Towards a plasma treatment of ocular surface infections

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The ocular surface is continuously exposed to microorganisms that can cause or aggravate infections like bacterial conjunctivitis and keratitis. In particular, the latter is considered an ocular emergency that requires immediate treatment to limit corneal morbidity and vision loss. In this contribution we present a study aimed at developing a plasma-based treatment of these infections, which exploits the bactericidal effects of the plasma. A low-power, atmospheric pressure plasma source specifically developed for plasma medicine applications has been used for the study [1]. In this source, a plasma is created ionizing a helium flow, mixed with ambient air, in the space between two grids. The effluent coming out of the most external grid, composed of helium enriched by reactive chemical species, is sent to the surface to be treated. *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. fumigatus* cultures, all possible agents of ocular infections, displayed a treatment duration-dependent inactivation, with decimal reduction times of tens of seconds for *E. coli*, *S. aureus*, and *A. fumigatus*, of 209 s for *P. aeruginosa*, and more than 300 s for *C. albicans*. To determine whether the treatment affected the viability of keratocytes and conjunctival fibroblasts, primary cells isolated from conjunctival or corneal tissues were cultured and treated for the same time intervals used with microorganisms (from 0 to 5 minutes). Cell viability, analyzed one hour after the treatment through the MTT test, was initially reduced for 5-minute treatments, unlike the case of 2-minute ones, but in all cases it significantly increased after 24 hours. The cells retained their typical morphology. A significant rise in intracellular Reactive Oxygen Species (ROS) levels was detected in all bacterial and fungal strains tested, as well as in kertocytes and fibroblasts. The burst of intracellular ROS was dampened by cell pretreatment with NAC, a ROS scavenger currently used in ophthalmic surgery. Treatment of *ex-vivo* human corneas for 2 minutes did not induce any change in the corneal stroma, but caused a partial epithelial cell detachment. Evaluation of DNA fragmentation (TUNEL test) performed in parallel did not reveal significant apoptotic effects in corneal tissues. *Ex vivo* human corneas infected with *E. coli*, *S. aureus* or *P. aeruginosa* were also studied. A 2-minute treatment significantly decreased the survival of microorganisms recovered from infected tissues, thus confirming the disinfectant power at the tissue level. The oxidative burst and functional recovery observed in cultured corneal cells was also confirmed. The formation of thymin dimers at DNA level, which would indicate a detrimental effect of UV radiation emitted by the plasma, was tested and no effect was detected. The results presented in this study indicate that a 2-minute treatment with our plasma source substantially inactivates ocular pathogens without causing significant tissue and DNA damage [2].

References

[1] Martines E., et al., New J. Phys. (2009), 11, 115014.
[2]Brun P., et al., “Disinfection of ocular cells and...”, accepted for publication on PLoS One.