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Nonequilibrium Atmospheric Pressure Dielectric Barrier Discharge in Ophthalmology

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ABSTRACT: In this article we report initial experiments applying nonequilibrium atmospheric pressure, pulsed, floating electrode dielectric barrier discharge in ophthalmology. We show that plasma can be applied to the eye both ex vivo in explanted pig eyes and *in vivo* in live rabbits and pigs. The results show effective sterilization below the threshold of significant damage to the cornea. This research is in the initial stages but is instrumental in the understanding of plasma's ability to become a novel therapeutic tool in ophthalmology.

KEY WORDS: eye sterilization, plasma sterilization, ophthalmology

I. INTRODUCTION

Celebrating over a decade of research, the field of Plasma Medicine continues to grow rapidly.[1–7] There are a increasing number of medical applications, including, for example, the development of antimicrobial coatings using both high- and low-pressure plasma discharges[8–12]; plasma treatment and preparation of medical textiles and fibers[13–15]; plasma-assisted stimulation of plant cell growth, seed germination, and other agriculture-related applications[4,16–22]; direct plasma decontamination of foodstuffs[23–26]; and the many medically relevant therapeutic treatment ideas such as plasma treatment of cancers,[1,27–42] treatment of inflammatory bowel disease,[43] skin treatment (primarily for sterilization),[44–46] and blood coagulation,[47–51] as well as many others. There even have been initial attempts at explaining the complex biochemical and biological mechanisms of nonequilibrium discharge interaction with living systems.[49,52–63] The focus of this work is the interaction of nonequilibrium atmospheric pressure dielectric barrier discharge

(DBD) with the surface and cells of the eye. There are important challenges in ophthalmology that plasmas may be able to address. The key challenges we are investigating are capillary coagulation during eye surgery,[64] sterilization of corneal tissue, which is an important challenge in many eye surgeries and drug injections, and treating uveal melanomas using plasmas.[65–69]

The conjunctiva, the thin, moist, transparent membrane covering the surface of the eye, serves as the primary protection of the ocular surface; it contains many capillary blood vessels that, under normal conditions, are barely visible. The conjunctival blood vessels are somewhat fragile and can easily break, especially during surgical intervention with a scalpel or a needle used for drug injection.[70–73] Subconjunctival hemorrhage can easily be prevented with plasma-assisted capillary coagulation.[43,47,48,50,51,74] Of course, the conjunctiva serves as a barrier against pathogenic organisms entering the eye.[75–77] Once the barrier is broken, plasma treatment may be used to inactivate pathogenic organisms on the surface of the eye to prevent infection. In this article we present effective sterilization of the eye both ex vivo with explanted pig eyes and *in vivo* in rabbits and pigs. We observe some level of damage at higher plasma doses, but there is clearly a dose where no visible damage to the eye is observed while effective and fast sterilization is achieved. Finally, we discuss some of the initials steps taken toward treatment of deep-tissue ailments of the eye, with an example of uveal melanoma treatment.

II. MATERIALS AND METHODS

A. Floating Electrode DBD

The floating electrode DBD (FE-DBD) plasma was constructed as we have described

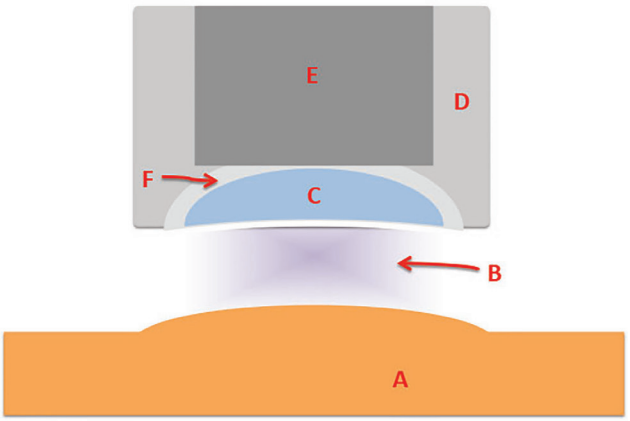

FIG. 1: Schematic of the plasma treatment probe on the surface of the eye: A) surface of the eye; B) plasma between the electrode and the eye; C) hard contact lens to mimic curvature of the eye; D) plastic electrode housing; E) high voltage metal electrode; and F) silver paste to provide good contact between the curved contact lens C) and the metal electrode E)

previously.[51,78,79] Simply, plasma is generated by the application of short pulses of ~2-μsec duration and ~30-kV amplitude to a quartz-covered metal electrode (Fig. 1). This way, plasma is generated between the surface being treated and the electrode. It is important to note that the living tissue, in this case tissue of the eye in a live animal, is used as a second active electrode and participates in the plasma generation. All the active species generated in plasma, such as hydrogen peroxide (H_2O_2), OH, and O_3, charges, and light comes in direct contact with the tissue being treated. Many groups previously showed that this is an effective antimicrobial treatment.[3,27,80–95]

To visualize the FE-DBD plasma treatment, we used an electrode we designed for ocular treatment and, as a second electrode, borosilicate glass coated with indium tin oxide (ITO) (Fisher Scientific). Figure 2 shows the setup with ITO-coated glass on the left and the eye treatment electrode on the right. In this case the voltage is turned off and

FIG. 2: Photograph of the plasma probe ~2mm away from the treatment surface (ITO-coated glass) with plasma turned off

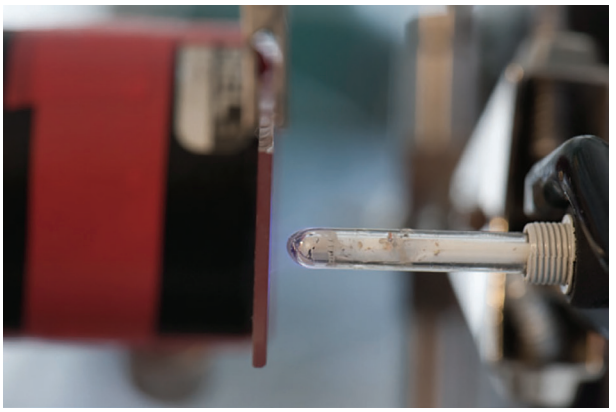

FIG. 3: Photograph of the plasma probe ~2mm away from the treatment surface (ITO-coated glass) with plasma turned on: very faint filaments are visible as compared to Figure 2

no plasma is visible. When the discharge is turned on while keeping the room lights on, the discharge is barely visible, as can be seen in Fig. 3.

The reader is urged to note the apparent uniformity of the discharge; of course, this pulsed plasma is nonuniform on the microscopic time and surface scales but to the eye appears quite uniform. In fact, in the dark the discharge can been seen through ITO-coated glass (see Fig. 4) and a fairly uniform treatment over the 1-second exposure time (the shutter is kept open for 1 second to accumulate sufficient light) can be observed. Since most treatments are on multisecond time scales, we can assume that most of the surface receives a uniform dose of plasma; however, this still remains an open question that needs to be answered in further studies.

B. Plasma Treatment of Agarose Gel

Agarose gels were prepared using agar powder (Fisher Scientific) and distilled water according to standard procedures. The agar powder and water solution was boiled on a hot plate until clear. While the solution was boiling, the stock solutions from the H_2O_2/horseradish peroxidase (HRP) assay kit were prepared according to the protocol provided by Life Technologies.

1. Amplex Red Reagent Stock Solution (10 mM)

Allow one vial of Amplex Red reagent (component A, blue cap) and dimethyl sulfoxide (component B, green cap) to warm to room temperature. Just before use, dissolve the contents of the vial of Amplex Red reagent in 60 μL of dimethyl sulfoxide. Each vial of Amplex Red reagent is sufficient for approximately 100 assays, with a final reaction volume of 100 μL/assay. Use the Amplex Red reagent stock solution on the same day it

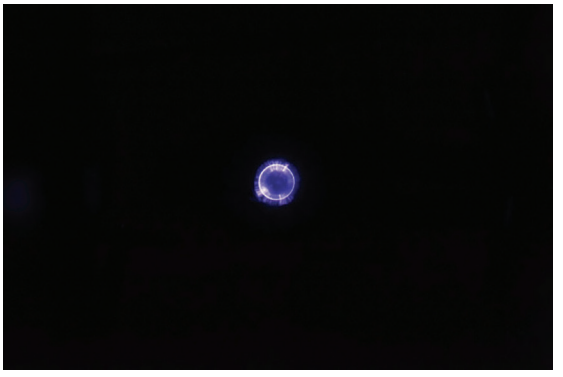

FIG. 4: Photograph of the plasma probe ~2mm away from the treatment surface (ITO-coated glass) with plasma turned on. The photo is made through the ITO glass. At the exposure time of 1 second one can clearly see uniform coverage of the treated surface

is prepared.

2. 1X Reaction Buffer

Add 4 mL of 5X Reaction Buffer (component C, white cap) to 16 mL of deionized water. This 20-mL volume of 1X reaction buffer working solution is sufficient for approximately 100 assays (100 µL each) with 10 mL excess for making stock solutions.

3. HRP Stock Solution (10 U/mL)

Dissolve the contents of the vial of HRP (component D, yellow cap) in 1.0 mL of 1X reaction buffer. After the assay, divide any unused HRP stock solution into single-use aliquots and store frozen at –20°C.

4. H_2O_2 Working Solution (20 mM)

Dilute the ~3% H_2O_2 (component E, red cap) into the appropriate volume of 1X reaction buffer. The actual concentration of H_2O_2 is indicated on the label. For instance, you can prepare a 20-mM H_2O_2 working solution from a 3.0% (0.88 M) H_2O_2 stock solution by diluting 22.7 µL of 3.0% H_2O_2 into 977 µL of 1X reaction buffer. Note that although the ~3% H_2O_2 stock solution has been stabilized to slow its degradation, the 20-mM H_2O_2 working solution prepared in this step is less stable and should be used within a few hours of preparation.

After the agar solution became clear, the contents were cooled to 60°C. The stock solutions from the Amplex Red H_2O_2/HRP kit were combined and added to the cooling agar solution and mixed thoroughly. Five milliliters of the solution was added to culture plates and cooled until solidified. Once the plates were cooled to room temperature, they were treated with FE-DBD plasma for 5, 10, and 15 seconds. Areas of plasma discharge were seen as pink spots. The diameters of these spots were marked and measured.

C. Plasma Treatment of the Eye

Figure 5 shows a schematic of the animal treatment protocol for live eye sterilization. For our treatments we administer a 100-µL drop of bacteria in phosphate-buffered saline onto the cornea (Figs. 5 and 6), attempting to keep the drop in the center while holding the animal. The plasma treatment is applied exactly as described above for a predetermined amount of time. After the treatment, we collect bacteria on a sterile cotton swab and then roll this swab over the surface of blood agar (Cardinal Health). Bacteria then are incubated at 37°C for 24 hours, after which the colony-forming units

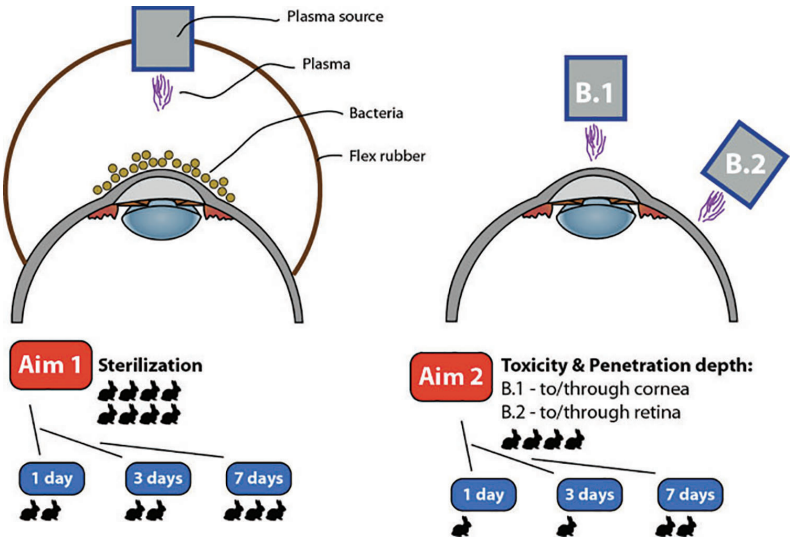

FIG. 5: Schematic of the animal treatment protocol setup, clearly identifying the treatment targets and survival times of the animals

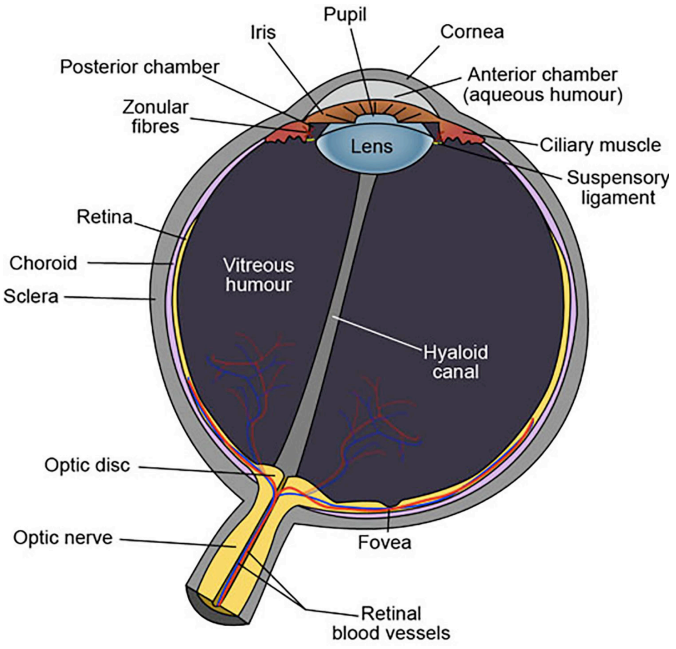

FIG. 6: Schematic of the eye , identifying all the areas of interest for the plasma treatment. Specifically in this work we treat cornea.(Adapted from https://pt.wikipedia.org/wiki/Ficheiro:Schematic_diagram_of_the_human_eye_pt.svg.)

are counted.

III. RESULTS AND DISCUSSION

A. Analysis of Plasma Treatment of Agarose Gel

We prepared agarose gel as described above and treated it with plasma the same way we later treated the eyes and as described above. Figure 7 shows a photograph of the agarose gel following 3 seconds of plasma treatment. The pinkish color of the dye clearly shows the area that received the treatment.

Figure 8 shows rather expected results: We observed an increase in the diameter of active species in the gel with increasing plasma treatment dose. Similar results were reported previously (by Dirks et al.,[25] for example) and we now frequently use this technique to validate the presence of reactive species in the treatment area. The size of the electrode (Fig. 2) is just less than 3 mm (copper part); however, the plasma extends beyond that, as can be seen from Figs. 3 and 4. Thus the spread of reactive species in agarose to >5 mm after just a few seconds of treatment is not unreasonable. While this is not the subject of this article, we plan to investigate in the future mechanisms of reactive species penetration through gels and tissues, focusing especially on the type of species.

B. Plasma Sterilization of Explanted Pig Cornea

Figure 9 shows a schematic of the treatment setup we used for all ex vivo and *in vivo*

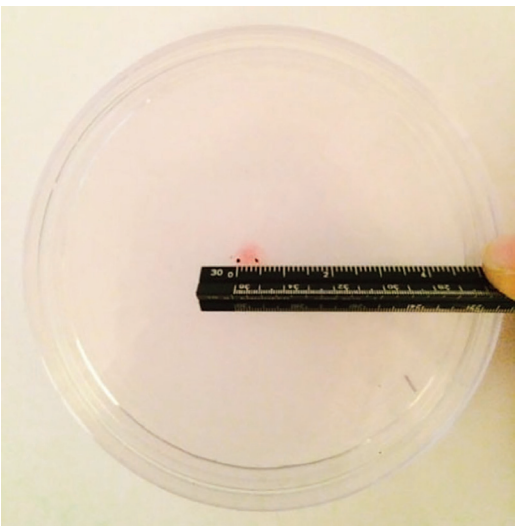

FIG. 7: Plasma treated agar plate with HRP showing diameter of plasma specie penetration.

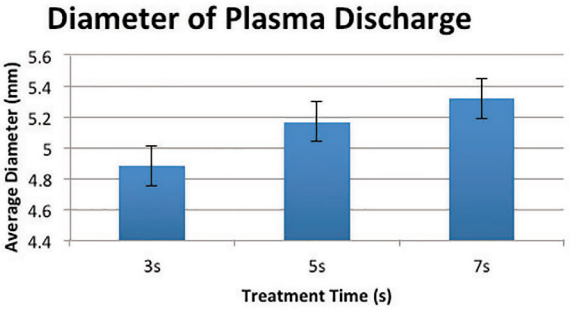

FIG. 8: Results of plasma discharge diameter test for treatment times of 3s, 5s, and 7s

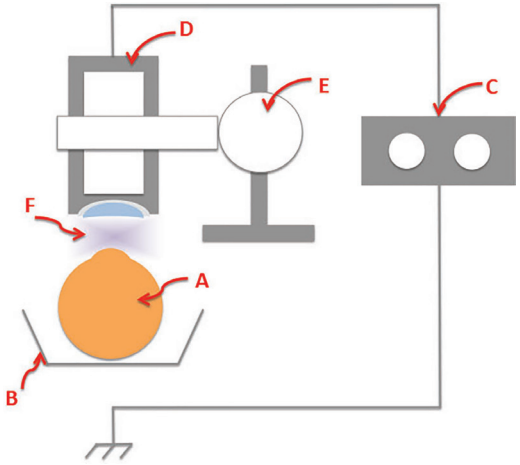

FIG. 9: Schematic of the plasma treatment of eye (same basic setup was used for both explanted eyes and live animals): A) explanted eye in a grounded petri dish (B) or the animal eye; C) high voltage pulsed power supply; D) eye treatment electrode as shown in Figure 1; E) Z-micropositioner (used with explanted eyes); and F) plasma generated between the powered electrode and the surface of the cornea

experiments reported here. Basically, the typical quartz-coveted copper electrode is connected to a microsecond-pulsed, high-voltage power supply through an ~1-m-long cable. The electrode itself is held by hand approximately 2 mm away from the surface. This is clearly not a precise technique and is heavily reliant on the operator, like many similar surgical techniques. We do plan to address this with a redesign of the probe, similar to the schematic presented in Fig. 5. The surgeon applying the treatment did not report having trouble maintaining a reasonably similar treatment distance of ~2 mm for all treatments.

Figure 10 visually explains the treatment protocol we used for the explanted pig

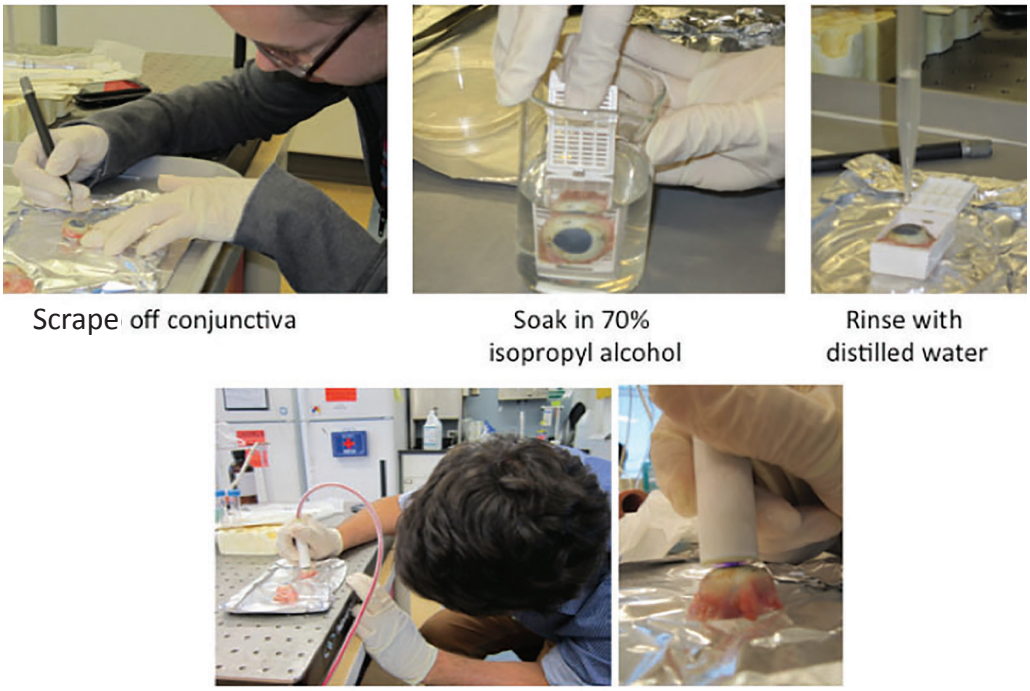

FIG. 10: Photographs of the treatment of the explanted pig eyes

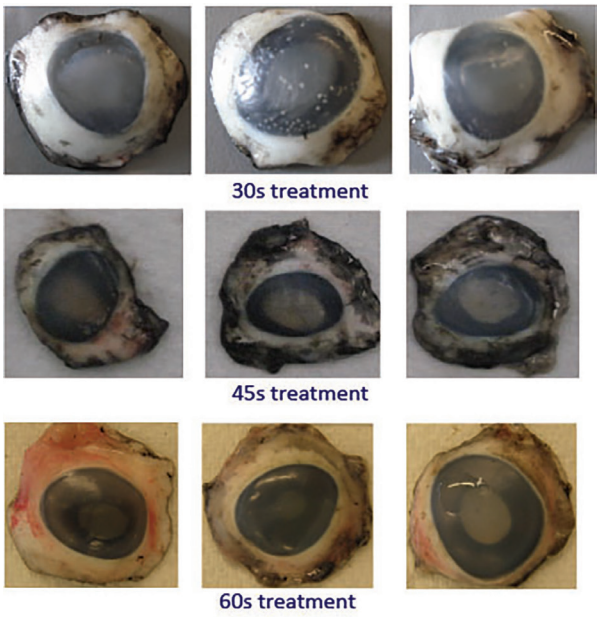

FIG. 11: Photograph of the explanted pig eyes after 30, 45, and 60 seconds of treatment showing no visible damage and no visible different between the treated samples

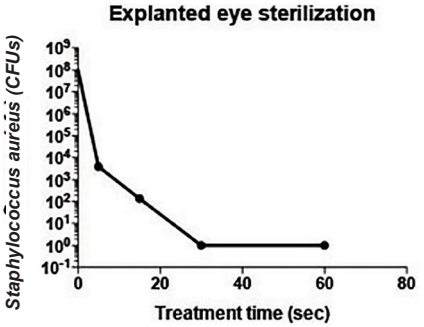

FIG. 12: Results of cornea sterilization of explanted pig eyes

eyes, purchased from a local butcher. First, we clean the eyes as best as we can in an attempt to minimize damage to the cornea. Then we soak the eyes in 70% isopropanol for 2 minutes to remove any existing bacteria. We follow this with rinsing and air-drying for 15 minutes under a fume hood, then we deposit a 100-µL drop of bacteria and, finally, perform plasma treatment followed by immediate swab and culture on blood agar.

Figure 11 shows a set of photographs of the explanted pig eyes after plasma treatment. The photos are taken usually within a minute following treatment, not exceeding 3 minutes for any of them. As can be seen, there is no visible effect on the cornea that can be observed by a camera. There is, however, slight drying that can be observed and that we noticed later in the rabbit and pig eyes. In the future we plan to perform histopathological analysis of the treated samples.

As can be seen in Fig. 12, we are able to achieve highly effective bacterial inactivation on the surface of the explanted eye. While this comes as no surprise—similar inactivation rates have previously been reported[51,96,97]—the reader will notice that these inactivation rates are significantly higher than what we report below for the *in vivo* treatments. We do not understand the mechanisms of such a notable difference in effect and plan to investigate this further in the future. Notably, a similar issue was observed in plasma-assisted sterilization of wounds in live animals; the bacterial reduction rated on *in vivo* wounds and other biological surfaces was much less than that on the model agarose gel surface.[25,45,51,57,96,98]

C. Plasma Sterilization of Live Rabbit Cornea

Similar to the schematic in Fig. 9, Fig. 13 shows a typical procedure in which the animal is held steady by the veterinarian and the plasma is applied by the surgeon.

Figure 14 shows results of plasma treatment of live rabbit cornea and inactivation of *Staphylococcus aureus* on the surface. From the initial concentration of ~10^6 colo-

FIG. 13: Photograph of rabbit undergoing plasma treatment. On this photograph the animal was euthanized prior to the treatment

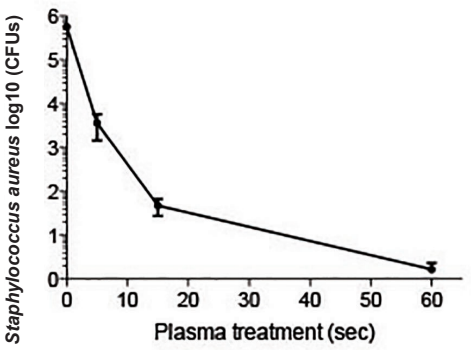

FIG. 14: Results of cornea sterilization of live rabbit

ny-forming units/mL, we quickly reduced the bacterial load by ~2 logs; further reduction requires a slightly higher dose.

D. Plasma Sterilization of Live Pig Cornea

Figure 15 shows the procedure for treatment of live pig cornea and Fig. 16 is a zoomed-

in view of the procedure. While one of the surgeons keeps the animal's eye open with tweezers, the second surgeon applies the plasma. Clearly, the treatment methodology needs to be optimized in the future: a few plasma filaments can be seen (Fig. 16) to jump to the metal tweezers. The surgeons, however, reported being quite comfortable using the probe and keeping the appropriate distance from the eye as they were instructed.

Figure 17 shows the eye once it was swabbed for bacterial counts and removed for analysis. As is quite apparent from Fig. 18, which is a zoomed-in view of Fig. 17, there is significant drying of the cornea following the 30-second plasma treatment.

Figure 19 shows a different animal (pig 3) being treated with plasma by a single surgeon. The operator is able to hold the eye open and perform the treatment comfortably.

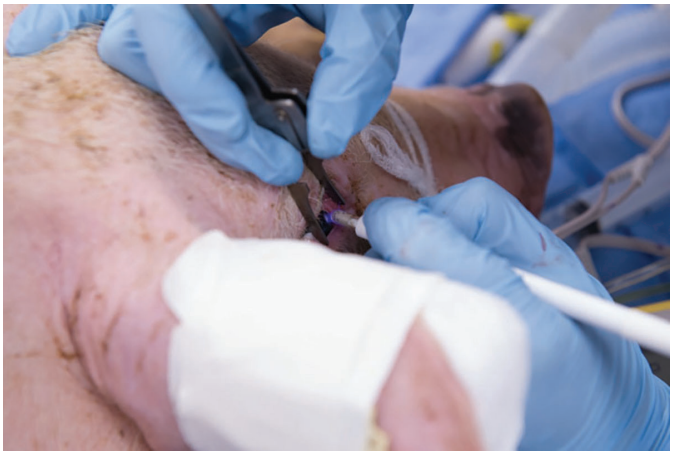

FIG. 15: Photograph of live pig (P1R, or Pig #1, right eye) undergoing treatment

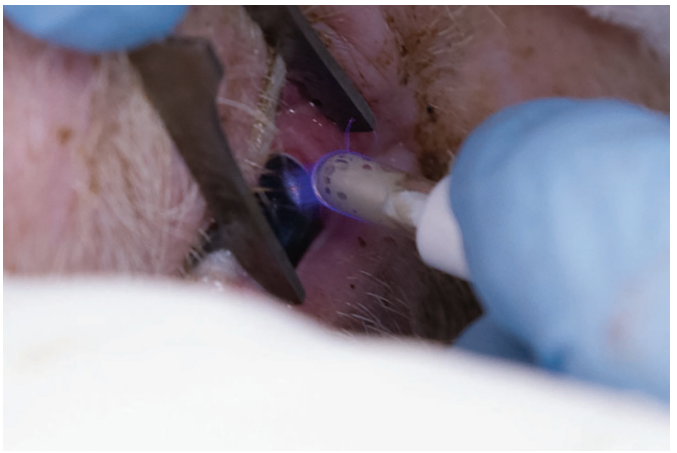

FIG. 16: Zoomed-in view of Figure 15, photograph of live pig (P1R, or Pig #1, right eye) undergoing treatment

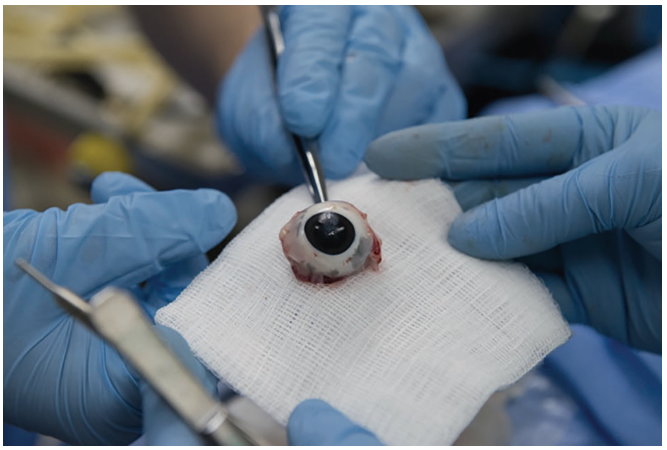

FIG. 17: Photograph of P1R eye removed for analysis. Shows the eye once it was swabbed for bacterial counts and removed for analysis. As is quite apparent from Figure 18, a zoomed-in view of Figure 18, there is significant drying of the cornea following the 30 sec plasma treatment

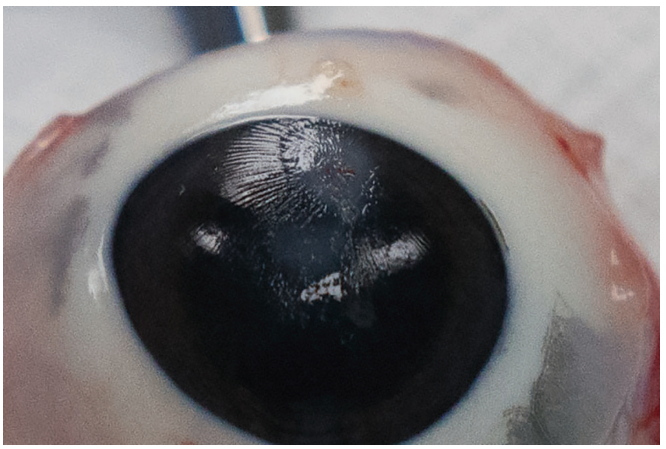

FIG. 18: Zoomed-in view of Figure 17

Similar to Fig. 17, in Figs. 21 and 22 we observe drying of the corneal surface following the 30-second plasma treatment. We did observe significantly less drying with the 15-second treatment (not shown), which leads us to believe that the effect is dose-dependent and can thus be optimized. We did this previously with treatment of mouse skin; we were able to achieve as much as 45 minutes of treatment without tissue damage.[51]

While the 15-second treatment led to less tissue drying, we did not observe significant sterilization (Fig. 23). We were able to achieve reduction of ~2 logs with the 30-second treatment regime. Based on our experience with treatment of live surfaces, we may attribute such low inactivation (compared with the results on agarose gel[51] or on explanted eyes reported above) to the treatment and collection procedure. As we noted,

FIG. 19: Photograph of live pig (P3L, or Pig #3, left eye) undergoing treatment

FIG. 20: Zoomed-in view of Figure 19

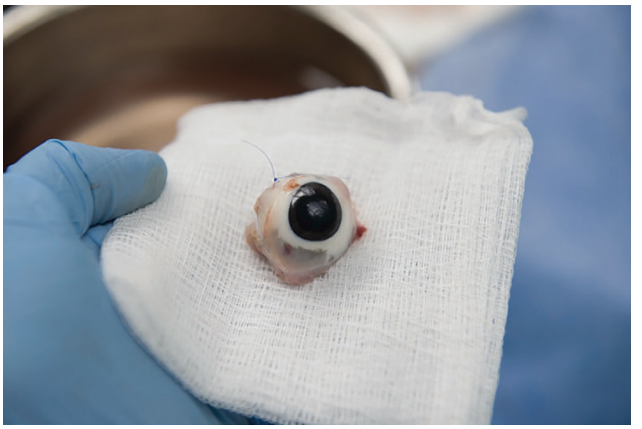

FIG. 21: Photograph of P3L eye removed for analysis

we apply a 100-µL drop to the surface of the cornea, which is already wet because the animal is alive. The drop quickly spreads over the eye. While we treat a ~5-mm circle with plasma (see agarose gel data above), the surface over which bacteria is spread and is collected from is much larger. We plan to modify the treatment, collection, and analysis protocol in the future to address this complication.

IV. CONCLUSION

In this article we show that treatment of a live animal cornea is possible with FE-DBD plasma without excessive damage to the cornea. Moreover, we show that this treatment

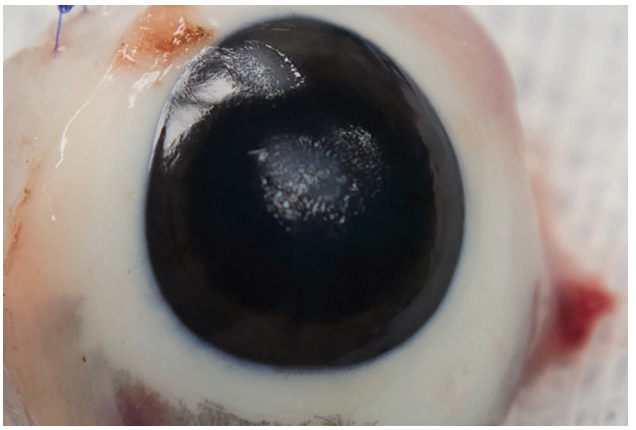

FIG. 22: Zoomed-in view of Figure 21

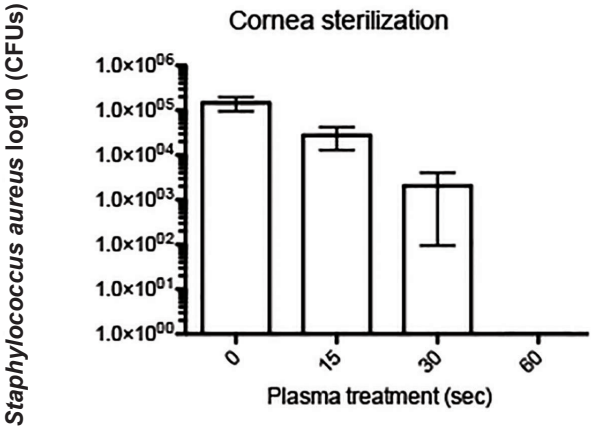

FIG. 23: Results of cornea sterilization of live pig

leads to effective reduction of pathogenic bacteria on the surface of the cornea. Bacteria here are used as a model for the treatment of live animal eyes. The key challenge is treating eye diseases, and this article presents our first step in this direction. Clearly, many unanswered questions must be resolved before plasma treatment of the eye becomes a medical product. As can be clearly observed (in Fig. 18, for example), there is apparent damage to the animal eye from higher doses of plasma. While this damage is not significant enough to break the delicate surface of the cornea, the animal is likely to suffer at least some level of discomfort. There is also an observed drying of the corneal surface (which can be addressed by adding saline, for example).

REFERENCES

1. Weltmann KD, Kindel E , von Woedtke T, Hahnel M , Stieber M , Brandenburg R. Atmospheric-pressure plasma sources: prospective tools for plasma medicine. Pure Appl Chem. 2010;82(6):1223–37.
2. Stoffels E. Cold atmospheric plasma for wound healing: in vitro assessment. First International Conference on Plasma Medicine (ICPM-1); October 15th - 18th, 2007; Corpus Christi, Texas .
3. Stoffels E. Gas plasmas in biology and medicine. J Physics D Appl Phys. 2006;39(16) http://iopscience.iop.org/0022-3727/39/16/E01.
4. Sera B, Gajdova I, Cernak M, Gavril B, Hnatiuc E, Kovacik D, Kriha V, Sláma J, Sery M, Spatenka P. How various plasma sources may affect seed germination and growth. 13th International Conference on Optimization of Electrical and Electronic Equipment (OPTIM); 24–26 May 2012. New York: IEEE; 2012. p. 1365–70.
5. Laroussi M. Low-temperature plasmas for medicine? IEEE Trans Plasma Sci. 2009;37(6):714–25.
6. Kong MG, Kroesen G, Morfill G, Nosenko T, Shimizu T, van Dijk J, Zimmermann JL. Plasma medicine: an introductory review. New J Phys. 2009;11:115012.
7. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, and Fridman A. Applied plasma medicine. Plasma Process Polym. 2008;5(6):503–33.
8. Yim JH, Fleischman MS, Rodriguez-Santiago V, Piehler LT, Williams, AA, Leadore JL, Pappas DD. Development of antimicrobial coatings by atmospheric pressure plasma using a guanidine-based precursor. ACS Appl Mater Interfaces, 2013;5(22):11836–43.
9. Cheruthazhekatt S, Cernak M, Slavicek P, Havel J. Gas plasmas and plasma modified materials in medicine. J Appl Biomed. 2010;8(2):55–66.
10. Pappas DD, Bujanda AA, Orlicki JA, Jensen RE. Chemical and morphological modification of polymers under a helium-oxygen dielectric barrier discharge. Surface Coatings Technol. 2008;203(5–7):830–4.
11. Ben Gadri R, Roth JR, Montie TC, Kelly-Wintenberg K, Tsai PPY, Helfritch DJ, Feldman P, Sherman DM, Karakaya F, Chen ZY. Sterilization and plasma processing of room temperature surfaces with a one atmosphere uniform glow discharge plasma (OAUGDP). Surface Coatings Technol. 2000;131(1–3):528–42.
12. Chan CM, Ko TM, Hiraoka H. Polymer surface modification by plasmas and photons. Surface Sci Rep. 1996;24(1–2):3–54.
13. Poll HU, Schladitz U, Schreiter S. Penetration of plasma effects into textile structures. Surface Coatings Technol. 2001;142–144:489–93.
14. Vohrer U, Muller H, Oehr C. Glow-discharge treatment for the modification of textiles. Surface Coatings Technol. 1998;98(1–3):1128–31.
15. Hocker H. Plasma treatment of textile fibers. Pure Appl Chem. 2002;74(3):423–7.
16. Park DP, Davis K, Gilani S, Alonzo C-A, Dobrynin D, Friedman G, Fridman A, Rabinovich A, Fridman G. Reactive nitrogen species produced in water by non-equilibrium plasma increase plant growth

rate and nutritional yield. Curr Appl Phys. 2013;13(Suppl 1):S19–29.
17. Henselová M, Slováková, M. Martinka, Zahoranová A. Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low-temperature plasma. Biologia. 2012;67(3):490–7.
18. Filatova I, Azharonok V, Shik A, Antoniuk A, Terletskaya N. Fungicidal effects of plasma and radio-wave pre-treatments on seeds of grain crops and legumes. Plasma for Bio-Decontamination Med Food Sec. 2012:469–79. Springer http://link.springer.com/chapter/10.1007/978-94-007-2852-3_36.
19. Bormashenko E, Grynyov R, Bormashenko Y, Drori E. Cold radiofrequency plasma treatment modifies wettability and germination speed of plant seeds. Sci Rep. 2012;2:741.
20. Zhuwen Z. Introduction of a new atmospheric pressure plasma device and application on tomato seeds. Agric Sci. 2011;2(1):23–7.
21. Huang M, Yin M. Effect of radio frequency discharge plasma on wheat's seed germination and seedling growth. J Shanxi Agric Univ (Nat Sci Ed). 2010;(5):393–7.
22. Dubinov AE, Lazarenko ER, Selemir VD. Effect of glow discharge air plasma on grain crops seed. IEEE Trans Plasma Sci. 2000;28(1):180–3.
23. Niemira BA. Cold plasma reduction of Salmonella and Escherichia coli O157:H7 on almonds using ambient pressure gases. J Food Sci. 2012;77(3):M171–5.
24. Niemira BA. Cold Plasma decontamination of foods. Annual Rev Food Sci Technol. 2012;3:125–42.
25. Dirks BP, Dobrynin D, Fridman G, Mukhin Y , Fridman A, Quinlan JJ. Treatment of raw poultry with nonthermal dielectric barrier discharge plasma to reduce Campylobacter jejuni and Salmonella enterica. J Food Prot. 2012;75(1):22–8.
26. Vleugels M, Shama G, Deng XT, Greenacre E, Brocklehurst T, Kong MG. Atmospheric plasma inactivation of biofilm-forming bacteria for food safety control. IEEE Trans Plasma Sci. 2005;33(2):824–8.
27. Park GY, Hong YJ, Lee HW, Sim JY, Lee JK. A global model for the Identification of the dominant reactions for atomic oxygen in He/O-2 atmospheric-pressure plasmas. Plasma Process Polym. 2010;7(3–4):281–7.
28. Kim JY, Kim SO, Wei Y, Li J. A flexible cold microplasma jet using biocompatible dielectric tubes for cancer therapy. Appl Phys Lett. 2010;96:203701.
29. Kim JY, Ballato J, Foy P, Hawkins T, Wei Y, Li J, Kim SO. Single cell level cancer therapy using a hollow optical fiber based microplasma. Small. 2010;6(14):1474–8.
30. Kim CH, Kwon S, Bahn JH, Lee K, Jun SI, Rack PD, Baek SJ. Effects of atmospheric nonthermal plasma on invasion of colorectal cancer cells. Appl Phys Lett. 2010;96(24):243701.
31. Morfill GE, Kong MG, Zimmermann JL. Focus on plasma medicine. New J Phys. 2009;11:115011.
32. Lee HJ, Shon CH, Kim YS, Kim S, Kim GC, Kong MG. Degradation of adhesion molecules of G361 melanoma cells by a non-thermal atmospheric pressure microplasma. New J Phys. 2009;11:115026.
33. Fridman G, Shereshevsky A, Jost M, Brooks A, Fridman A, Gutsol A, Vasilets V, Friedman G. Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines. Plasma Chem Plasma Process. 2007;27(2):163–76.
34. Fridman G, Kalghatgi S, Sensenig R, Shereshevsky A, Stoddard R, Friedman G, Gutsol A, Vasilets V, Fridman A, Brooks AD. Non-equilibrium dielectric barrier discharge plasma promoting apoptotic behavior in melanoma skin cancer cells. In: Plasma assisted decontamination of biological and chemical agents. Çesme, Turkey: NATO Advanced Study Institute (ASI); 2007.
35. Stoffels E, Flikweert AJ, Stoffels WW, Kroesen GMW. Plasma needle: a non-destructive atmospheric plasma source for fine surface treatment of (bio)materials. Plasma Sources Sci Technol. 2002;11(4):383–8.
36. Zimmermann U, Friedrich U, Mussauer H, Gessner P, Hamel K, Sukhorukov V. Electromanipulation of mammalian cells: fundamentals and application. IEEE Trans Plasma Sci. 2000;28(1):72–82.
37. Weaver JC. Electroporation of cells and tissues. IEEE Trans Plasma Sci. 2000;28(1):24–33.
38. Pakhomov AG, Murthy PR. Low-intensity millimeter waves as a novel therapeutic modality. IEEE Trans Plasma Sci. 2000;28(1):34–40.

39. Dev SB, Rabussay DP, Widera G, Hofmann GA. Medical applications of electroporation. IEEE Trans Plasma Sci. 2000;28(1):206–23.
40. Changjun L, Baoyi W, Zishu W, Hong Z. Cell deformation and increase of cytotoxicity of anticancer drugs due to low-intensity transient electromagnetic pulses. IEEE Trans Plasma Sci. 2000;28(1):150–4.
41. Vandamme M, Robert E, Pesnel S, Barbosa E, Dozias S, Sobilo J, Lerondel S, Le Pape A, Pouvesle JM. Antitumor effect of plasma treatment on U87 glioma xenografts: preliminary results. Plasma Process Polym. 2010;7(3–4):264–73.
42. Pesnel S, Vandamme M, Lerondel S, LePape A, Robert E, Dozias S, Barbosa E, Pouvesle JM. Antitumor effect of plasma exposure: preliminary results in a mouse model. 2nd International Conference on Plasma Medicine (ICPM-2); 16–21 March 2009; San Antonio, Texas.
43. Chakravarthy K, Dobrynin D, Fridman G, Friedman G, Murthy S, Fridman AA. Cold Spark discharge plasma treatment of inflammatory bowel disease in an animal model of ulcerative colitis. Plasma Med. 2011;1(1):3–19.
44. D'Souza J, Kumar V, Kamath R. Study of bacteria causing skin infections in school going children in Udupi district; cross-sectional study. Int J Pharmacol Toxicol. 2014;2(2):50–2.
45. Dobrynin D, Wu A, Kalghatgi S, Park S, Shainsky N, Wasko K, Dumani E, Ownbey E, Joshi S, Sensenig R. Live pig skin tissue and wound toxicity of cold plasma treatment. Plasma Med. 2011;1(1):93–108.
46. Lloyd G, Friedman G, Jafri S, Schultz G, Fridman A, Harding K. Gas plasma: medical uses and developments in wound care. Plasma Process Polym. 2010;7(3–4):194–211.
47. Choi J, Mohamed AAH, Kang SK, Woo KC, Kim KT, Lee JK. 900-MHz Nonthermal atmospheric pressure plasma jet for biomedical applications. Plasma Process Polym. 2010;7(3–4):258–63.
48. Kuo S, Tarasenko O, Chang J, Popovic S, Chen C, Fan H, Scott A, Lahiani M, Alusta P, Drake J. Contribution of a portable air plasma torch to rapid blood coagulation as a method of preventing bleeding. New J Phys. 2009;11:115016.
49. Kalghatgi SU, Fridman G, Cooper M, Nagaraj G, Peddinghaus M, Balasubramanian M, Vasilets VN, Gutsol A, Fridman A, Friedman G. Mechanism of blood coagulation by nonthermal atmospheric pressure dielectric barrier discharge plasma. IEEE Trans Plasma Sci. 2007;35(5, Part 2):1559–66.
50. Raiser J, Zenker M. Argon plasma coagulation for open surgical and endoscopic applications: state of the art. J Physics D Appl Phys. 2006;39(16):3520.
51. Fridman G, Peddinghaus M, Ayan H, Fridman A, Balasubramanian M, Gutsol A, Brooks A, Friedman G. Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air. Plasma Chem Plasma Process. 2006;26(4):425–42.
52. Yasuda H, Miura T, Kurita H, Takashima K, Mizuno A. Biological evaluation of DNA damage in bacteriophages inactivated by atmospheric pressure cold plasma. Plasma Process Polym. 2010;7(3–4):301–8.
53. Walsh JL, Iza F, Janson NB, Law VJ, Kong MG. Three distinct modes in a cold atmospheric pressure plasma jet. J Phys DAppl Phys. 2010;43(7):075201.
54. Shashurin A, Shneider MN, Dogariu A, Miles RB, Keidar M. Temporary-resolved measurement of electron density in small atmospheric plasmas. Appl Phys Lett. 2010;96(17):171502–3.
55. Machala Z, Chladekova L, Pelach M. Plasma agents in bio-decontamination by dc discharges in atmospheric air. J Phys DAppl Phys. 2010;43(22):222001.
56. Jiang N, Cao ZX. Experimental studies on an atmospheric pressure He plasma jet. Acta Phys Sinica, 2010;59(5):3324–30.
57. Yost A, Dobrynin D, Friedman G, Fridman A, Fridman G. Cold plasma inactivation of bacteria on dry or wet surfaces: mechanisms of DNA mutation and peroxidation of phospholipids. In: Drexel University Research Day; April 23rd, 2009; Philadelphia, PA.
58. Sato T, Ochiai S, Urayama T. Generation and transport mechanisms of chemical species by a post-discharge flow for inactivation of bacteria. New J Phys. 2009;11:115018.
59. Nosenko T, Shimizu T, Morfill GE. Designing plasmas for chronic wound disinfection. New J Phys. 2009;11:115013.

60. Dobrynin, D, Fridman G, Friedman G, Fridman A. Physical and biological mechanisms of direct plasma interaction with living tissue. New J Phys. 2009;11:115020.
61. Dobrynin D, Fridman G, Lelkes P, Barbee K, Cooper M, Ayan H. Brooks A. Shereshevsky A, Balasubramanian M, Zhu P, Freedman B, Kelly C, Azizkhan-Clifford J, Kalghatgi S, Friedman G, Fridman A. Mechanisms of plasma interaction with living tissue. In: Drexel University Research Day; April 17th, 2007; Philadelphia, PA.
62. Zhang R, Wang L, Wu Y, Guan Z, Jia Z. Bacterial decontamination of water by bipolar pulsed discharge in a gas-liquid-solid three-phase discharge reactor. IEEE Trans Plasma Sci. 2006;34(4):1370–4.
63. Yurgelenas YV, Wagner HE. A computational model of a barrier discharge in air at atmospheric pressure: the role of residual surface charges in microdischarge formation. J Phys D Appl Phys. 2006;39(18):4031.
64. Vargo JJ. Clinical applications of the argon plasma coagulator. Gastrointest Endosc. 2004;59(1):81–8.
65. Egan KM, Seddon JM, Glynn RJ, Gragoudas ES, Albert DM. Epidemiologic aspects of uveal melanoma. Surv Ophthalmol. 1988;32(4):239–51.
66. Folberg R, Rummelt V, Parys-Van Ginderdeuren R, Hwang T, Woolson RF, Pe'er J, Gruman LM. The prognostic value of tumor blood vessel morphology in primary uveal melanoma. Ophthalmology. 1993;100(9):1389–98.
67. Singh AD, Topham A. Incidence of uveal melanoma in the United States: 1973–1997. Ophthalmology. 2003;110(5):956–61.
68. Kujala E, Mäkitie T, Kivelä T. Very long-term prognosis of patients with malignant uveal melanoma. Investigative Ophthalmol Vis Sci. 2003;44(11):4651–9.
69. McLean LCIW, Foster WD, Zimmerman LE. Uveal melanoma: location, size, cell type, and enucleation as risk factors in metastasis. Hum Pathol. 1982;13(2):123–32.
70. Bahar I, Weinberger D, Dan G, Avisar R. Pterygium surgery: fibrin glue versus Vicryl sutures for conjunctival closure. Cornea. 2006;25(10):1168–72.
71. Starck T, Kenyon KR, Serrano F. Conjunctival autograft for primary and recurrent pterygia: surgical technique and problem management. Cornea. 1991;10(3):196–202.
72. Bahar I, Weinberger D, Gaton DD, Avisar R. Fibrin glue versus vicryl sutures for primary conjunctival closure in pterygium surgery: long-term results. Curr Eye Res. 2007;32(5):399–405.
73. de Bustros S, Glaser BM, Johnson MA. Thrombin infusion for the control of intraocular bleeding during vitreous surgery. Arch Ophthalmol. 1985;103(6):837–9.
74. Balasubramanian M, Peddinghaus M, Fridman G, Sebastian A, Fridman A, Gutsol A, Brooks A, Friedman G. Blood conservation and coagulation by non-thermal dielectric barrier discharge technology. Transfusion. 2007;47(2):6A.
75. Robinson CM, Zhou X, Rajaiya J, Yousuf MA, Singh G, DeSerres JJ, Walsh MP, Wong S, Seto D, Dyer DW. Predicting the next eye pathogen: analysis of a novel adenovirus. MBio. 2013;4(2):e00595–12.
76. Tronsmo A, Raa J. Life cycle of the dry eye rot pathogen Botrytis cinerea Pers. on apple. J Phytopathol. 1977;89(3):203–7.
77. Ritterband DC, Shahn M, Seedor JA. Colletotrichum graminicola: a new corneal pathogen. Cornea. 1997;16(3):362–4.
78. Cooper M, Fridman G, Staack D, Gutsol AF, Vasilets VN, Anandan S, Cho YI, Fridman A, Tsapin A. Decontamination of surfaces from extremophile organisms using nonthermal atmospheric-pressure plasmas. IEEE Trans Plasma Sci. 2009;37(6):866–71.
79. Kalghatgi SU, Fridman G, Fridman A, Friedman G, Morss-Clyne A. Non-thermal dielectric barrier discharge plasma treatment of endothelial cells. Conf Proc IEEE Eng Med Biol Soc. 2008;2008:3578–81.
80. Vaze ND, Gallagher MJ, Sin P, Fridman G, Vasilets VN, Gutsol AF, Anandan S, Friedman G, Fridman A. Inactivation of bacteria in flight by direct exposure to nonthermal plasma. IEEE Trans Plasma Sci. 2010;38(11):3234–40.
81. Scholtz V, Julak J, Kriha V. The Microbicidal Effect of low-temperature plasma generated by corona discharge: comparison of various microorganisms on an agar surface or in aqueous suspension. Plasma

Process Polym. 2010;7(3–4):237–43.
82. Cooper M, Fridman G, Fridman A, Joshi SG. Biological responses of Bacillus stratosphericus to floating electrode-dielectric barrier discharge plasma treatment. J Appl Microbiol. 2010;109(6):2039–48.
83. Vasilets VN, Gutsol A, Shekhter AB, Fridman A. Plasma medicine. High Energy Chem. 2009;43(3):229–33.
84. Pompl R, Jamitzky F, Shimizu T, Steffes B, Bunk W, Schmidt HU, Georgi M, Ramrath K, Stolz W, Stark RW, Urayama T, Fujii S, Morfill GE. The effect of low-temperature plasma on bacteria as observed by repeated AFM imaging. New J Phys. 2009;11:115023.
85. Martines E, Zuin M, Cavazzana R, Gazza E, Serianni G, Spagnolo S, Spolaore M, Leonardi A, Deligianni V, Brun P, Aragona M, Castagliuolo I. A novel plasma source for sterilization of living tissues. New J Phys. 2009;11:115014.
86. Dobrynin D, Fridman G, Rieger J, Wynosky M, Rest R, Mukhin Y, Fridman A. Elimination of B. anthracis spores using dielectric barrier discharge plasma. IEEE International Conference on Plasma Science (ICOPS) 2009 Abstracts.
87. Gutsol A, Vaze N, Arjunan K, Gallagher M, Yang Y, Zhu J, Vasilets V, Fridman A. Plasma for air and water sterilization. NATO Advanced Study Institute (ASI), Plasma Assisted Decontamination of Biological and Chemical Agents; 2007; Çesme, Turkey.
88. Narsetti R, Curry RD, McDonald KF, Clevenger TE, Nichols LM. Microbial inactivation in water using pulsed electric fields and magnetic pulse compressor technology. IEEE Trans Plasma Sci. 2006;34(4):1386–93.
89. Laroussi M, Minayeva O, Dobbs FC, Woods J. Spores survivability after exposure to low-temperature plasmas. IEEE Trans Plasma Sci. 2006;34(4):1253–6.
90. Hayashi N, Guan W, Tsutsui S, Tomari T, Hanada Y. Sterilization of medical equipment using radicals produced by oxygen/water vapor RF plasma. Japan J Appl Phys. 2006;45(10S):8358.
91. Goree J, Liu B, Drake D, Stoffels E. Killing of S. mutans Bacteria using a plasma needle at atmospheric pressure. IEEE Trans Plasma Sci. 2006;34(4):1317–24.
92. Cheng C, Liu P, Xu L, Zhang L, Zhan R, Zhang W. Development of a new atmospheric pressure cold plasma jet generator and application in sterilization. Chin Phys. 2006;15(7):1544.
93. Boudam MK, Moisan M, Saoudi B, Popovici C, Gherardi N, Massines F. Bacterial spore inactivation by atmospheric-pressure plasmas in the presence or absence of UV photons as obtained with the same gas mixture. J Phys D Appl Phys. 2006;39(16):3494.
94. Baxter HC, Campbell GA, Richardson PR, Jones AC, Whittle IR, Casey M, Whittaker AG, Baxter RL. Surgical instrument decontamination: efficacy of introducing an argon:oxygen rf gas-plasma cleaning step as part of the cleaning cycle for stainless steel instruments. IEEE Trans Plasma Sci. 2006;34(4):1337–44.
95. Abramzon N, Joaquin JC, Bray J, Brelles-Mariño G. Biofilm destruction by RF high-pressure cold plasma jet. IEEE Trans Plasma Sci. 2006;34(4):1304–9.
96. Fridman G, Brooks AD, Balasubramanian M, Fridman A, Gutsol A, Vasilets VN, Ayan H, Friedman G. Comparison of direct and indirect effects of non-thermal atmospheric pressure plasma on bacteria. Plasma Process Polym. 2007;4:370–5.
97. Ayan H, Fridman G, Staack D, Gutsol A, Vasilets V, Fridman A, Friedman G. Heating effect of dielectric barrier discharges for direct medical treatment. IEEE Trans Plasma Sci. 2009;37(1):113–20.
98. Dobrynin D, Kalghatgi S, Fridman G, Wu A, Podolsky E, Barbee K, Brooks A, Friedman G, Fridman A. Toxicity of low temperature plasma treatment of living tissue. Presented as a poster at the Drexel IEEE Graduate Forum, Technical Poster Symposium; May 4th, 2009; Philadelphia, PA.