Material conformity and bactericidal properties of high-frequency-pulse-modulated and low-frequency-pulse-excited plasmas

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Abstract. Plasma sterilization attracts an increasing attention as an alternative method for chemical sterilization. In this study, we investigate plasma sterilization for practical applications, particularly in dentistry and oral surgery [1]. Helium-diluted oxygen was excited by a dielectric barrier electrode at normal atmospheric pressure. Control of the neutral gas temperature was performed under the plasma sterilization. The relation between the intensity of the spectral emission from the excited oxygen atoms and bactericidal effect was investigated using Bacillus stearothermophilus and opportunistic infection bacterium. A comparison is performed with a low-frequency wide-gap discharge. Degradation and material conformity were investigated using the Tyvek unwoven fabric for the sterile package and soft-silicone resin, methyl-methacrylate powder filler used in the dental surgery.

1. Introduction
Plasma sterilization attracts an increasing attention for the prevention of infections in hospitals. Conventional schemes of sterilization include gas sterilization using ethylene oxide and autoclave, pressurized steam, and cleaning with a liquid sterilizing agent. Although ethylene oxide gas sterilization is widely used in the sterilization of medical equipment, in the 1980s, concerns for carcinogenic properties and DNA damage were reported. A strict protocol is followed in the application of this scheme [2]. The application of a high-temperature pressurized steam is limited to heat-resistive materials. Liquid agents, such as 2–20% glutaraldehyde, provide not only a disinfection of pathogenic bacteria but also coagulation of proteins. The residual liquid agent may be a strong irritant to the skin and esophageal surface.

In plasma sterilization, the main role in the bactericidal effect is performed by short-life radicals, using the oxidation and hydrogenation activities of the free-bond and ultra-violet (UV) emissions; no harmful chemical compounds are required. In this study, we present a comparative study of sterilization using helium mixed with oxygen or nitrogen controlled separately, and material conformity to the sterile package, soft silicone forming material, poly-siloxane, and granular bone cement, methyl-methacrylate.

In this experiment, we developed a low-cost power sources to be used in a simple structure of dielectric barrier electrodes, reported for the first time in 1987 by Kanazawa et al [3]. The sterilization
characteristics of this original atmospheric-pressure glow device were validated by the authors’ group in 2001–2003. Since then, numerous applications to surface modification and film synthesis have been reported [4].

The first contribution to the sterilization of pathogenic bacteria was reported in 1996 by Laroussi. Inactivation of various pathogenic microorganisms including viruses and pathogenic proteins was observed in influenza and bovine spongiform encephalopathy [5,6]. Although a model for the effective cause of sterilization has been proposed, such as UV radiation, and oxygen and nitrogen oxide radicals, various experimental schemes have been reported involving the electrode design and frequency, excitation wave-form, and power supply [7–10]. Plasma sterilization needs active oxygen or nitrogen. These agents react with chemical compounds; therefore, it is important to evaluate effects on materials by plasma as well as the bactericidal effect [11]. Only a few studies reported the material conformity and sterilization effect under a controlled power reduction of radical diffusion by the medical equipment in the sterilization process [12].

2. Experimental methods

2.1. Equipment
Helium-diluted oxygen and nitrogen were excited between dielectric barrier electrodes (planar parallel electrodes), excited with a high-frequency power supply or low-frequency pulse inverter at normal atmospheric pressure. Spectral emission of the reactive gas was observed. The relation between the intensity of oxygen radicals and sterilization was confirmed. A diffused glow discharge is difficult to occur when oxygen is used; helium carrier gas with a large electron diffusion leads to a stable glow discharge. The experimental results show that helium dilution is important for the production of dissociated atoms and excitation to the higher level. In the collisional excitation process, Penning excitation, energy transfers from excited helium atoms to excite oxygen atoms or hydroxyl radicals. This process is generally used in a discharge lamp and gaseous discharge laser, in order to decrease the breakdown voltage.

2.1.1. High-frequency discharge device. Figure 1 shows a schematic of the employed system. The helium-diluted mixture is excited between the dielectric barrier electrodes by a modulated radio-frequency power supply at 27.12 MHz (maximum 1 kW). Water cooled the electrode block, a rectangular aluminum parallelepiped with a width, depth, and thickness of 150, 50, and 20 mm, respectively, with a complex perforation, maintained at 20°C. The surface of the electrode was covered with a transparent quartz plate (230 mm × 115 mm, thickness: 3 mm). The main role of these dielectric barrier plates is to prevent the transfer of arc discharge and excitation of the radicals, referred to as non-thermal excitation. In this experiment, the discharge volume is only 3 mm, surrounded by the glass plate and polymer sheet.

![Figure 1. Pulse-modulated high-frequency discharge at normal atmospheric pressure, and schematic.](image)

The design of the diffuser is very important, as the homogeneity of the excitation is determined by the gas flow. The helium-diluted oxygen gas was introduced by a glass diffuser with 10 equally spaced...
perforations, with a diameter of 1 mm, attached at the back of the discharge volume. A similar gas leak plate with equally spaced perforations was placed on the front end of the discharge volume. The gas flow was controlled with a piezo-activated mass flow controller, from 1 mL/min to 5 L/min.

2.1.2. Low-frequency-pulse-inverter plasma device. A pulse-inverter power supply was developed for the low-frequency excitation (50 kHz, maximum 150 W). The electrode had a width, depth, and thickness of 150, 36, and 12 mm, respectively, with a complex perforation for water cooling. The maximum discharge volume had a square shape with dimensions of 150 mm × 36 mm, and gap of 20 mm.

The true input power was estimated by the digitized current waveform multiplied by the voltage, as shown in figure 2. In this experiment, the electrode gap was 15 mm, and the gas flow rate was 3 L/min; no significant change was observed by the change of the gas flow rate and mixture.

![Figure 2](image-url)

Figure 2. Waveforms for the estimation of power deposition. (a) Current–voltage waveform. (b) Electric power and accumulated energy.

From the origin, a zero-cross point of the voltage wave form, the integration over one period, from approximately -0.00000920 to 0.00000910 per second gives the average power:

\[
P = \frac{1}{T} \int_{-0.0000092}^{0.0000091} [I(t) \cdot V(t)] dt
\]

\[
= \frac{1}{0.0000183} \cdot 0.00052192 \approx 28.5
\]

This calculation revealed that most of the input electric power is consumed as reactive electric power, attributed to the charging current to the capacitance of the dielectric barrier.

2.1.3. High-frequency plasma jet device. Figure 3 shows the plasma jet device. The helium-diluted gas mixture is excited with a high-frequency power source at 13.56 MHz, 400 W. The high-frequency power source is an auto-matching servo-device without reflection. The plasma jet device consists of two electrode blocks with the dielectric barrier forming a nozzle at the outlet of the flowing gas. Two aluminum blocks were placed at the opposite sides. The temperature was controlled at 20°C by the circulation of water in the channel. The electrode block had a depth, length (parallel to the gas flow), and thickness of 50, 70 and 20 mm, respectively. The surface of the electrode was covered by a quartz plate with a thickness of 1 mm to prevent the transfer of arc discharge. The discharge gap was 1.5 mm. The gap was narrowed to 0.5 mm at the opening, in order to increase the flow velocity. Figure 3 shows the electrode of the plasma jet device.
Figure 3. Lateral view of the plasma jet device and schematic of the experimental setup.

The discharge plasma can be observed through the lateral barrier. A biological indicator was passed under the opening of the electrode, at a distance of 2.4 mm, moving at a speed of 3 mm/min. The spectral emission at 309 nm was observed from the opening.

The gas flow was controlled with a piezo-activated mass-flow controller, from 50 mL/min to 5 L/min, and a moisture of distilled water was loaded diverting a part of the gas mixture, approximately 3 mol%.

2.2. Diagnostics
The spectral emission was observed with a Raman spectrometer, MonoSpec18, Thermo Vision Colorado, equipped with a thermoelectrically cooled charge-coupled device (CCD) camera system ST-6V, SBIG (focal length: 156 mm). The optical probe was placed at the end of the outlet of the plasma, aimed to observe a band peak in the vicinity of 309 nm proportional to the excited state of the OH radicals. The gas temperature was measured with an optical sensor (FL-2000, ANRITSU, Japan) inserted into the discharge volume, which was not influenced by the high-frequency electric field.

2.3. Biological indicator
Spore forming bacteria are used as a biological indicator for the validation of the autoclave, Geobacillus stearothermophilus, ATCC 7953, sampled from Attest TM 1262 (3M Co., figure 4(a)) inoculated on a filter paper (8.6×10^5 colony forming unit (CFU)). The carrier was dismounted from a sterile cylindrical plastic package in a sterile environment; only the carrier was inserted into the plasma. After the experiment, the carrier was mixed with a recovery medium with a pH indicator, and incubated in a sterile cylindrical package for 48 h, conforming to the protocol prescribed by 3 M. The temperature of the incubator was 56°C for Geobacillus stearothermophilus. One biological indicator was set at the test.

An opportunistic infection refers to a disease caused by weak infectious microbes, which is normally prevented by the natural immunity. Various microbes can cause opportunistic infection. In this experiment, the biological indicator of selected microbes was prepared in the following manner. After the culturing in a soybean casein digest (SCD, Becton Dickinson), 0.01 mL was cultured on a solid agar culture medium. One scoop of a platinum inoculation loop was diluted in distilled water. The density was adjusted to 10^6 CFU; the inoculated water was then placed on a glass plate with dimensions of 18 × 18 × 0.15 mm^3, dried in a clean room, and covered with a sterile package of Tyvek, non-woven polyethylene terephthalate sheet with a thickness of 0.15 mm covered with a transparent film (figure 4(b)). The selected samples are Gram negative: Escherichia coli ATCC8739, Salmonella enteritidis, Gram positive: Staphylococcus aureus ATCC6538, and yeast fungus: Candida albicans ATCC10231. After the exposure, the biological indicators were immersed in a recovery medium, SCD, 100 mL, and incubated for seven days at 30–35°C (25°C in a Candida albicans case). The sterility after the plasma treatment was judged by the turbidity of the recovery medium. The influence of
experimental conditions, such as air humidity in the drying process and conservation time, is investigated with an untreated control. Although the turbidity and pH detection show only whether the sterilization process was successful, the colony counting assessment could provide better information. The colony counting was performed with *Geobacillus stearothermophilus* and *Bacillus atrophaeus*, discussed in other reports.

**Figure 4.** Biological indicator. Commercially available biological indicators (a) *Attest*™ 1262 and (b) *Tyvek*: non-woven fabric of polyethylene. (c) Exposure to the atmospheric-pressure plasma discharge.

### 3. Materials, methods, and experimental results

#### 3.1. High-frequency plasma

In the atmospheric pressure discharge, the thermal conductance between the discharge volume and cooled surface is limited. The neutral gas temperature at high frequencies, 13.56 MHz and 27.12 MHz, increases in the wide gaps, which may be desirable from the viewpoint of the treatment. A control scheme of the temperature was developed. Using pulse modulation, the neutral gas temperature of 400°C became as low as 95–100°C. This operation was realized with a gap of 3 mm, electric power of 670 W, pulse width of 10 μs, and duty cycle of 50%. The flow rate of the gas mixture was 1.5 L/min, while that of oxygen was 1 mL/min. The results are shown in table 1. Negative means that the successful sterilization of 3M-type *Attest*™ 1262, 10⁶ CFU, was successfully inactivated. Positive corresponds to the observation of the propagation. One biological indicator was set at the test. In the pulse modulated high-frequency discharges, the discharge is cut-off before the electron energy is conducted or thermalized to heavy ions and neutrals. The results indicate that the best pulse width was 10 μs.

| Pulse width (μs) | Temperature (°C) | Processing time (one pass, minutes) |
|-----------------|------------------|-----------------------------------|
| ON              | OFF              | 0.5     | 1.0     | 1.5     | 2.0     | 2.5     | 3.0     | 3.5     |
| 30              | 30               | 134     | +       | -       | -       | -       | -       | -       |
| 20              | 20               | 125     | +       | +       | -       | -       | -       | -       |
| 15              | 15               | 115     | +       | -       | -       | -       | -       | -       |
| 10              | 10               | 90      | +       | -       | -       | -       | -       | -       |
| 7               | 7                | 65      | +       | +       |        |        |         |         |

The neutral gas temperature was fixed at 90°C, and the biological indicator was exposed through a sterile package. The right-hand side of table 2 shows the control in the hot-gas environment without the exposure to the plasma.
Table 2. Opportunistic infection bacteria, exposure through the sterile package.

| Species                          | Plasma treatment (min) | Treatment at 90°C (min) |
|----------------------------------|------------------------|-------------------------|
|                                  | 1  3  5  10  15        | 1  3  5  10  15         |
| *Escherichia coli* ATCC8739 (1.6×10⁷) | - - - - -             | + + - - -             |
| *Salmonella enteritidis* (3.5×10⁷) | - - - - -             | + + + - -             |
| *Staphylococcus aureus* ATCC6538 (4.7×10⁷) | + + - - -             | + + + + +             |
| *Candida albicans* ATCC10231 (5.1×10⁶) | - - - - -             | + - - - -             |

Power source: 670 W, ON: 10 μs, OFF: 10 μs, He: 1.5 L/min, O₂: 1 mL/min

3.2. Low-frequency discharge

3.2.1. Sterilization experiment. Table 3(a)(b) shows the results of the sterilization experiment. The gas mixture was He (3 L/min) and O₂ or N₂ (3 mL/min). In this experiment, the gap was 15 mm. Although the gap is wider than that of the high-frequency discharge, the neutral gas can be maintained at a lower temperature of approximately 60°C. The number of the trial is indicated by the symbols. Although the nitrogen case shows an unknown opportunistic dependence, it should be noted that a certain number of the experiment indicated a grey area, such as “ - + - “, corresponding to a partially complete sterilization of the 10⁻⁶-th order in 5 min.

Table 3. Antibacterial effect at a low frequency; comparison between oxygen and nitrogen mixture.

(a) Sterilization experiment (direct exposure)

| Flow rate (L/min) | Time (min) |
|-------------------|------------|
| He                | O₂         | N₂         | 5     | 10     | 15     |
| 3                 | 0.003      | 0          | +     | +      | +      |
| 3                 | 0          | 0.003      | +     | +      | +      |

(b) Sterilization experiment (exposure inside a small syringe)

| Flow rate (L/min) | Time (min) |
|-------------------|------------|
| He                | O₂         | N₂         | 30    | 35     | 40     | 45     | 60     |
| 3                 | 0.003      | 0          | +     | +      | +      | -      | -      | -      |
| 3                 | 0          | 0.003      | +     | -      | +      | +      | -      | -      |

In the low-frequency device, the discharge was excited by a non-sinusoidal pulse voltage. The electron energy exceeds the dissociative ionization energy of the working gas, oxygen; the so-called advanced oxidation is triggered with a lower thermal heating effect.

The band spectrum in the vicinity of 309 nm was observed for a 0.003/3-mixture with oxygen. This peak was emitted by the OH radicals; no peak was observed with nitrogen.

3.2.2. Surface treatment of polymer materials in dentistry. A low-frequency discharge was excited between the dielectric barrier electrodes, at a distance of 20 mm. Soft-silicone impressions of a back teeth, Exafine® and Exahigh-flex®, GC Japan, were analyzed.

The power input was operated at 150 W. The neutral gas temperature increased to approximately 80°C. After 10 min of exposure, the surface of the impression material was studied with a reflective Fourier-transform infrared (FTIR) photometer. A typical example of the change in the Exafine® sample is shown in figure 5. The transparencies of the surfaces, in the Exafine® and Exahigh-flex® samples, exhibited significant decreases at 1,500 cm⁻¹ and 2,800 cm⁻¹. These changes were localized at the surface; no significant change was observed in the cut-out samples, and no significant change in the form was induced by the plasma exposure.
Figure 5. Plasma treatment of an impression material Exafine® (GC). (a) Impression material. (b) FTIR diagrams; control without a plasma treatment, and (c) after a plasma treatment, at 150 W and 50 kHz, for 10 min.

The results of the sterilization experiment suggest that the helium-diluted oxygen low-frequency plasma can be a candidate for the precaution of infection by viral hepatitis and human immunodeficiency virus (HIV), through the impression material.

Further, the effect of the plasma exposure to the granular bone cement resin was investigated. In general, these powders are stored or kept open for a subsequent use without a sterilization process, as a chemical sterilization may reduce the biological conformity. Two 0.16-g scoops of bone cement included in Super-Bond® were measured and expanded in a sterile petri dish (figure 6(a)). Super-Bond® is a self-curing adhesive resin cement including 4-META, MMA/TBB: 4-methacryloyloxyethyl trimellitate anhydride; Methyl methacrylate; Tri-n-butyl borane polymerization agent. Immediately after the exposure to the helium-diluted oxygen plasma, MMA monomer was mixed to form poly (methyl methacrylate) (PMMA) solid (figure 6(b)). The surface hardness was measured using a Vickers hardness tester. The average hardness of the control is 17.2 HV (Vickers Hardness), whereas the granular bone cement after the 10 min of plasma exposure formed a solid with a decreased hardness of 15.4 HV. This change may be in a permissible range as a tooth filling material, corresponding to an increase in the flexibility. This result reveals that surface phenomena, such as oxidation or addition of carbonyl groups, occurred on the filler.

Figure 6. Plasma treatment of a granular acrylic resin. (a) Plasma treatment of a granular acrylic resin under atmospheric pressure. (b) Polymerized powder mixture (left) without treatment and (right) plasma-treated.
3.2.3. **High-frequency plasma jet device sterilization experiment.** We performed a sterilization using the plasma jet device and sterility assessment with the biological indicator, *Geobacillus stearothermophilus*, and pH detection. Table 4 shows the results of the sterilization experiment. The sterilization was performed in a helium carrier without a gaseous mixture, but with the water vapor with a density at the saturated water vapor pressure at 20°C, approximately 3 mol%. The detection of the oxygen-containing functional groups was performed using X-ray photoelectron spectroscopy (XPS).

| Discharge medium | O₂   | No mixture | 50 mL/min | No mixture | 50 mL/min |
|------------------|------|------------|-----------|------------|-----------|
| He (5 L/min)     | -    | +          | +         | +          |
| He (2.5 L/min) + Ar (2.5/min) | -    | +          | +         | +         |

The band spectrum in the vicinity of 309 nm was observed for a mixture with oxygen. This peak was emitted by the OH radicals generated from the migrated water molecules, without mixture; no peak was observed with oxygen or water vapor mixture.

4. **Conclusion**

A plasma sterilization was realized at the normal atmospheric pressure. The performances were compared for three different types. In each experiment, a 6Log₁₀-level sterilization of the biological indicators, *Geobacillus stearothermophilus* ATCC7953, was achieved in 3–15 min, which may be a reasonable waiting time in practical applications. In the high-frequency discharge, the biological indicator was sterilized. The thickness of the object was limited by the discharge gap of 3 mm. This thickness was determined by the control of the neutral gas temperature. The low-frequency discharges, where a larger object can be inserted into the discharge gap of 20 mm, require a prolonged processing time. The low-frequency-pulse-excited device and high-frequency plasma jet device can overcome the discharge volume limit, as shown with the high-frequency discharge device.

The plasma sterilization process introduces oxidation or addition of carbonyl groups on the surface of polymer materials. The evaluation of the damage, or benefit of this effect, require further studies. Soft-silicone impression resin and granular PMMA were treated using a low-frequency discharge; an evaluation of the material conformity was performed. The sterility of such medical product is guaranteed by the factory. In general, one unit of the bone cement can be used several times. It should not be discarded; the remaining part is stored, and could be used in a subsequent treatment. A sterilizer for such a small amount of medical material may find a place in the market. In dentistry or oral surgery, such sterilizer could be employed as a precaution against the risk of droplet infection, owing to the infectious contaminations of blood or saliva. The bactericidal effect and material conformity require further studies, for a practical use.

A gas-plasma-based approach is useful for the sterilization of fragile medical devices, in particular, in the reprocessing of endoscopes. The sterilization characteristics need further evaluations with different types of microorganisms [13].

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