Cold atmospheric plasma as a therapeutic tool in Medicine and Dentistry

C. Y. Koga-Ito[1] , R. S. Pessoa[2] and K. G. Kostov[3]

[1] *Department of Environment Engineering, Institute of Science and Technology, São Paulo State University (UNESP), São José dos Campos, Brazil*
[2] *Department of Physics, Technological Institute of Aeronautics, São José dos Campos, Brazil*
[3]*Department of Physics, Guaratinguetá Faculty of Engineering, São Paulo State University (UNESP), Guaratinguetá, Brazil*

Abstract: Our group has been working on the applications of cold atmospheric plasma (CAP) in Medicine and Dentistry for more than a decade, aiming to explore its wide range of synergistic biological effects. The use of CAP for the treatment of infectious diseases has been explored by using in vitro, ex vivo and in vivo methodologies, testing both direct CAP application and plasma-activated liquids. Effective and safe protocols to the treatment of chronic wounds, dental caries, periodontitis, and oral candidiasis were investigated.

Keywords: Cold plasma, plasma activated liquids, infectious diseases, Medicine, Dentistry.

1. General

Cold plasmas (CAPs) operated at atmospheric pressure are capable of generating different active agents such as reactive oxygen and nitrogen species (RONS), energetic (UV) photos, and charged particles. The contact of CAP with living tissues induces a wide range of simultaneous biological effects (antimicrobial, anti-inflammatory, tissue repairing and antineoplastic), thus making the plasma a promising therapeutic tool in the medical area.

Exploring these potentialities, our group has been working on the application of CAP in Medicine and Dentistry for more than a decade. The use of CAP for the treatment of infectious diseases has been explored by using in vitro, ex vivo and in vivo methodologies, testing both direct CAP application and plasma-activated liquids.

2. Exploring the potentialities of non-thermal atmospheric pressure plasma jets

CAP was proven to be effective against several microbial species. The first study of our group reported the inhibitory effect of Helium CAP generated inside Petri dishes on bacterial suspensions [1]. Significant reduction of viable cells of both *Staphylococcus aureus* and *Escherichia coli* was observed, with evidences of cell wall damages detected by scanning electron micrograph.

After, Helium non-thermal atmospheric pressure plasma jets (He-APPJs) were focused by our group. APPJ has the advantage of generating cold plasma plumes not confined by electrodes, which is interesting for biomedical applications. He-APPJ showed antimicrobial effect on the Gram-positive bacterium *Enterococcus faecalis* (ATCC 29212), the Gram-negative bacterium *Pseudomonas aeruginosa* (ATCC 15442) and the fungus *Candida albicans* (SC 5314). It could be observed that *E. faecalis* was the most susceptible species, whereas *C. albicans* was the more resistant one [2].

Considering the wide antimicrobial spectrum, CAP showed to be particularly interesting for the treatment of infectious diseases. Importantly, our studies evidenced that CAP showed inhibitory effects not only on planktonic cells but also on biofilms, that are highly organized microbial communities. Biofilm formation is one of the most relevant virulence factor of pathogenic microorganisms. Besides, biofilms are proven to be more resistant to external factors, such as the antimicrobials when compared to planktonic cells.

It has been reported that Helium-CAP was able to reduce the overall viability of biofilms related to chronic wounds composed by methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Enterococcus faecalis* after 5 minutes exposure, with low cytotoxicity and genotoxicity to a mouse fibroblastic cell line (3T3) [3]. Ongoing in vivo studies evaluating infected experimental wounds in mice and rabbits show improved tissue repair after He-CAP exposure for 5 minutes.

In Dentistry, the simultaneous antimicrobial, anti-inflammatory, and tissue repairing effects of CAP are particularly useful for the treatment of periodontitis. Periodontitis is a highly prevalent oral disease and has a very complex pathogenesis. Our group reported the inhibitory effects of Helium CAP on *Porphyromonas gingivalis*/*Streptococcus gordonii* and *P. gingivalis* HW24D-1 mature biofilms, with low cytotoxicity to human gingival fibroblasts [4]. Also, low genotoxicity to keratinocytes (OBA-9) was detected [5]. The effective protocol was tested in vivo using a model of experimental periodontitis induced in c57bl/6 mice. It could be observed that CAP exposition for 5 minutes tends to improve periodontal tissue recovery, evaluated by mineral tissue improvement and Type I collagen percentage [4].

Besides, CAP shows the potential to be used for the decontamination of caries lesions in minimally invasive techniques. Argon CAP produced by kINPen09™ showed inhibitory effects on multispecies cariogenic biofilms formed by *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus gordonii* on hydroxyapatite discs [5]. Ongoing study on the effect of Helium CAP on these biofilms also shows promising results.

The antifungal effect against medically relevant species was also detected, and CAP emerges as a potential therapeutic alternative for recalcitrant fungal infections. It is interesting to highlight that fungal infections are one of the most challenging issues in Medicine, due to the increasing antifungal resistance and few therapeutical options.

In this context, CAP was able to reduce *Trichophyton rubrum* growth, germination and adherence to nail. Fungal suspensions exposed to plasma jet for 10 and 15 min were not able to infect nail specimens in a ex vivo infection model [6].

Candidiasis, is a fungal infection that occurs frequently among immunocompromised patients and can cause superficial lesions that might evolve to life-threatening disseminated infections. The inhibitory effect of Helium-CAP was reported against *Candida albicans* (SC 5314 and ATCC 18804) and five clinical isolates from denture stomatitis lesions [7]. Helium-CAP was able to modulate *Candida albicans* virulence traits, reducing the filamentation rate in almost 40 times, the adherence to epithelial cells and biofilm formation [8]. Importantly, the CAP protocol showed low cytotoxicity to Vero cells [9]. In vivo study on the Helium-CAP treatment of experimentally induced oral candidiasis showed simultaneous anti-inflammatory and inhibitory effect on fungal morphogenesis, evidenced by marked reduction in tissue invasion [9].

It is important to highlight that the adaptation of plasma device to intra-oral application was an essential experimental step. A small plasma jet could be launched from a long flexible plastic tube end. This tube could be easily manipulated so that the plasma jet could be precisely directed to the target inside the oral cavity [10].

3. Exploring the potentialities of activated liquids

More recently, the applications of plasma-activated liquids (PAL) have also been explored by our group. The use of exposed liquids has numerous advantages in clinical application. The PAL was prepared in a gliding arc plasma system. The antimicrobial effect of direct application [11] or nebulized plasma-activated water (PAW) was detected for *Staphylococcus aureus* and *Escherichia coli* [12]. Interestingly, the nebulized PAW maintained the physicochemical properties in tube lengths from 0.1 up to 1.0 m [12].

The plasma-activated deionized water (pH 2.5), plasma-activated distilled water (pH 2.5 and 3), and plasma-activated tap water (TAP 3.5) showed the best inhibitory effects on *Escherichia coli*, with low cytotoxicity to oral keratinocytes (NOK) [13].

4. Conclusions

Cold plasmas operated at atmospheric pressure (CAP) showed inhibitory effects on biofilms formed by pathogenic bacterial and fungal species. The in vitro, in situ and in vivo studies point out to the promising application of CAP for several infectious diseases. Moreover, the low cytotoxicity and genotoxicity of CAP against several mammalian cell lines have been proven, providing further evidence for clinical application.

The plasma activated liquids, applied directly or nebulized, also show potent antimicrobial activity and open new perspectives for treatment of infectious diseases.

5. Acknowledgements

The authors acknowledge financial support from The São Paulo State Research Foundation (FAPESP, grants 2016/07196-6 and 2019/05856-7) and Brazilian National Council for Scientific and Technological Development (309762/2021-9).

6. References

[1] K.G. Kostov, V. Rocha, C.Y. Koga-Ito, M.A. Algatti, R.Y. Honda, M.E. Kayama, R.P. Mota, Surface Coatings Technology, **204**, 18 (2010).

[2] T.M.C. Nishime, A. C. Borges, C.Y. Koga-Ito, M. Munemasa, L.R.O. Hein, K.G. Kostov, Surface Coatings Technology, **1**, 19 (2017).

[3] M.A.C. Oliveira, G.M.G. Lima, T.M.C. Nishime, A.V.L. Gontijo, M.B.R.C. Menezes, M.V. Caliari, K.G. Kostov, C.Y. Koga-Ito, Applied Sciences, **11**, 5441 (2021).

[4] G.M.G. Lima, A.C. Borges, T.M.C. Nishime, G.F. Santana-Melo, K.G. Kostov, M.P. Mayer, C.Y. Koga-Ito, Molecules, **26**, 5590 (2021).

[5] G.M.G. Lima, C.F.L. Carta, A.C. Borges, T.M.C. Nishime, C.A.V. Silva, M.V. Caliari, M.P. Mayer, K.G. Kostov, C.Y. Koga-Ito, Applied Sciences, **12**, 7247 (2022).

[6] A.C. Borges, T.M.C. Nishime, S.M. Rovetta, G.M.G Lima, K.G. Kostov, G.P. Thim, B.R. Menezes, J.P.B. Machado, C.Y. Koga-Ito, Mycopathologia, **184**, 585 (2019).

[7] K.G. Kostov, A.C. Borges, C.Y. Koga-Ito, T.M.C. Nishime, V. Prysiazhnyi, R.I. Honda, IEEE Transactions on Plasma Science, **43**, 770 (2015).

[8] A.C. Borges, T.M.C. Nishime, K.G. Kostov, G.M.G. Lima, A.V.L. Gontijo, J.N.M.M. Carvalho, R. I. Honda, C.Y. Koga-Ito. Clinical Plasma Medicine, **7**, 9 (2017).

[9] A.C. Borges, G.M.G. Lima, T.M.C. Nishime, A.V.L. Gontijo, K.G. Kostov, C.Y. Koga-Ito. Plos One, **13**, 0199832 (2018).

[10] K.G. Kostov, T.M.C. Nishime, M. Machida, A.C. Borges, V. Prysiazhnyi, C.Y. Koga-Ito. Plasma Processes and Polymers, **12**, 1383 (2015).

[11] W. Chiappim, A.G. Sampaio, F. Miranda, M. Fraga, G. Petraconi, A. Silva Sobrinho, K.G. Kostov, C.Y. Koga-Ito, R. Pessoa. Water, **13**, 1480 (2021).

[12] W. Chiappim, A.G. Sampaio, F. Miranda, M. Fraga, G. Petraconi, A. Silva Sobrinho, P. Cardoso, K.G. Kostov, C.Y. Koga-Ito, R. Pessoa. Plasma Processes and Polymers, **210001**, 14 (2021).

[13] A.G. Sampaio, W. Chiappim, N.V. Moreira, B. Botan Neto, R. Pessoa, C.Y. Koga-Ito. International Journal of Molecular Sciences, **23**, 13893 (2022).