Comparison of microbiological effects in long fine-lumen tubes by low and atmospheric pressure plasmas

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Inadequate cleaning and disinfection of reprocessed endoscopes have been reasonable factors of infectious complications during endoscopic procedures. The decontamination of long fine-lumen tubes as occurred in endoscopes by low and atmospheric pressure plasmas is shown. Furthermore, the antimicrobial effect of different plasma setups is comparable due to a new established and standardized contamination and recovery procedure.

Key words: bacterial spores; non-thermal plasma; polytetrafluoroethylene (PTFE); decontamination; multicentre trials

Introduction

Minimal invasive treatment using tubes such as catheters and endoscopes is well-established. Since the introduction of flexible endoscopy into medical practice, many cases of infectious complications involving bacteria, fungi and viruses have been linked to endoscopic procedures. Inadequate cleaning and disinfection during the reprocessing of the instruments have been reasonable factors as well as insufficient final rinsing and incomplete drying of the endoscope or contaminated flushing equipment for the air/ water-channel. Flexible endoscopes are thermo-labile and cannot withstand heat sterilization processes. Common disinfection processes like ethylene oxide or hydrogen peroxide vapour as well as formaldehyde are more or less effective, but require long contact and aeration times. Furthermore, these processes use toxic and explosive substances. Therefore, the development of new methods for the sterilization of thermo sensitive devices especially with long fine lumen is very important. A promising possibility is the decontamination by plasma discharge treatment. Various plasma setups have been developed. However, due to the complexity of plasma techniques and technologies, setups and parameters, it is impossible to compare their antimicrobial efficacy by single experiments. A standardization of microbiological parameters is necessary to attribute the observed effects solely to the plasma efficacy.

This work is based on round robin tests with and without plasma treatment by three institution (the Fraunhofer IGB Stuttgart, Germany; the HygCen GmbH Schwerin, Germany and the INP Greifswald, Germany) and the publications by Maucher et al. (2011) and Schnabel et al. (2012).

Materials and Methods

Endospores of *B. atrophaeus* (ATCC 9372) and of *G. stearothermophilus* (ATCC 7953) as standardized biological indicator for sterilization processes were chosen.

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Polytetrafluoroethylene (PTFE) tubes (Saint-Gobain Performance Plast, Germany) with an inner diameter of 2 mm, outer diameter of 3 mm, and a length of 1–1.2 m (1 m length was used as positive reference, without plasma treatment, or 1.20 m, according to the required length for the used plasma treatment composition) were used as test tubes.

The contamination of the test tubes with endospores and the recovery of reference bacteria and of survival bacteria after plasma plasma treatment was realized by a newly developed cycle method. This so called IGB-cycle method is described in Maucher et al. (2011). The defined endospore suspension with 10^8–10^9 cfu ml^{-1} was pumped for a period of 5 minutes (flow rate of 15 ml min^{-1}) for contamination or 50 ml tryptic soy broth for 20 min (flow rate of 30 ml min^{-1}) for recovery from a storage flask through the test tube and back to the flask. Venting was assured by a filter. After plasma treatment, the collected suspension contains the endospores detached from the test tube and suspended in the broth. The resulting amounts of colony forming units (cfu) were quantified according to, for instance, EN 14561 and TS/ISO 15883-5.

The INP decided to use a coaxial DBD setup. The setup consists of a grounded, metallic inner and a spiral outer electrode for the presented studies described in Schnabel et al. (2012). The inner electrode is moved in translatory motion to avoid local damages of the tube wall. The microbial decontamination was done with moistened air (1.5 slm and a relative humidity of 65%). The high voltage was generated by a car ignition coil from the amplified sine-wave signal of a signal generator (‘‘AFG 3022 B’’ Tektronix, Cologne, GmbH Germany). The process parameters for the decontaminating plasma treatment of three tubes were an Uss of 9.0 kV, a f of 3.0 kHz and a P of 8.0 W. The all in all treatment time for the decontamination of the specimen was 14 min and the inner temperature ranged from 50 to 60 °C.

The decontamination of the specimen at Fraunhofer IGB has been done using low pressure plasma glow discharge. The plasma treatments have been done with a coaxial arrangement of the electrodes with or without an inner electrode as described in Schnabel et al. (2012). The grounded electrode is a wire of stainless steel (diameter 0.8mm) and located along the axis within the lumen of the contaminated PTFE tube. This specimen is located within a conduit of polymethylmethacrylate (PMMA). The inner diameter of this conduit (6mm, wall thickness 1mm) is slightly larger than the outer diameter of the specimen. The hot electrode does consist of an adhesive copper strip (supplier 3M Deutschland GmbH, width 10 mm), which is wounded in a helical manner around the PMMA conduit. The power source is a RF generator (ENI) HPG-2 with 190 kHz. The vacuum is created at one end of the specimen by a rotary vane pump (Alcatel 2012A) resulting in a residual pressure of 2 mbar. The other end is connected to massflow controllers (MKS). The pressure (Vacubrand DVR5) at the entrance of gas flow into the specimen is 15 mbar, at the exit 4 mbar. The applied powers and treatment times have been at 15 W and 20 min.

Results and Discussion

The focus was on the comparability of the antibiological efficacy affected by different plasma techniques conducted by different laboratories. Furthermore, the recovery of plasma treated specimen by three different laboratories was compared. Overall six comparative experiments were carried out. The contamination of all test tubes was performed according to the IGB-cycle-method described above. HygCen performed the contamination for the experimental series 2, 3, and 6. IGB performed the contamination in series 5 and INP in series 4. The recovery experiments were done by all participating laboratories using the earlier explained recovery procedure. The plasma treatment was realized by the working groups of the IGB (low pressure plasma) and of the INP (atmospheric pressure plasma).

The round robin tests 1–3 are preliminary performed to investigate different microbial techniques for contamination and recovery of bacterial load in long fine lumen tubes. During these tests a standardized procedure was established for the comparability of antimicrobial efficacy of different plasma techniques independent on performer, microorganism, and laboratory. The first round robin test showed strong differences in contamination and recovery as measured by the participating laboratories. The second experimental series showed significant improvement in the uniformity of the results. The detected residues of the plasma treated specimen were comparable for each plasma configuration itself and for both techniques. The final standardization was reached by the third test. Moreover, the results for the plasma treated specimen showed a rising decontamination by plasma with reductions of 3.0–4.3 $\log_{10}$ cfu per specimen.

Finally, the establishment of the IGB-cycle-method for untreated and plasma treated specimen was received within three experimental series. The used conditions led to the best comparable results and furthermore to an upward decontamination of *B. atrophaeus* endospores in long fine lumen tubes. To verify the reproducibility of the methodology, the round robin tests 4–6 were performed to show the possibility of standardization of the IGB-cycle-method. Therefore, the microbial contamination was done by the INP (4th series), the IGB (5th series), and with endospores of *G. stearothermophilus* (6th series, contamination by HygCen).

The treatment with plasma resulted in *B. atrophaeus* endospore reductions from 3.2 $\log_{10}$ cfu per specimen as a minimum to 6.2 $\log_{10}$ cfu per specimen as a maximum. A reduction factor of 5.3 $\log_{10}$ cfu per specimen for the inactivation of spores of the biological indicator *G. stearothermophilus* could be achieved. Both plasma techniques lead to very good decontamination results that lay underneath the detection limit of 0 cfu per specimen.

Six series of experiments were required to assure a widespread standardization in the problematic areas related to verification of decontamination performance. The first important step is the procedure used to prepare the contaminating agent and the condition of the specimen. Secondly, the methodology used for contamination and its uniformity must be investigated. Finally the procedure for recovery has to be adjusted to assure reproducible and quantifiable results.

The advantage of our experimental workflow is the comparability of the microbicidal efficacy of different plasma setups by a standardized contamination and recovery used for all compared specimen. The problem of comparability of various plasma techniques was described by Ehlbeck et al. (2011).

Conclusion

Due to the complexity of plasma techniques and technologies, setups, and parameters, it is impossible to compare their antimicrobial efficacy by single experiments. A standardization of microbiological parameters is necessary to attribute the observed effects solely to the plasma efficacy. Therefore the specimen, microorganism, load, contamination, and recovery method as well as initial concentration must be well defined. Moreover, the commonly used and established challenge test is not suitable for non-thermal plasma decontamination investigations. In this work we showed a new and innovative procedure for the investigation of plasma techniques used for decontamination of long fine lumen of thermo-labile tubes and the possibility to get comparable results which rely on the antimicrobial effects of plasma. Furthermore three new plasma setups for the decontamination of PTFE-tubes were developed. Moreover, the effort which is needed to achieve comparable results in the round robin tests demonstrated the difficulties to compare microbiological results of different research groups in literature. Beside the standardized testing procedures also the amount and kind of data describing the test procedures is insufficient for comparability. Therefore, more work is needed to ensure in future the correct evaluation of data of different researchers.

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References

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., von Woedtke, Th., Brandenburg, R., von dem Hagen, T. and Weltmann, K.-D. (2011) Low temperature atmospheric pressure plasma sources for microbial decontamination. *J. Phys. D: Appl. Phys.* 44: 013002 (18pp)

Maucher, T., Schnabel, U., Volkwein, W., Koehnlein, J., Winter, J., Weltmann, K.-D., Trick, I. and Oehr, C. (2011) Assembly of Standardized Test Specimen for Microbial Quantification of Plasma Sterilization Processes of fine PTFE Tubes as used in Thermo Sensitive Medical Devices like Flexible Endoscopes. *Plasma Process. Polym.* 8, 200-207

Schnabel, U., Maucher, T., Köhnlein, J., Volkwein, W., Niquet, R., Trick, I., Stieber, M., Müller, M., Werner, H.-P., Ehlbeck, J., Oehr, C. and Weltmann, K.-D. (2012) Multicentre Trials for Decontamination of Fine-Lumen PTFE Tubes Loaded with Bacterial Endospores by Low and Atmospheric Pressure Plasma. *Plasma Process. Polym.* 9, 37-47