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Novel biological indicator using DNA-labeled microbeads for evaluating nonthermal plasma sterilization

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Nonthermal atmospheric-pressure plasma (NTP) has emerged as a promising sterilization technology due to its ability to generate reactive species in atmospheric air. These species can damage bacteria, for example, by causing oxidative damage to cell membranes, proteins, and nucleic acids, which can lead to effective sterilization. In addition to chemical effects, physical factors such as ultraviolet radiation, electric field-induced disruption, and shock waves contribute to microbial inactivation. Unlike conventional sterilization methods such as autoclaving or chemical disinfectants, NTP operates at room temperature and atmospheric pressure. Its versatility and safety profile enable it to be used in various applications, including medical, food, and indoor environments [1].

Plasma-activated water (PAW), which retains reactive species, enables flexible application in liquid-based systems and has shown promise in agriculture, food safety, and wound treatment [2]. Importantly, NTP and PAW do not require hazardous chemicals.

Despite its advantages, evaluating the sterilization efficacy of NTP systems remains a challenge. Traditional biological assays using microorganisms require cultivation and aseptic techniques, which can take several days to yield results. Chemical indicators, while faster, often fail to reflect actual microbial inactivation because they are generally tailored to single sterilization modalities and may respond to plasma exposure without confirming sterilization. Conventional indicators lack the flexibility to accommodate the diverse range of plasma discharge configurations and reactive species profiles.

To address these challenges, we have proposed a novel biological indicator based on DNA-labeled magnetic microbeads for rapid and visual evaluation of plasma sterilization. This method utilizes the principle that plasma-induced reactive species degrade DNA molecules [3]. When exposed to PAW, DNA degradation alters the surface properties of the microbeads. Under a magnetic field, intact DNA-labeled microbeads aggregate at the center of a hydrophilic concave glass plate, while microbeads labeled with degraded DNA spread uniformly due to their hydrophilicity [4]. This behavior enables direct and visual discrimination of DNA integrity without the need for complex instrumentation.

Figure 1 shows an example of the sterilization experiments. A dielectric barrier discharge (DBD) reactor consisting of a fluid reservoir with a high-voltage liquid electrode and a dielectric bottom was used to sterilize *E. coli* and generate PAW. A grounded metal-mesh electrode was placed beneath the reactor. DBD was generated between the dielectric bottom and the mesh. The setup can sterilize 10^9 CFU/mL of *Escherichia coli* (*E. coli*) within 30 seconds of DBD exposure. PAW was generated by exposing deionized water or TE buffer to the DBD.

The DNA-labeled microbeads were suspended in each PAW prepared by different DBD exposure times. By comparing the sterilization efficacy with the DNA degradation observed using the visualization method, PAW generated using a diluted TE buffer showed results consistent with the temporal *E. coli* sterilization trends. This indicates that the use of radical scavengers, such as diluted TE buffer, allowed for controlled DNA degradation, thereby enhancing the sensitivity and tunability of the indicator.

This method provides a rapid, simple, and cost-effective approach to evaluate plasma sterilization performance. It enables direct visual detection within one minute of incubation and can be digitized using image processing techniques. This approach addresses the critical need for efficient tools to assess sterilization. It opens new avenues for research and application in plasma-based technologies, including point-of-care diagnostics, food safety monitoring, and on-site sterilization validation.

References

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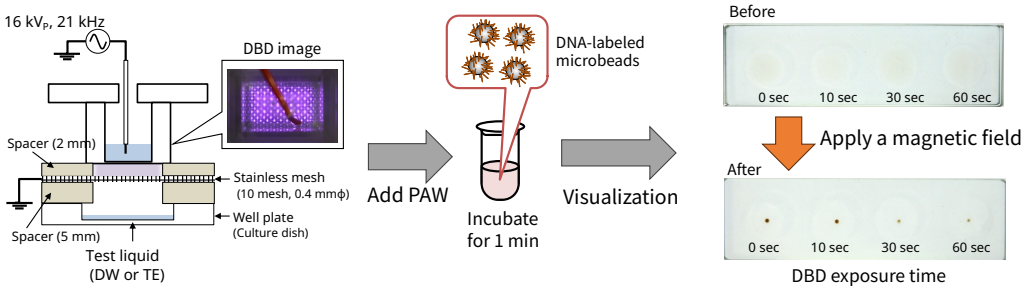

Figure 1. Experiments using DNA-labeled microbeads to evaluate the sterilization capability of the DBD reactor. The visualization result shown here was obtained using 1/1000 diluted TE for PAW.