reported recently, atmospheric pressure plasma plume, jet, glow discharge torch (APGD-t), CP brush, CP needle, floating-electrode DBD (FEDBD), CP jets, micro-plasma jets and considerably more. However, not all CP sources have been proven valuable tools for biomedical applications, and further biological characterisation is needed to prove their potential.

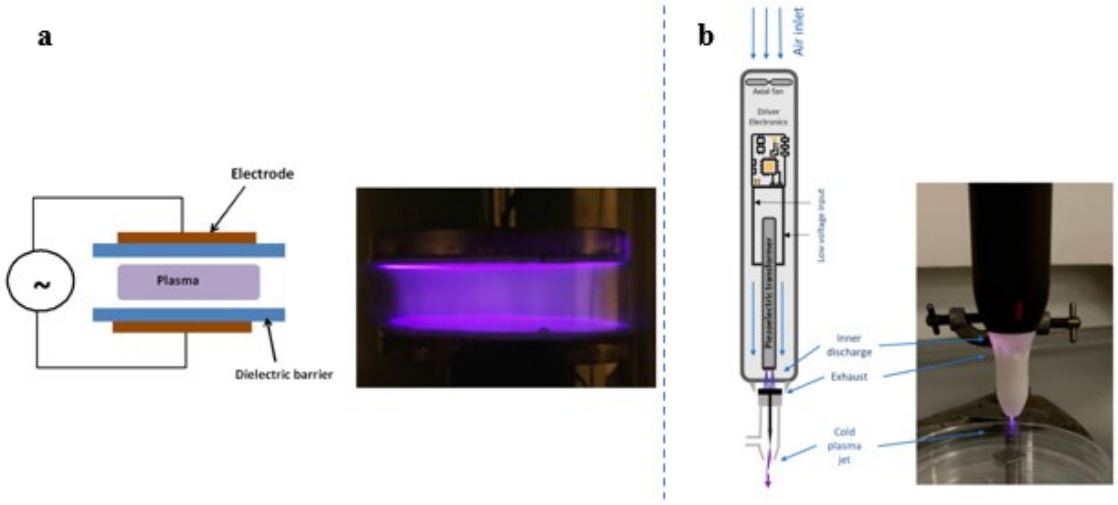

Figure 11.1: Schematic view (left) and photograph (right) of a) an atmospheric pressure diffuse plasma generated by a dielectric barrier discharge (DBD) adapted from Laroussi, 2018 and a schematic view (left) and a photograph (right) of b) a handheld CP Jet nozzle system with attached nozzle against a nonconductive surface adapted from Gonzalez-gonzalez et al., 2021.

Despite CP technology being a new principle in the medical field, its applications in drug delivery have experienced considerable growth. Drugs are currently being used as effective means to improve health and increase longevity. Drug delivery can be described as the transportation process of a therapeutic agent with appropriate pharmacokinetics to achieve the desired effect. Medications can be administered into the body by swallowing (through the gastrointestinal tract), inhalation, absorption through the skin, or intravenous injection (Chamundeeswari et al., 2018).

However, some of the existing methods for drug delivery pose significant drawbacks. For example, drug administration by swallowing leads to reduced absorption and

bioavailability of the drug as it moves through the gastrointestinal tract. More recently, direct drug administration to infected or at risk body areas has been attempted. However, local administration of drugs with sustained release can be achieved through nanostructures acting as the drug's delivery vehicles at the desired site and, more recently, by localised, non-invasive plasma treatment, which offers the possibility of controlled drug release at the molecular level (Heinlin et al., 2011).

Over the last two decades, many researchers in the field of plasma medicine demonstrated that CPs could be applied for clinical and biomedical applications (beyond decontamination) to improve drug delivery and surpass the limitations of pain, electric shock, patient discomfort, skin deformation and irritation observed with the application of traditional methods. Plasma medicine is being extensively investigated for various therapeutic applications in dermatology, dentistry, infection control and oncology. This chapter focuses on the direct or indirect application of CPs for enhancing drug delivery.

11.2 Cold atmospheric plasma applications for transdermal drug delivery

The transdermal delivery system of drugs is an attractive method for painless, non-invasive delivery and sustainable release through the skin to the blood circulation. CP techniques have proven their potential as transdermal drug delivery approaches to improve absorption rate of various drugs.

CP treatments only affect the surface of different types of materials without causing alteration of the physical, chemical, mechanical, electrical, and optical properties of their interior (Tabares and Junkar, 2021). Thus, several research studies aimed to treat dermatological problems, such as skin wounds and infections, by plasma which is

relatively easy to achieve by adjusting the discharge and plasma parameters (Gan et al., 2021; Mai-Prochnow et al., 2014; O'Connor et al., 2014). However, efficient transdermal drug delivery remains a challenge mainly due to the skin's barrier properties, namely *stratum corneum* (SC), the highly lipophilic outermost layer of the epidermis. CP applications have been focused on their effects on the skin barrier as an approach for drug delivery in clinical and biomedical applications beyond a disease treatment. For instance, it was found that a 9-channel plasma jet array treatment promoted transdermal delivery of patent blue V, which is a synthetic triphenylmethane hydrophilic dye with a molecular weight (MW) of 1159.427 Da used in medicine as a dye to colour lymph vessels (Lv et al., 2021). The authors investigated the effect of the jet array under 7 kV, 7 kHz and 9 kV, 9 kHz treatment for 3 and 6 min on porcine ear skin using He or a mix of He with 0.5% oxygen (O_2) working gas composition. Their results revealed the potential of using CP jet array treatment for enhancing transdermal drug delivery with drug penetration across the skin being enhanced between 2 and 110 times after the plasma treatment. It also increased with treatment time as well as when the working gas was a mixture of He/O_2. A limitation when a high applied voltage was used (9 kV with pulse frequency at 9 kHz, 6 min) is that it caused an increased heating of the skin. However, lower voltage treatments did not have the same undesirable effect. The authors concluded that the increased density of the ROS flux observed with treatment time and the 0.5% O_2 incorporation into the working gas enhanced the penetration efficiency of the drug. A potential mechanism for this could be the intracellular lipid layer's oxidation, causing instability of the SC's structure due to the presence of unsaturated lipids and cholesterol. Moreover, further studies showed a beneficial transdermal delivery of topical anesthetic cream with lidocaine (MW: 234.34

Da) and prilocaine (MW: 220.316 Da) as a pre-treatment prior to CO_2 laser treatment for post-acne scars (Xin et al., 2021b) and galantamine hydrobromide (MW: 368.3 Da) used as a drug for Alzheimer's disease treatment (Shimizu et al., 2016). Even though the exact mechanism for increasing skin permeability upon CP treatment is yet to be identified, Van der Paal et al. (2019) proposed that CP generated reactive species induce lipid oxidation of the intracellular lipid layers. This effect leads to cross-linkages between the SC's anchored lipids and subsequent formation of nanopores thus, facilitating the increased transdermal permeation of drug molecules.

One of the three pathways that allow a drug to penetrate the SC is the intracellular route – together with the intercellular route and follicular route – where the drug must diffuse across the keratinocytes found in the lipid matrix and then be transported straight to the dermis. Recently, Lee et al. (2021) used an atmospheric-pressure, Ar-plasma jet device to treat keratinocytes for 10, 30, and 60 s and proved that CP treatment significantly increased the transfer of high-MW fluorescein-dextrans (70 and 150 kDa). The increased transmission observed was due to the plasma-induced nitric oxides into the Ar-treated HaCaT cells that can regulate the junctions anchoring the cells of the second skin barrier and lead to increased permeability. Moreover, the fluorescent signals obtained showed a significantly increased intensity of the high-MW molecules in the Ar-treated HaCaT cells compared with the untreated cells, suggesting that Ar-plasma increases the permeation of molecules over 1 kDa that are difficult to penetrate the skin. As we have already discussed, the first barrier of the skin is SC, while the second skin barrier, located below SC, mainly consists of a group of proteins able to form a strong barrier that can inhibit penetration of external agents (Elias, 2005). In another study, it was demonstrated that Ar-plasma

treatment for 5 min using a DBD plasma jet on HaCaT cells modulated the function of E-cadherin which is involved in cell-to-cell interactions, and its modulation can affect the skin's permeability (Lee et al., 2018). This is in line with the Schmidt et al. (2020) study, that investigated the effect of CP treatment both *in vitro* on keratinocytes and *in vivo* on murine skin. Plasma treatment was carried out using an atmospheric pressure Ar-plasma jet kINPen Med at a frequency of 1 MHz and a constant distance of 8 mm. *In vitro* experiments revealed how the plasma-derived ROS of hydrogen peroxide, nitrite, nitrate, or hypochlorous acid act on skin cells, while it is known that short-lived species (such as hydroxyl radicals, superoxide anion etc.) deteriorate in the culture media (Bekeschus et al., 2017). The authors found that plasma-derived reactive species modify the junctional network by affecting expression levels of transmembrane proteins, which promotes tissue oxygenation and oxidation of SC-lipids. Following this, *in vivo* plasma treatment caused histological changes to the murine skin leading to increased levels of curcumin (MW: 368.38) within the SC, but plasma treatment did not show to affect curcumin permeation in the epidermal region.

11.3 Cold atmospheric plasma applications in oncology – cancer treatment

In this section, special consideration will be given to the most recent approaches of CP as an efficient method for promoting drug delivery against numerous carcinogenic cells as a new growing field in plasma medicine called "plasma oncology". Although this is a relatively new field, with the first results reported by Kieft et al. (2004), the increasing number of research articles showing the successful promotion of drugs in treating a broad spectrum of different tumour cells has fuelled the hope for CP to be used as a new therapy

for cancer. Several studies outlined in this section show that CP can be used as a novel physical drug delivery tool against tumour cells *in vitro*.

11.3.1 *In vitro* CP applications for drug delivery in cancer cell lines

The development of efficient and safe drug delivery methods to substitute the well-established techniques of electroporation and sonoporation remains challenging. CP has been identified as a promising method to substitute these techniques since the anticancer effects of plasma seem to be uniform and are not restricted to a particular type of tumour. It has been shown that direct and indirect CP treatment can be used as a new physical drug delivery tool for low and high-MW molecules into human cervical cancer HeLa cells and murine breast carcinoma 4T1 cells (Vijayarangan et al., 2020). In the work of Vijayarangan et al. (2020) a He-plasma jet DBD reactor was employed to treat cells directly with a constant capillary-to-cells distance set at 11 mm or indirectly with plasma-activated media (PAM) generated at high voltage pulses (14 kV, 100 Hz pulse frequency) after 100 s. Interestingly, the uptake efficiency in 4T1 cells after CP treatment increased. Furthermore, the internalisation efficiency revealed a size-dependent uptake into 4T1 cells with the highest molecular weight FITC-Dextran of 150 kDa showing a successful but lower delivery into the cells than the same agent, at lower-MW of 4 and 70 kDa. These results revealed that CP induced uptake could be more challenging for higher MW compounds, while similar observations were made in a previous study of the same research group for HeLa cells (Vijayarangan et al., 2018). In addition, Vijayarangan et al. (2020) confirmed the successful delivery of doxorubicin (MW: 543.4 Da) into 4T1 cells with direct CP treatment, an agent used as chemotherapy medication to treat various types of cancer.

However, in the same study, when cells were treated indirectly, with PAM alone or in combination with electric fields, no cellular uptake was observed compared to direct CP treatment. The increased drug uptake observed after direct CP treatment was due to the disruption of the transient plasma membrane by the reactive oxygen and nitrogen species (RONS) produced which lasted for tens of minutes, allowing the efficient uptake of small and high-MW substances into HeLa and 4T1 cells.

The work of Vijayarangan et al. (2020) revealed the great potential of CP for increased cell uptake and sparked new opportunities for combined protocols. Xu et al. (2016) investigated the biological effects of CP treatment on multiple myeloma cells (LP-1 MM). They found that plasma treatment could be applied as a new strategy to overcome the resistance of myeloma cells to chemotherapy medications, which is a considerable challenge. Following CP treatment using a plasma jet device at 10 kHz pulse frequency and a fixed distance of 2.0 cm for 30 and 40 s, myeloma cells revealed an increased sensitivity in bortezomib (MW: 384.237 Da) first-line drug in myeloma chemotherapy. The combined treatment of CP and bortezomib on LP-1 cells showed a significantly decreased cell viability compared with each treatment used alone, indicating the synergistic effect and the higher delivery of the chemotherapeutic agent into the cells after plasma treatment. The higher sensitivity of myeloma cells was previously found by Xu et al. (2012) that could be due to the downregulation of the *CY1A1* gene expression that accelerates the bortezomib metabolism in myeloma cells. Further investigation is needed to prove if CP treatment could decrease the CYP1A1 expression leading to improved sensitivity to bortezomib. Direct or indirect CP treatment can demonstrate different sensitivity in different cancer cell lines. Glioblastoma (GBM) is the most common type of malignant brain tumour in adults. To

elucidate the effect of CP treatment, Köritzer et al. (2013) analysed glioblastoma cell lines LN18, LN229 and U87MG. They used a CP device based on Surface Micro Discharge technology where the electrode for plasma production was placed at the top inside a closed box, and plasma was produced at 8.5 kV voltage and 1 kHz pulse frequency in ambient air. Remarkably, CP treatment for 30 to 180 s restored the responsiveness of resistant LN18 glioma cells towards therapy with the first-line chemotherapeutic drug temozolomide (TMZ) compared to treatment with TMZ (MW: 194.151 Da) alone. This synergistic effect indicates a high likelihood of CP treatment in improving the delivery of chemotherapeutic drugs across the blood-brain barrier to reach and treat tumour cells, revealing the high potential of CP in cancer therapy. In another interesting study, a He-CP jet device with two electrodes was used as a promising technique to promote immune checkpoint blockade (ICB) therapy against cancer by treating a patch with hollow-structured microneedles acting as microchannels to promote the release of tumour associated antigens and CP reactive species (Chen et al., 2020). The authors demonstrated that 4 min of CP treatment led to a synergistic effect of CP treatment and ICB therapy integrated with the hollow microneedles relying on the transdermal delivery of CP's reactive species and immune checkpoint inhibitors as anti-programmed death-ligand 1 antibody (aPDL1) into the target tumour cells. Furthermore, it is known that CP can allow gene transfer since 2005, when Ogawa et al. used an atmospheric DBD plasma device and successfully transferred plasmid DNA encoding green fluorescence protein (GFP) into neuronal and HeLa cells. The study of Chen et al. (2020) provided a suitable minimally invasive technique for cancer treatment. In essence, the above results demonstrate the enhanced effect of CP and regular chemotherapeutic agents to minimise drug resistance in cancer cell lines and increase drug

delivery on site. However, further *in vivo* experiments should be carried out to tweeze out the beneficial effects of CP treatment on different tumour cell types and pave the way for medical applications.

11.3.2. Anticancer effect of CP in combination with Nanoparticles (NPs) for delivering drugs

Another novel approach to controlling drug delivery in modern medicine is using NPs as drug delivery carriers to overcome the drawbacks of commercial delivery systems. NPs have numerous advantages, such as improving hydrophobic drug delivery, reducing drug degradation in the gastrointestinal tract, sustaining and triggering the release, and many more. Therefore, the application of CP for delivering anticancer drugs using nano-vehicles has revolutionised cancer treatment and is of high interest (Xu et al., 2012). In addition to traditional chemotherapy and radiotherapy, the synergistic action of NPs loaded with chemotherapeutic agents together with CP technology has shown their potential in cancer therapy (Cheng et al., 2014; Zhu et al., 2016). It has been exhibited that CP treatment coupled with polymeric NPs led to an *in vitro* synergistic inhibition of breast cancer cell growth and downregulation of metastasis-related gene expression (VEGF, MMP9, MMP2, MTDH), which are involved in minimising drug resistance and can promote drug uptake (Zhu et al., 2016). Gold nanoparticles (Au-NPs) can be used as drug delivery carriers due to their low toxicity to normal cells and selective toxicity to specific cancer cell lines (Connor et al., 2005; Patra et al., 2007). Indeed, He et al. (2018) observed that when Au-NPs were used alone, in agreement with other reports, low cytotoxicity was revealed against cancer cells (Connor et al., 2005). Although, when Au-NPs were used in

combination with a DBD plasma treatment for 30s at high voltage (75 kV), increased synergistic cytotoxicity and enhanced uptake of Au-NPs on U373MG glioblastoma cells were revealed. The authors indicated that the long-lived reactive species did not play a major role in the enhanced uptake of AuNP, suggesting that physical effects play a minor role, while chemical effects induced by direct and indirect exposure to CAP appeared as the primary mediator due to increased endocytosis observed. Kong et al. (2011), in their review of the interaction of CP and drug-loaded NPs with cells, documented that NPs may favourably deposit near cancerous cells instead of healthy cells due to their different mechanical properties (Iyer et al., 2009). More recently, the same effect has been observed by Manaloto et al. (2020) by visualising the glioma cell morphology using spectral imaging. Images of *in vitro* brain cancer cells U373MG after the combined treatment of CP and Ag-NPs demonstrated morphological changes (losing the astrocyte shape) with a higher distribution of Ag-NPs in cancerous cells. Even though the exact mechanism of the combined effect of CP and NPs is as of yet poorly understood, the existing findings in the field show an enhanced selective permeability through the induction of membrane disruption of CP species leading to facilitated intracellular diffusion of NPs towards diseased sites within a tissue (Kong et al., 2011).

To date exciting progress has been made in plasma oncology, but many challenges remain for the successful development of cancer therapy for different types of cancer. A synopsis of the main findings of the existing CP mediated cancer drug delivery studies can be found in Table 11.1.

Table 11.1: List of research papers presenting the *in vitro* effects of CP treatment for promoting drug delivery in various cancer cell lines.

Plasma source and gas	Plasma treatment	Cell types (lines)	Results	Reference
Plasma jet (He)	Direct	Breast cancer cells (MDA-MB-231)	Synergistic anticancer effect of 60s CP and drug-loaded NPs against breast cancer cell growth due to increased cellular uptake of drug-loaded NPs, Down-regulation of metastasis-related gene expression (VEGF, MMP9, MMP2, MTDH) led to decreased drug resistance	Zhu et al., 2016
Plasma jet gun based on DBD (He)	Direct (hybrid)	Human cervical cancer cells (HeLa)	Permeabilisation of propidium iodide in the cells was up to seven times higher after CP treatment with 1.000, 10.000, and 100.000 pulses at 10 Hz for 100 s Drug delivery observed through the formation of 6.5 nm diameter pores Plasma induced permeabilisation was dependent on endocytosis	Vijayarangan et al., 2018

Plasma jet gun based on DBD (He)	Direct (hybrid)	Human cervical cancer (HeLa) and murine breast carcinoma cells (4T1)	High uptake levels in both HeLa and 4T1 cells (100s of CP treatment), High MW molecules of FITC-Dextran were successfully delivered into 4T1 cells after 100s of CP treatment	Vijayarangan et al., 2020
	Indirect		Increased Doxorubicin uptake and more efficient delivery when the drug was added in PAM after CP treatment	
Plasma jet (He)	Direct	Human cervical cancer cells (HeLa)	Dextrans with MW of 3 and 10 kDa were delivered in HeLa cells only in the plasma-treated area	Leduc et al., 2009
High-voltage DBD atmospheric plasma system (ambient air)	Direct	Human glioblastoma multiforme cells (U373MG)	Increased cellular uptake of Ag-NPs after the synergistic treatment with a low dose of 0.07 μg/ml Ag-NP in combination with 25s CAP at 75 kV	Manaloto et al., 2020

High-voltage DBD atmospheric plasma system (ambient air)	Direct and Indirect	Human brain glioblastoma cancer cell (U373MG-CD14)	Increased uptake and accumulation into U373MG cells after 30s of CP treatment (direct and indirect), Chemical effects and endocytosis were the major uptake mechanisms	He et al., 2018

11.4 Development of controlled releasing surfaces by cold plasma modification for drug delivery

CP has the capacity to change the surface characteristics of different materials and create surfaces able to absorb therapeutic agents and release them in a controlled way to patients. The use of plasma treatment to control and improve the rate of drug delivery of bioactive agents has created new opportunities for the development of drug delivery systems.

In terms of drug delivery, surface modification of materials for biomedical use by cold plasma has become a field of high scientific interest due to the numerous advantages and the selectivity of this technique, exhibiting great potential for various applications. CP can be efficiently applied to enhance the surface properties of these biomaterials without the drawbacks of the traditional surface modification techniques, e.g. machining, grinding, and chemical grafting, and can modify the surface characteristics only in a few nanometres depth without affecting the bulk attributes of the materials (Reyna-Martínez et al., 2018). The desired alterations obtained by CP surface modification on various biomaterials range from improving surface adhesion and wettability, achieving sterilisation, as well as biocompatibility and bioactivity by changing the surface's chemical composition to allow the immobilisation or controlled release of drugs and bioactive molecules (Yoshida et al.,

2013). In the present section, CP treatments of different biomaterials will be discussed to define their potential use for the successful administration of drugs or bioactive molecules. Polymers are widely used in various biomedical applications such as medical devices, surgical implants, and prosthetic biomaterials and are among the new drug carriers for effective drug delivery. Interestingly, Labay et al. (2015) used a corona discharge CP device at ambient air to load polypropylene (PP) hernia meshes with ampicillin as a new method to treat possible post-surgery infections. Open hernia repair is a very common surgical operation where the hernia is pushed back into the abdomen, and the abdominal wall becomes fortified using stitches or a synthetic mesh. A surgical mesh is a medical implant that supports damaged tissue around hernias as it heals, and significant physio-chemical differences among available meshes can be found. A biomedical implant refers to an artificial functional organ that can fully restore the injured natural organ or tissue of the body without causing any adverse effects (Stloukal et al., 2017). One of the main advantages of CP is that it can be used to manipulate the surface properties of PP meshes-implants and improve their wettability, limiting the efficient drug loading. In this approach, Labay et al. (2015) demonstrated that after 3.5 s of CP functionalisation, the wettability of the PP meshes significantly increased, leading to a 3-fold higher loading (59.5%) capacity and 84.6% total release of ampicillin, which could be useful apropos of local treatment. After plasma functionalisation, a progressive increase in surface roughness was revealed that did not affect the fibroblast (CRL-1658) adhesion following the findings of Pandiyaraj et al. (2009) on PP films after long CP treatment for 2 – 10 min. This is an important finding as the microstructure of the biomedical material is important in promoting the initial attachment to the surface and the subsequent proliferation of the cells. It is generally

accepted that cell adhesion is greater on rough surfaces, but the adhesion of cells may vary depending on the cell line. More recently, Zahedi et al. (2021) experimented with developing PP meshes loaded with betaine hydrochloride that can be exploited as wound dressings for the controlled drug delivery in diabetic wounds. In particular, the PP meshes were functionalised using direct plasma treatment at ambient air conditions and then CP was applied for 30 s to allow the polymerisation of the polyethylene glycol (PEG) using an Argon-plasma bubble reactor to fix and delay drug release. The amount of loaded betaine on the plasma-treated meshes reached almost 80%, while HPLC analysis revealed an *in vitro* drug release of up to 10%. The *in vivo* results of this study presented the wound healing potential of the CP-treated PP meshes on rat skin, where the modified meshes induced faster tissue regeneration and accelerated wound closure compared to the control group.

Zhu et al. (2015) demonstrated that a DBD CP system using He (working gas) could be applied to modify the surface of electrospun scaffolds to increase the adsorption of vitronectin and poly(lactic-co-glycolic) acid (PLGA) microspheres loaded with bovine serum albumin (BSA) as a bioactive compound. Their results revealed a significantly higher cell proliferation on CP-treated scaffolds with embedded BSA-loaded microspheres after 7 days. Furthermore, CP treatment decreased the hydrophobicity of the scaffolds leading to high adsorption of vitronectin while displaying interconnected porous topography with homogenous distribution of PLGA microspheres that released the encapsulated bioactive factor and promoted chondrogenesis. There is a high demand for novel strategies to improve the articular cartilage's poor tissue regeneration, and this study provided a new strategy through the synergistic effect of CP and bioactive compound

loaded microspheres and electrospinning for tissue regeneration. Numerous studies presented the prospect of using CP treatment to control the release kinetics of the drug for various applications, including CP-treated biodegradable porous silicon microparticles (pSi MPs) loaded with camptothecin, an anticancer agent (McInnes et al., 2016), electrospun poly(e-caprolactone) (PCL) mats loaded with dopamine to form polydopamine coatings (Xie et al., 2012), partially dissolvable CP-treated polymer microneedles (MNs) for the efficient delivery of drugs and vaccines (Lee et al., 2015; Nair et al., 2015), and Calcium phosphate (CaP) ceramics loaded with antibiotics to prevent infections (C. Canal et al., 2016) or coated with a biodegradable copolymer to control the subsequent drug delivery of simvastatin (Cristina Canal et al., 2016).

Plasma surface modification has been extensively used as an effective technique to promote drug delivery and controlled drug release for numerous applications in plasma medicine. However, some issues remain. In order to achieve successful surface modification by CP treatment, it generally requires an in-depth knowledge of physics, chemistry, and engineering of the surfaces and a robust biological background to explain the effects of plasma modification prior to *in vivo* practical application.

Conclusions

This chapter introduces the fundamentals of CP, state of the art and primary challenges in the field of CP mediated drug delivery. Given that at present drug delivery has many limitations, CP could be a suitable alternative method to circumvent at least some of these limitations in a number of diseases (e.g. skin infections, cancer). However, even though various atmospheric CP sources for biomedical applications have been described in the literature, most of them have only been characterised by *in vitro* cell biology, and as of yet the number of *in vivo* studies is limited. In the future, CP-based therapies are anticipated to become common practice in medicine as plasma treatment proves to be a simple, rapid, cost-effective, and substrate-independent and can significantly reduce the level of the required drugs for therapy by improving their overall drug delivery. Although, to ensure this, it is necessary to develop flexible and modular plasma devices that can be employed in plasma medicine to treat variable target areas at clinical settings or even at home. To overcome these challenges and meet the requirements, CP research should continue to evolve and help translate the *in vitro* results into clinical applications by gaining more in-depth insights into the mechanisms involved in plasma-induced effects in tissues.

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