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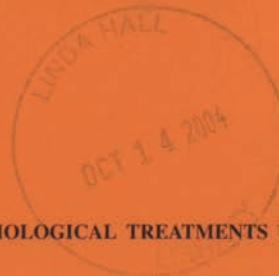
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The Effects of UV Irradiation and Gas Plasma Treatment on Living Mammalian Cells and Bacteria: A Comparative Approach

Edward A. Sosnin, Eva Stoffels, Michael V. Erofeev, Ingrid E. Kieft, and Sergey E. Kunts

Abstract—Living mammalian cells and bacteria were exposed to irradiation from narrow-band UV lamps and treated with a nonthermal gas plasma (plasma needle). The model systems were: *Chinese Hamster Ovary* (CHO-K1) cells (fibroblasts) and *Escherichia Coli* bacteria. UV irradiation can lead to cell death (necrosis) in fibroblasts, but the doses that cause such damage are much higher than those needed to destroy *Escherichia Coli*. The usage of UV radiation in combination with active oxygen radicals lowers the UV dose sufficient to kill the cells. However, in any case the fibroblasts seem to be fairly resistant to UV radiation and/or radicals. Possibly, the lamps may be used for decontamination of infected wounds. The most important active species in an atmospheric plasma are the radicals; the role of UV is less pronounced. Treatment of CHO-K1 cells with the plasma needle can lead to cell necrosis under extreme conditions, but moderate doses cause only a temporary interruption of cell adhesion. Plasma needle may be used for fine tissue treatment (e.g., controlled cell removal without inflammation) and also for bacterial decontamination.

Index Terms—Bacterial decontamination, fibroblast cells, plasma treatment, UV radiation.

I. INTRODUCTION

NONTHERMAL plasmas operate at room or slightly elevated temperatures. Treatment with such plasmas has been demonstrated to be a powerful method of inactivating microorganisms [1]–[5]. Plasma treatment has many advantages in comparison with other bacterial inactivation methods, such as dry heat or hot steam sterilization, irradiation by UV/gamma rays and other techniques [1]. Plasma decontamination is usually fast, efficient, and safe in terms of thermal, chemical, or irradiation damage.

Nonthermal plasmas are easiest to generate under reduced pressures (less than 1 mbar) in specially designed vacuum reactors. In the last decennia, these plasmas found a broad spectrum of applications in material technology. The idea of employing low-pressure plasmas for bacterial inactivation was introduced long ago [2]. Primarily, it was assumed that the key agent in plasma sterilization is the chemistry (highly reactive unstable

species—radicals). However, Soloshenko *et al.* [3] showed that also the 160–220-nm UV radiation plays an important role in low-pressure plasma sterilization.

Nowadays, a general trend has arisen both in material processing and biotechnology: vacuum reactors are often being replaced by atmospheric plasma sources. There are already various principles and designs, which deliver plasmas operating at temperatures not higher than several tens of degrees. Examples include atmospheric jets [5], dielectric barrier discharges [6], and microplasmas [7]. These new developments allow treatment to materials, which can withstand neither high temperature nor low pressure (e.g., biological tissues). Anti-bacterial properties of atmospheric nonthermal plasmas are already well established [4], [5]. The inactivation of microorganisms proceeds faster than at low pressures; it is ascribed to radical interactions with the membrane, while the role of UV is less important [4].

In parallel to plasma sterilization, treatment with UV radiation alone is a subject of extensive investigations [8]–[14]. Effective bacterial inactivation was demonstrated for many kinds of bacteria and viruses; various sources were used and the wavelength dependence was determined [12]–[14]. Furthermore, the synergy of active oxygen radicals and UV was studied [15]. For this purpose, photosensitizers like H_2O_2 were used. This particular molecule produces OH, peroxy, and hydroperoxy radicals upon irradiation. It was shown that the joint action of radicals and photons facilitates bacterial inactivation.

In the mentioned studies, UV and plasma treatment was performed *ex vivo*. One is reluctant to apply these media to living tissues because of the risk of cellular damage by UV [16] and radicals [17]. However, we have noticed that the lethal doses for bacteria are surprisingly low. Therefore, in this paper, we investigate the possibility of *in vivo* treatment, selective bacterial inactivation without harming the cells. For this purpose, one has to establish what doses of UV or plasma exposure can be applied without extensive tissue damage. We study the reaction of living mammalian cells to UV radiation, using narrow-band UV lamps (excilamps) and *Chinese Hamster Ovary* (CHO-K1) cells in culture as a model system. These cells are fibroblasts, a basal cell type, which is involved in many processes, e.g., wound repair. We compare the effects of UV radiation on cells with data on UV bacterial deactivation. Furthermore, the case when irradiated cells are immersed in a hydrogen peroxide solution (a photosensitizer) is considered.

Finally, we present some facts on *in vivo* treatment by means of a novel nonthermal plasma source (the plasma needle [7]). A fundamental study has been undertaken to identify all possible

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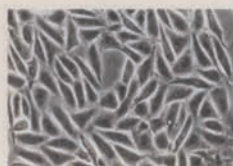


Fig. 1. CHO-K1 cells in culture, used for UV and plasma treatment. Untreated sample. Cells are about 30 μm long.

responses of living objects exposed to the plasma. This may lead to development of new techniques, like disinfection of tissues or removal of cells without inflammatory response, and on a longer time scale to new methods in the health care.

II. EXPERIMENTAL SETUP

A. Cell Culture Preparation

CHO-K1 cells were cultured in flasks containing Ham's F-12 medium with stable L-glutamin (Bio Whittaker, Europe) containing 9.0% of Fetal Bovine Serum [(FBS), Biochrom, AG] and 0.45% of Gentamycin (10 mg/ml, Biochrom AG). The cells were stored in an incubator at 37 °C with 5% CO₂. To prepare samples for UV treatment, the cells were trypsinized (0.05% Trypsin/0.02% EDTA solution in PBS, Biochrom AG) and transferred into Petri dishes. For plasma treatment, the same procedure was followed, but the cells were transferred onto sterilized object glasses (26 × 10 × 1 mm, adapted to fit into the plasma chamber) and placed in multiwell dishes. The cells were incubated for 2 or 3 days, or until nearly confluent (see Fig. 1).

Just before treatment, the medium was removed and the samples were rinsed twice with phosphate buffered saline (PBS). During irradiation or plasma exposure the cells were covered with a thin film of PBS (typically 0.3 mm) in order to prevent them from drying.

B. Bacterial Sample Preparation

A pure culture of *Escherichia Coli*, belonging to the main species of the enterobacteria group, was used. *E. Coli* is one of the most resistant species within enterobacteria group, so it is frequently used in a study of UV disinfection and sanitation, performed at the Scientific Research Institute of Balneology (Tomsk, Russia). The *E. Coli* was supplied by the American National Academy, and had a series number K12 ATCC 25 922.

The culture of *E. Coli* was supported on beef-extract agar (BEA) and kept at the temperature of 4 °C. The optimal concentration of microbial dredge was found based on the method of multiple dilutions.

C. Ultraviolet Radiation Sources

Irradiation was performed using two low-pressure capacitive discharge UV lamps developed at Laboratory of Optical Radiation, High Current Electronics Institute, Siberian Branch of the Russian Academy of Science [18]–[22]. These lamps are confined in quartz tubes (38-mm inner diameter and 300-mm-long silica tube with 80% transparency at $\lambda = 200$ nm). The first of these lamps is a new excimer lamp, filled with Xe and Br₂

mixture (Type XeBr_LERA.5); the active radiating species is XeBr*. Excimer lamps (or so-called excilamps) are a subclass of electric discharge lamps emitting in UV or vacuum UV spectral ranges. Excilamps provide UV irradiance up to 10 mW/cm² and their efficiency is typically 7%–30%. The main feature which distinguishes excilamps from other UV sources is that their spectra consist of narrow bands (the band halfwidth rarely exceeds 5–8 nm). The B→X band emission adds to as much as 80%–90% of the total radiation of excilamp. By varying the composition of the working mixture it is possible to select a required wavelength. Therefore, excilamps are suited to study wavelength-specific effects of UV radiation on various objects, including microorganisms. The XeBr-excimer lamp employed in our experiments produces a spectrum with an emission peak at $\lambda \sim 282$ nm and weak D → X and C → A bands. As confirmed in our previous experiments [14], the XeBr-lamp is most efficient in sterilization. This is because the inactivation of bacteria is mainly related to the DNA/RNA damage, and the maximum absorption of DNA and RNA occurs at the photon wavelength between 240 and 300 nm.

The second light source is a low-pressure lamp (Type LLERA.5) filled with iodine vapor [19]. The Iodine lamp produces a spectrum with a narrow peak of I* emission at $\lambda \sim 206$ nm.

D. Low-Temperature Plasma

A small-size nonthermal plasma (*plasma needle*) has been developed for the purpose of studying plasma interactions with living species [7]. A plasma needle is an atmospheric glow initiated under helium atmosphere by applying radio-frequency (about 10 MHz) electric voltage to a sharp metal pin. The plasma operates under gentle conditions; the voltage needed to sustain the discharge is low (200–400 V peak-to-peak), and the electric power consumption is only 10–300 mW. The size of the glow is 0.1–1 mm. It has been previously established that the gas temperature in the plasma is at most a few degrees above the room temperature. Also, energy fluxes from the plasma have been determined for different operation parameters, using a calibrated probe. The total energy flux (radiation, radical, and ion contribution) ranges from 0 to 2 W/cm². The plasma can be applied to heat-sensitive materials or human skin (see Fig. 3) without any thermal damage or pain sensation. From spectral measurements, it follows that this plasma is a poor UV source. Most of the emission (helium lines, nitrogen, and oxygen bands) lies within the visible range. Some N₂ bands are detected between 300 and 400 nm, no emission can be found below 300 nm (see typical spectra in Fig. 4). Possibly, the plasma could emit radiation in the vacuum UV range (<100 nm, originating from the helium transitions), which we cannot detect using the present equipment. However, these short-wavelength photons cannot propagate outside the plasma; they are efficiently cut off by air and water (absorption coefficient in water is 10⁷ m⁻¹ at 100 nm).

The experimental arrangement for cell treatment is shown in Fig. 5. The chamber was filled with helium at the flow rate of 2 l/min. Flat samples were placed on the bottom of the chamber, where an externally manipulated stage is installed. The distance between the sample surface and the needle tip was adjusted by means of another manipulator.

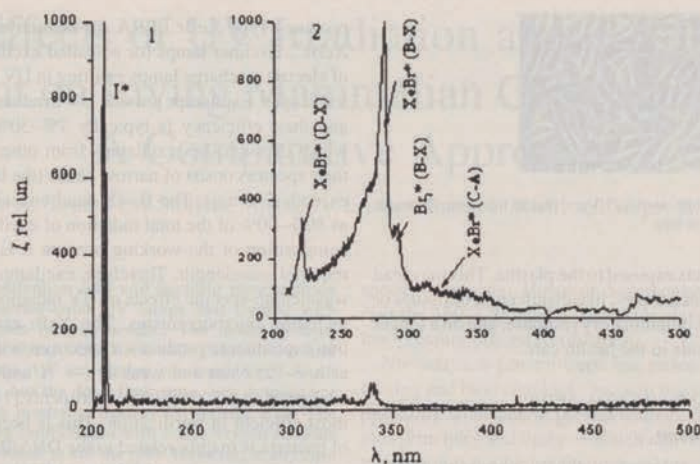


Fig. 2. Emission spectra (range 200–500 nm) from two different UV light sources: (1) iodine lamp and (2) XeBr-excilamp.

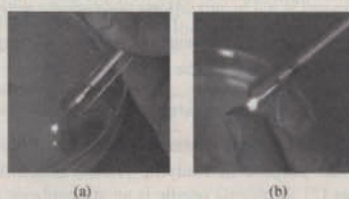


Fig. 3. Plasma needle: plasma is created at the tip of a sharp metal needle. (a) Treatment of cells in culture placed in a Petri dish (for precise positioning another setup was used, see Fig. 5). (b) Glow spreads over the surface of the tissue.

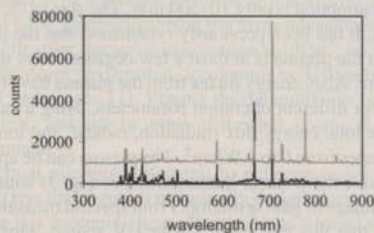


Fig. 4. Typical spectra of the plasma: in pure helium (gray line, given an offset of 10 000 counts) and in a mixture of 90% helium and 10% nitrogen (black line). Latter case, atomic helium lines are suppressed and some N_2 bands emerge below 400 nm.

E. Sample Treatment

For UV treatment, Petri dishes were placed at a 5.5-cm distance from the UV lamp. At this distance, the heating effect of substrate was minimized, while the irradiance could be sufficiently high. The uniformity in the radiation dose over the surface of the sample was about 10%. UV radiation was dosed by varying the treatment time between 2 and 5 min, while the lamp irradiance was kept constant at 4 mW/cm² for the XeBr lamp and 3 mW/cm² for the I-lamp. All experiments were repeated at least three times.

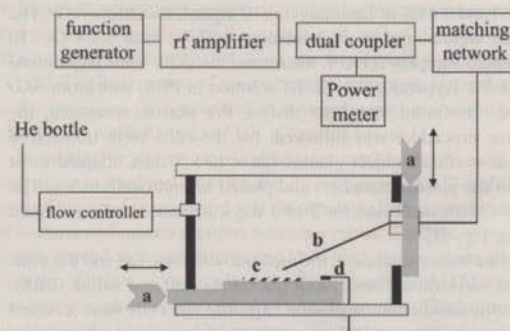


Fig. 5. Experimental arrangement for cell treatment with the plasma needle, rectangular metal/plastic box filled with helium. (a) Manipulators to move the sample and to adjust the needle—sample distance, (b) needle, (c) sample, and (d) thermocouple to control the temperature.

UV treatment of *E. Coli* bacteria was performed in Tomsk; Petri dishes with *E. Coli* cultures were irradiated from several seconds to several minutes at a 5-cm distance and at different levels of UV irradiance (up to 10 mW/cm²). Each exposure was repeated four to five times. For control, several dishes were left not irradiated. The number of survivors was determined by the method of bacterial inoculation in dense media (the Koch method of bacterial inoculation in agarized media in Petri dishes). The inoculations were cultivated at the temperature of 20 °C–23 °C. After 72 h, the number of surviving microorganisms was determined.

During plasma treatment, the object glasses containing the CHO-K1 cells were placed on the moving stage at the bottom of the chamber. A plasma needle was brought to a 1.5-mm distance to the sample; visually, the glow was just touching the surface of the PBS solution. Typical treatment time was 1 min, during which the sample was moved by the manipulator over a typical distance of 1 cm. This produces a typical “track” of

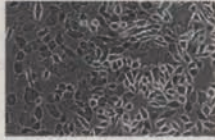


Fig. 6. CHO-K1 sample irradiated by the iodine lamp at 0.6 J/cm^2 . Most of the cells are necrotic.

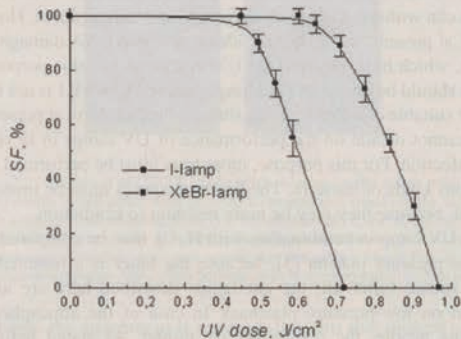


Fig. 7. Survival curve of CHO-K1 after treatment with UVB (■) and UVC (●) radiation. Cells were irradiated at 37°C .

plasma-treated cells, which can be easily recognized under the microscope. Individual cells on this track were irradiated for 1 to 10 s.

F. Observation

After treatment, the samples were observed under a light microscope. Trypan blue (0.5% in physiological saline, Biochrom) was applied in order to distinguish between dead and living cells. Trypan blue penetrates only the cells with membrane leakage (dead cells) and colors the whole cytoplasm blue; the living cells remain colorless. Dead and living cells were counted and the fraction of survivors was determined. The surviving fraction (SF) is defined by $N/N_0 \cdot 100\%$, where N_0 is the initial number of cells and N the number of surviving cells. Mean values were calculated from numbers found in five different places of a Petri dish.

In some cases (mainly after plasma treatment), no trypan blue was applied, but the samples were incubated for several hours in order to study the long-term post-treatment phenomena.

III. RESULTS AND DISCUSSION

As expected, high UV doses result in cell damage and death by necrosis (rupture of cell membranes). It can be seen in Fig. 6, where a sample treated with the iodine lamp at 0.6 J/cm^2 and subsequently stained with trypan blue contains a mixture of dead and surviving cells. The dependence of the fraction of SF on the irradiation dose is displayed in Fig. 7. The most striking feature is the presence of a threshold. The process of inactivation begins only after some critical dose of radiation, which is about 0.5 J/cm^2 for the iodine and 0.7 J/cm^2 for the XeBr lamp. All cells within the irradiated area are dead when the dose exceeds

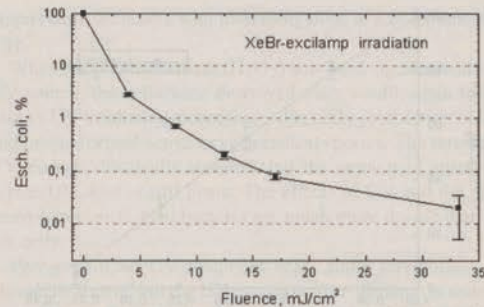


Fig. 8. Survival curve of *Escherichia coli* irradiated with UVB (XeBr-excilamp).

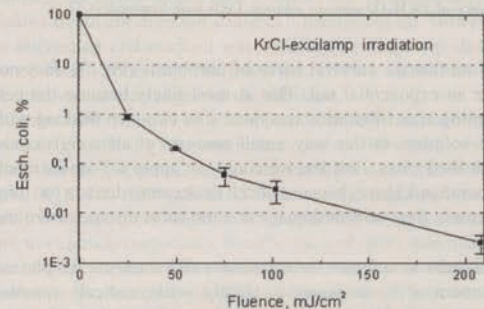


Fig. 9. Survival curve of *Escherichia coli* after irradiation with a KrCl-excilamp (222 nm).

0.7 J/cm^2 for iodine and 1 J/cm^2 for XeBr lamp. The UV dose (threshold and lethal dose) is lower for UVC (I-lamp) than for UVB (XeBr lamp). This can be easily understood because absorption of UV radiation by lipids in cell membranes is highest for $\lambda < 230 \text{ nm}$ [23] and the irradiated power of the iodine lamp lies mainly in this range (Fig. 2).

The presence of the threshold dose for cell deactivation (Fig. 7) makes these results significantly different from the ones obtained for deactivation of *Escherichia Coli* and other bacteria, widely reported in the literature [10]–[15]. For comparison, we include typical bacterial survival curves, resulting from the irradiation of *E. Coli* with XeBr and KrCl ($\lambda = 222 \text{ nm}$) excilamps (both developed in the High Current Electronics Institute, Tomsk). These are given in Figs. 8 and 9, respectively. First of all, one can immediately see that UV doses needed to deactivate *E. Coli* are much lower than the ones that cause necrosis in fibroblasts. Besides, already very low UV doses reduce the bacterial population by two decades. At higher doses, the killing rate is somewhat tempered, and the survival curves display exponential tails. Decontamination to 0.01% of the original population is achieved by UV doses, which are still too low to affect fibroblasts (at least not on the short term, or on a typical time scale of 2 days during which CHO-K1 cells can be maintained in the incubator). This shows that fibroblasts are extremely resistant to UV. Irradiation with UV lamps may become a method of selective bacterial decontamination of wounds without killing the body cells that strive to repair the wound.

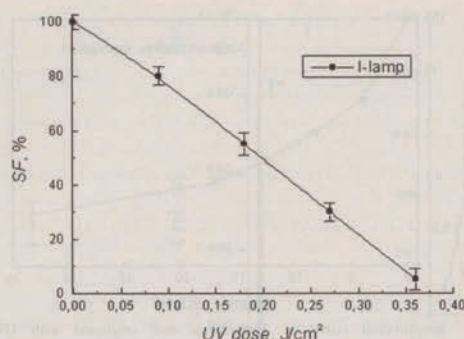


Fig. 10. Survival curve of CHO-K1 after irradiation with the iodine lamp in presence of 3% H_2O_2 aqueous solution. Cells were irradiated at 37 °C.

Note that the survival curve of fibroblasts (Fig. 7) does not have an exponential tail. This is most likely because the cell counting after irradiation was done after two-step flushing with PBS solution. In this way, small amounts of alive cells could be flushed away. Besides, we could not apply UV doses much higher than 1 J/cm², because of cell desiccation due to a too long treatment time or heat damage at a too short distance from the lamp.

In order to simulate the conditions characteristic for plasma treatment, it is necessary to supply active radicals simultaneously with UV irradiation. This can be performed using a photosensitizer, such as H_2O_2 , which produces reactive oxygen species upon UV irradiation. Possibly, in the current experiment, the conditions were similar to those described by Soloshenko *et al.* [3], who performed effective bacterial inactivation in a low-pressure plasma. However, it is difficult to compare the exact UV doses, because a low-pressure plasma emits also photons with a shorter wavelength (<200 nm). These photons are normally lost by absorption in air at ambient pressure, but they can reach the surface when the sterilized object is surrounded by plasma under low-pressure conditions.

We used radiation with $\lambda = 206$ nm (I-lamp), which is not efficiently absorbed in air or water. A 3% aqueous H_2O_2 solution was applied to the CHO-K1 culture just before irradiation; the layer of fluid was about 1-mm thick. The H_2O_2 solution alone (without lamp irradiation) also has some toxic effects on cells, because small amounts of reactive oxygen species are released by decomposition of H_2O_2 by ambient light. However, the cells could survive in this environment for several minutes. The UV lamp in Fig. 10 was applied to cell/ H_2O_2 samples for up to 5 min. The last points, corresponding to long treatment times, may be influenced by natural toxicity of peroxide solution. However, when the UV was applied, we observed a major increase of the killing rate, even at short treatment times. Fig. 10 demonstrates that the usage of H_2O_2 together with UVC lowers the lethal radiation dose by a factor of two (compare with Fig. 7). Moreover, there is no threshold in the inactivation curve; the fraction of dead cells increases approximately linearly with increasing UV dose.

Similar studies of the effect of 1% H_2O_2 in combination with 254-nm radiation on bacteria were described by other authors

[15]. They reported an efficient reduction of bacterial population; the UV doses needed to inactivate 99.99% of microorganisms were in some cases even ten times lower than in absence of a photosensitizer.

From the above results, it can be concluded that the effect of UV (alone or in combination with active radicals) on fibroblasts is by far not so drastic as on bacteria. The rate of killing cells is much lower than that for *E. Coli* (compare linear scales in Figs. 7 and 10 with logarithmic scales in Figs. 8 and 9), and the cells can withstand relatively large UV and radical doses. However, at present, we cannot decide on possible DNA damage in cells, which have survived the UV treatment. For this purpose, cells should be observed for a long time, and CHO-K1 is not the most suitable cell line for such studies. Furthermore, at present, we cannot decide on the performance of UV lamps in *in vivo* disinfection. For this purpose, more tests must be performed on various kinds of bacteria. The bacterial spores must be investigated, because they may be more resistant to irradiation.

A UV lamp in combination with H_2O_2 may be compared to a low-pressure plasma [3], because the latter is a reasonable UV source (after all, the excilamps described here are also based on low-pressure plasmas). In case of the atmospheric plasma needle, the conditions are milder. As stated before, a plasma needle is a poor UV emitter. The plasma-related effects are rather of chemical nature (radicals). It is known that radicals alone are also capable of inactivating bacteria, though not as efficient as in combination with UV. Recently, bacterial inactivation with the plasma needle was performed on *E. Coli* [24]. The reduction of bacterial population by two decades (99%) was achieved within 1–2 min at the power level of about 10 mW. This dose does not cause any reaction in CHO-K1, which again confirms the particularly high sensitivity of *E. Coli* bacteria to UV/plasma treatment. Therefore, we are confident that it is possible to find plasma conditions under which the bacteria are eliminated, but cells remain alive. When higher plasma doses are applied (>10 mW), interesting cell reactions are induced. We shall describe some general features of plasma interactions with CHO-K1 cells.

Plasma treatment of living cells can have many consequences. Naturally, a high dose leads to cell death (necrosis). Typically, necrosis occurs when the plasma power is higher than 0.2 W and the exposure time is longer than 10 s (per treated spot). In terms of energy dose, this corresponds to 20 J/cm², which is very high. However, even upon such harsh treatment the cells are not disintegrated, but they retain their shape and internal structure (see Fig. 11).

When the power and treatment time were substantially reduced (to 50 mW and 1 s per spot), no necrosis was observed. The cells remained negative to trypan blue. In Fig. 12, it can be seen that the cells (partly) detach from the sample surface. Characteristic voids are created in the sheet of cells [Fig. 12(a)]. The partly detached cells assume a rounded shape [Fig. 12(b)]. Careful observation of treated samples showed that detachment is not an instantaneous reaction; it can occur within seconds or even up to 1 min after treatment. Viability of detached (rounded) cells was studied using assays described elsewhere [25]. Here, we can state that the interruption of cell adhesion is a temporary effect. The cells remain unharmed and after several (2 to



Fig. 11. Necrotic cells after treatment with the plasma needle at 0.3 W. These cells are stained with trypan blue (the membranes are damaged).

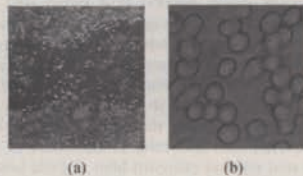


Fig. 12. Treatment of CHO-K1 cells by the plasma needle at low power (50 mW) results in cell detachment. (a) Plasma-treated spot (track) in the sample, with a typical void resulting from cell detachment. (b) Close-up of the edge of the treated area, showing partly detached cells. When cell adhesion is interrupted, they assume a rounded shape (not spread over the surface like in Fig. 1). This situation is temporary; the contact between cells is restored within several hours.

4) hours the attachment is restored. It seems that plasma treatment induces a temporary disturbance in the cell metabolism, which is expressed (among others) by loss of adhesion. The reactive agent, responsible for this behavior has not been identified yet. It was checked that radio-frequency electric fields and static electricity do not induce such effects. Detachment is most likely caused by plasma species, which are in direct contact with the cell (under PBS solution). The role of active radicals cannot be ruled out. However, these are probably not the oxygen species created in photosensitized UV treatment with H_2O_2 , because in the latter case no cell detachment was observed. In the near future, we shall investigate whether positive ions from the plasma can trigger this specific cell reaction.

Cell detachment without severe damage is a refined way of cell manipulation. The loosened cells can be removed from a tissue, but as they are still alive, no inflammatory response should be induced. The area of plasma action is always well defined; the influenced cells are strictly localized and the borders between affected and unaffected zones are very sharp. This demonstrates the locality and precision of plasma treatment.

IV. CONCLUSION

The effect of UVB and UVC radiation on CHO-K1 cells in culture was studied. These cells are fibroblasts, a cell type that is important in tissue repair. The UV radiation was supplied by two novel lamps, which are characterized by a narrow-band spectrum with 80%–90% of the irradiated power lying in the UVB and UVC range, respectively. The UV dose that leads to necrosis in fibroblasts is more than ten times higher than the one needed for deactivation of *E. Coli* bacteria. The deactivation curve for CHO-K1 cells displays a rather high threshold of 0.5–0.7 J/cm²; above this value, the number of survivors decreases approximately linearly with increasing UV dose. The lethal UV doses (no survivors) for the fibroblasts are 1 J/cm² for UVB and 0.7 J/cm² for UVC radiation. In contrast, the threshold for inactivation of *E. Coli* (if existing) is extremely low, and the number

of survivors decreases with increasing dose in a bi-exponential way.

When the photosensitizer (H_2O_2) was used together with the UV source, the cells were destroyed more readily than in the case of UV irradiation alone; this is due to the joint action of UV and *in situ* formed active oxygen radical species. The threshold UV dose is drastically lowered, but the amount of survivors versus UV dose is still linear. The effects of UV and the photosensitizer on *E. Coli* bacteria are much more drastic than on the cells.

Photosensitized UV treatment bears some resemblance to plasma treatment, but the UV emission from the lamp is usually more intense than plasma emission. We treated the fibroblast cells with a nonthermal plasma, which is an efficient source of radicals, but a poor source of UV. Under low-power conditions plasma treatment does not cause cell necrosis at all. However, an interesting cell reaction was observed, a temporary disturbance in cell adhesion. The loosened cells are viable and can be removed or rearranged on the substrate.

In the view of future *in vivo* applications, UV treatment may provide a method to decontaminate tissues. However, we realize that the presented results are valid for one kind of bacteria (*E. Coli*). Although *E. Coli*, as a gram-negative type, is fairly resistant, there might be other species that are more difficult to eliminate; especially, the efficiency of spore deactivation must be investigated. Therefore, additional studies are needed to assess the performance of UV decontamination. Plasma treatment offers possibilities of sophisticated tissue modification, like local removal or manipulation of cells without damage to the tissue. However, before the UV lamp and plasma techniques may be implemented, extensive studies of long-term effects of UV and plasma on living tissues must be performed. These studies should involve assays for possible DNA damage and *in vivo* tests on animals.

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REFERENCES

- [1] S. Lerouge, M. R. Wertheimer, and L. H. Yahia, "Plasma sterilization: A review of parameters, mechanisms, and limitations," *Plasma Polym.*, vol. 6, pp. 175–188, 2001.
- [2] S. J. Fraser, R. B. Gillette, and R. L. Olson, "Sterilizing process and apparatus utilizing gas plasma," U.S. Patent 3 948 601, Apr. 1976.
- [3] I. A. Soloshenko, V. V. Tsiolko, V. A. Khomich, A. I. Shchedrin, A. V. Ryabtsev, V. Yu. Bazhenov, and I. L. Mikhno, "Sterilization of medical products in low-pressure glow discharges," *Plasma Phys. Rep.*, vol. 26, pp. 792–800, 2000.
- [4] M. Laroussi, "Non-thermal decontamination of biological media by atmospheric pressure plasmas: Review, analysis and prospects," *IEEE Trans. Plasma Sci.*, vol. 30, pp. 1409–1415, Aug. 2002.
- [5] H. W. Herrmann, I. Henins, J. Park, and G. S. Selwyn, "Decontamination of chemical and biological warfare (CBW) agents using atmospheric pressure plasma jet," *Phys. Plasmas*, vol. 6, no. 5, pp. 2284–2289, 1999.
- [6] B. Gellert and U. Kogelschatz, "Generation of excimer emission in dielectric barrier discharges," *J. Appl. Phys.*, vol. B52, pp. 14–21, 1991.
- [7] E. Stoffels, A. J. Flikweert, W. W. Stoffels, and G. M. W. Kroesen, "Plasma needle: A nondestructive atmospheric plasma source for fine surface treatment of (bio)materials," *Plasma Sources Sci. Technol.*, vol. 11, pp. 383–388, 2002.

- [8] J. C. H. Chang, S. F. Ossoff, D. C. Lobe, M. H. Dorfman, C. M. Du-mais, R. G. Qualls, and J. D. Johnson, "UV inactivation of pathogenic and indicator micro-organisms," *Appl. Environ. Microbiol.*, vol. 49, pp. 1361-1365, 1985.
- [9] G. D. Harris, V. D. Adams, D. L. Sorensen, and M. S. Curtis, "Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of bacteria," *Wat. Res.*, vol. 21, pp. 687-692, 1987.
- [10] J. K. Race and M. Ewell, "Examination of peak power dependence in the UV inactivation of bacterial spores," *Appl. Environ. Microbiol.*, vol. 67, pp. 5830-5832, 2001.
- [11] J. G. Anderson, N. J. Rowan, S. J. MacGregor, R. A. Fouracre, and O. Farish, "Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light," *IEEE Trans. Plasma Sci.*, vol. 28, pp. 83-87, Feb. 2000.
- [12] S. A. Craik, D. Weldon, G. R. Finch, J. R. Bolton, and M. Belosevic, "Inactivation of *Cryptosporidium Parvum* oocysts using medium- and low-pressure ultraviolet radiation," *Wat. Res.*, vol. 35, pp. 1387-1398, 2001.
- [13] N. Giese and J. Darby, "Sensitivity of microorganisms to different wave-lengths of UV light: Implications on modeling of medium pressure UV systems," *Wat. Res.*, vol. 34, pp. 4007-4013, 2000.
- [14] E. A. Sosnin, L. V. Lavrent'eva, M. R. Yusupov, Y. V. Masterova, and V. F. Tarasenko, "Inactivation of *Escherichia Coli* using capacitive discharge excilamps," in *Proc. 2nd Int. Workshop Biological Effects of Elec-tromagnetic Fields*, Rhodes, Greece, 2002, pp. 953-957.
- [15] K. F. McDonald, R. D. Curry, R. E. Cleverger, B. J. Brazos, K. Un-klesbay, A. Eisenstark, S. Baker, J. Golden, and R. Morgan, "The devel-opment of photosensitized pulsed and continuous ultraviolet decontam-ination techniques for surface and solutions," *IEEE Trans. Plasma Sci.*, vol. 28, pp. 89-96, Feb. 2000.
- [16] J. E. Cleaver, "Repair processes for photochemical damage in mam-malian cells," *Adv. Rad. Biol.*, vol. 4, pp. 1-75, 1974.
- [17] V. Vallyathan, V. Castranova, and X. Shi, Eds., *Oxygen/Nitrogen Rad-icals: Cell Injury and Disease*. New York: Kluwer, 2003.
- [18] E. A. Sosnin, M. V. Erofeev, V. F. Tarasenko, and D. V. Shitz, "Capacitive discharge excilamps," *Instrum. Exper. Tech.*, vol. 45, pp. 118-119, 2002.
- [19] E. A. Sosnin, M. V. Erofeev, A. A. Lisenko, V. F. Tarasenko, and D. V. Shitz, "Capacitive discharge excilamps performance investigations," *Optical J. (St. Petersburg)*, vol. 69, pp. 77-80, 2002.
- [20] M. V. Erofeev, E. A. Sosnin, V. F. Tarasenko, and D. V. Shitz, "Efficient XeBr-excimer lamp excited by capacitive discharge," *Atmos. Oceanic Opt.*, vol. 13, pp. 862-864, 2000.
- [21] M. I. Lomaev, V. S. Skakun, E. A. Sosnin, V. F. Tarasenko, and D. V. Shitz, "Effective sealed-off excilamps excited by capacitive discharge," *Tech. Phys. Lett.*, vol. 25, pp. 27-32, 1999.
- [22] M. I. Lomaev, V. S. Skakun, E. A. Sosnin, and V. F. Tarasenko, "Oper-ating mixtures of HF capacitive discharge excilamps," RU Patent 2 154 323, July 1998.
- [23] Yu. A. Vladimirov and A. Ya. Potapenko, "Physics and chemistry of photobiological processes," *High School Publishing*, 1989.
- [24] R. E. J. Sladek, E. Stoffels, R. Walraven, P. J. A. Tielbeek, and R. Kool-hoven, "Plasma treatment of caries: A novel method in dentistry," *IEEE Trans. Plasma Sci.*, vol. 32, pp. 1540-1543, Aug. 2004.
- [25] E. Stoffels, I. E. Kieft, and R. E. J. Sladek, "Superficial treatment of mam-malian cells using plasma needle," *J. Phys D., Appl. Phys.*, vol. 36, pp. 2908-2913, 2003.



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