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Fungal Sterilization Using Microwave-Induced Argon Plasma at Atmospheric Pressure

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Abstract The main aim of this study was to investigate the sterilization effects of microwave-induced argon plasma at atmospheric pressure on paper materials contaminated with fungi. Plasma-treated filter papers showed no evidence to an unaided eye of burning or paper discoloration due to the plasma treatment. All fungi were perfectly sterilized in less than 1 sec, regardless of strains. These results indicate that this sterilization method for paper materials is easy to use, requires significantly less time than other traditional methods and different plasma sterilization methods, and is also nontoxic.

Key words: Fungi, sterilization, paper materials, microwave-induced argon plasma, atmospheric pressure

Ancient paper materials, such as history books and various documents, which are stored in libraries and museums, are an important part of our cultural heritage. They represent an important responsibility for libraries, archives, and museums, which are the guardians of our history and of the evolution of philosophical and scientific thinking [23]. Although the preservation of paper materials is important, the growth of microorganisms on paper materials destroys the paper, causes stains, and can be responsible for the loss of paper strength [7, 23]. In addition, microbial contamination can cause diseases among the staff of archives or restoration workshops [2, 7, 23]. For these reasons, sterilization methods for paper materials are required. Although traditional methods for the sterilization of microorganisms, such as dry or moist heat and gas, are dependable and well understood, faster and less damaging methods are necessary for the sterilization of paper materials [20].

A new sterilization method in the field of protection and conservation of paper materials could be plasma treatment, which is a well-established technique in a number of other processes, such as plasma cleaning, etching, and coating [7]. Sterilization by microwave plasma possesses a number of advantages compared with known dry heat, steam autoclave, and gas (ethylene oxide) techniques [12]. It has been suggested that microwave plasma can be used for killing bacteria and viruses. The advantage of microwave plasma sterilization [4] is that it is not only capable of killing bacteria and viruses, but also of removing the dead bacteria and viruses (pyrogens) from the surface of the objects being sterilized [5].

The purpose of this study is to describe a new method, the use of microwave-induced argon plasma at atmospheric pressure for the sterilization of paper materials contaminated with fungi, and to present some experimental results regarding the sterilization of some selected fungi.

A 2.45 GHz, waveguide-based, microwave-induced argon plasma system was used to generate plasma at atmospheric pressure. A schematic diagram of this system is shown in Fig. 1A. This system (Fig. 1B) consists of a 1 kW magnetron power supply commonly used in a microwave oven, an applicator including a tuning section, which is required to reduce the reflected power, and a nozzle section made of quartz. The plasma generated at the end of the nozzle is formed by the interaction of the high electrical field, generated by the microwave power, between the waveguide aperture and the gas nozzle. Argon was chosen as the working gas for this plasma system

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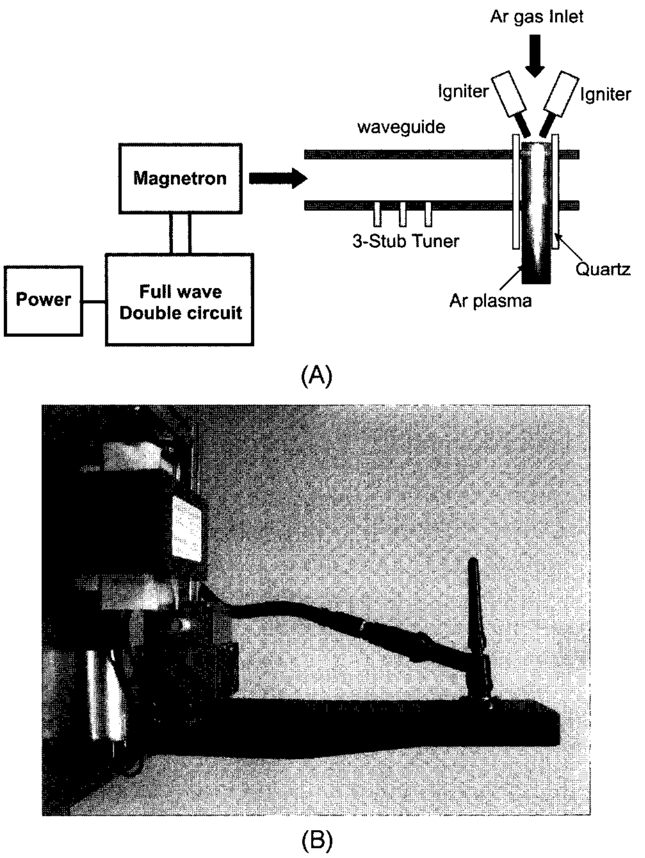

Fig. 1. Microwave-induced argon plasma system.
(A) Schematic diagram; (B) A 2.45 GHz microwave-induced argon plasma system.

because its lack of chemical activity, was expected to ensure the absence of any specific effects on the microorganisms.

Four fungal strains, *Aspergillus niger* [Yonsei Medical Center (YMC) 0100], *Cladosporium cladosporiodes* (YMC 0099), *Penicillium citrinum* (YMC 0253), and *Chaetomium* sp. (YMC 0176), which were identified by their mycological characteristics [13, 16, 18], were used. They were isolated from indoor dust and air, and kept as stock cultures in the laboratory. All fungal strains were maintained on potato-dextrose agar (Difco, Detroit, MI, U.S.A.) slants supplemented with 30 μg/l chloramphenicol (Wako Pure Chemical, Tokyo, Japan) in order to suppress any bacterial growth [10, 21].

For the sterilization test, all fungal strains were suspended in a 0.9% saline solution. The suspensions of fungal spores were inoculated on the sterilized filter papers (10 mm×10 mm×0.75 mm) in Petri dishes, and allowed to dry at room temperature for 1 h. Prior to the sterilization test, the filter papers inoculated with fungi were removed from the Petri dishes and placed in front of a nozzle from which plasma was blown. All strains were exposed to the plasma for 1, 2, 3, 4, 5, 10, 20, and 30 sec. After plasma treatment, the filter papers were transferred into a screw-cap tube containing 2 ml of 0.9% saline, shaken by a vortex for 1 min, and the strains of fungi in the saline spread on potato-dextrose agar. The number of recovered colonies (colony-forming unit, CFU) of fungi was counted after 7 d of incubation at 25°C.

Among the 4 strains, the morphology of *Penicillium citrinum*, one of the most frequently detected strains in contaminated paper materials, was observed by a scanning electron microscope (SEM, S-800, HITACHI, Tokyo, Japan).

Microwave plasmas are generated without any electrodes [6, 8, 17]. Thus, the plasma can be excited in various applications, and can provide a stable, continuous plasma stream over a large range of gas pressures [14]. The microwave plasma generated by the atmospheric pressure gas discharge is a source of electrons, ions, excited atoms and molecules, active free radicals, and UV radiation. These factors qualify microwave plasma as a unique sterilization agent for various applications [14] and materials, including the field of protection and conservation of paper materials. The main advantage of microwave plasma in paper treatment is the possibility of cleaning and sterilization with no decrease in paper strength [23].

In this study, all fungi were perfectly sterilized in less than 1 sec regardless of the strain, as shown in Fig. 2, and the sterilization effect on the spores of *Penicillium citrinum* by the microwave-induced argon plasma was confirmed by SEM. Figure 3 shows SEM images of *Penicillium citrinum*. Figure 3(A) shows normal (control) spores, which have a globular shape with no plasma exposure, while Fig. 3(B), (C), and (D) show the effects of 1, 3, and 10 sec of plasma exposure, respectively. Spores of *Penicillium citrinum* were rapidly damaged in their cell walls, showed a significant reduction in size, and exhibited transformed and amorphous morphologies. They were reduced to smaller structures and microscopic debris after 10 sec of exposure.

However, plasma-treated filter papers showed no evidence to an unaided eye of burning or paper discoloration due to

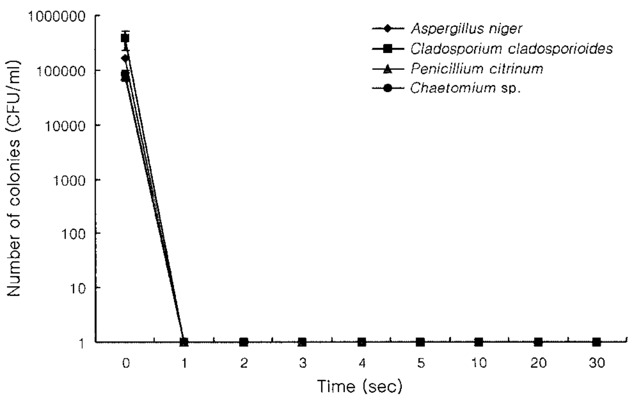

Fig. 2. Sterilization effect on fungi by a microwave-induced argon plasma system at atmospheric pressure.

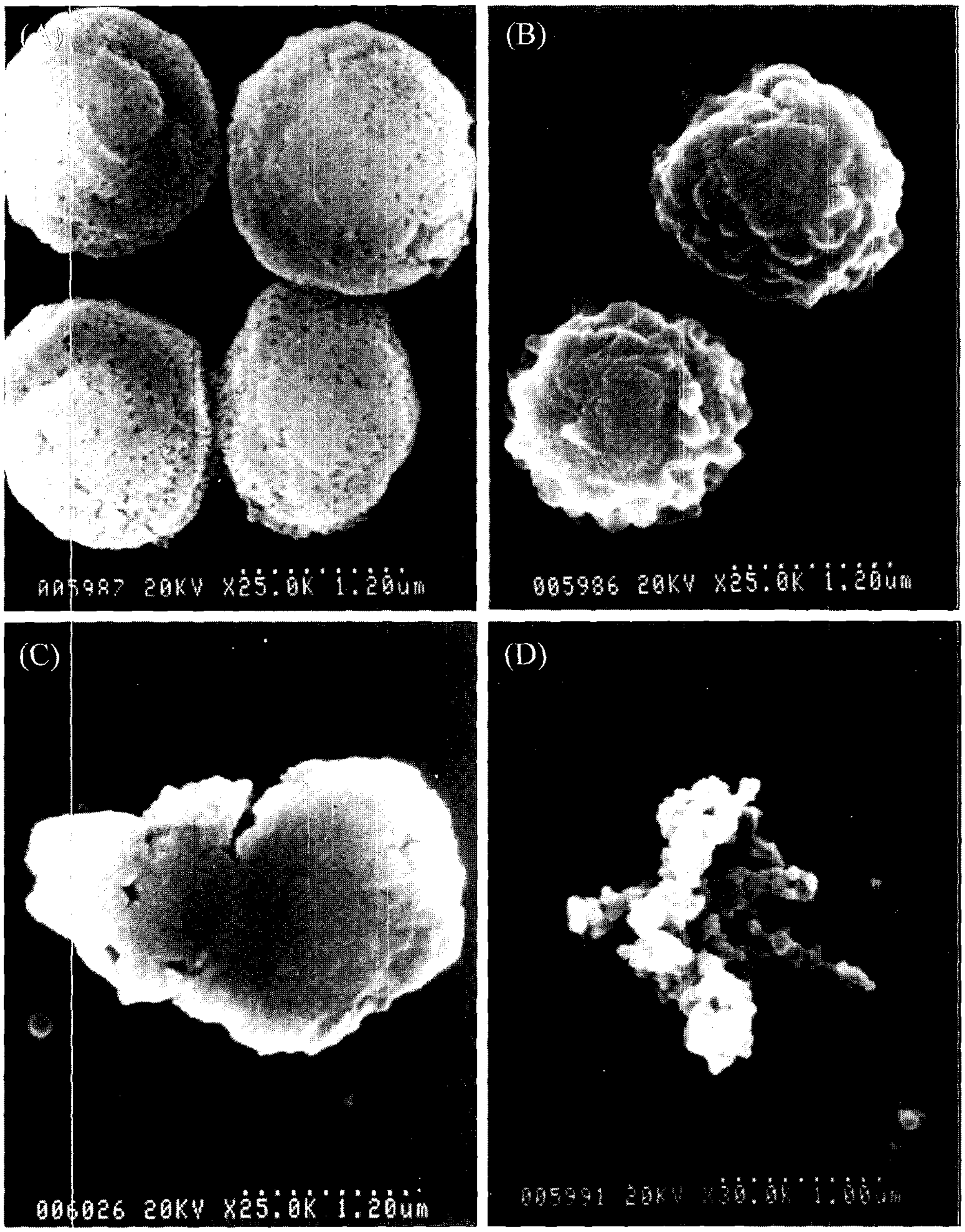

Fig. 3. Scanning electron microscope (SEM) photographs of *Penicillium citrinum*.
(A) Untreated control, (B) spores exposed to t=1 s, (C) t=3 s, and (D) t=10 s of microwave-induced argon plasma at atmospheric pressure.

the plasma treatment. These results demonstrate the possibility of microwave-induced argon plasma at atmospheric pressure to sterilize paper materials, such as books and documents, contaminated with fungi.

In general, sterilization is based on either a physical or chemical process or both, and the microorganisms can be inactivated by heat, radiation, or chemical treatment [1, 3, 9]. Inactivation of bacteria and fungi in materials is as important as the removal of the killed bacteria and fungi or their debris from the materials being sterilized [5].

The process of enzyme treatment to inactivate microorganisms involves first softening of the cell wall of the microorganisms. When the wall is collapsed, the DNAs of the microorganisms are destroyed. The sterilization process with microwave plasma is based on a similar principle. The activated free radicals first weaken the cell wall of microorganisms by reacting with hydrocarbon bonds. As the process continues, the microwave plasma removes the outer layer of the microorganisms [5]. At this point, either the cell wall bursts out or its internal structure is destroyed by the activated free radicals and UV light. This process was confirmed by SEM images in this study. Figure 3 shows the images of spores ruptured and damaged by microwave plasma. This result indicates that the sterilization effect on the microorganisms is related to the activated free radicals.

As seen in Figs. 3(B), 3(C), and 3(D), another sterilization process of microwave plasma similar to plasma etching is the erosion of the microorganisms by etching to form volatile compounds through slow combustion with oxygen atoms or radicals emanating from the plasma. These SEM images show a significant reduction in the size of the fungi, and exhibit transformed and amorphous morphologies. This result demonstrates that the sterilization effect of plasma on the microorganisms is caused by a strong etching process, when the microorganisms are exposed to this microwave plasma at atmospheric pressure.

The other sterilizing mechanism of microwave plasma is the direct destruction by UV light of the microorganism's genetic material. UV radiation is known to penetrate into the cell walls of microorganisms causing disruption of unsaturated bonds, particularly purine and pyrimidine components of nucleoproteins [5, 14]. Moreover, UV light is also known as an antimicrobial agent, particularly for surface applications [5]. In this study, the amount of UV light generated by microwave-induced argon plasma was measured indirectly by using a radiometer/photometer (IL1400A, International Light, Inc., Newburyport, MA, U.S.A.) with a solar blind vacuum photodiode. The amount of UV light ranged from 65 mW/cm^2 (minimum) to 94 mW/cm^2 (maximum). This result demonstrates that a large amount of UV light was generated by plasma, and that the generated UV light was involved in the sterilization of microorganisms. Furthermore, UV light generated by plasma enhanced the etching process.

A sterilization test was further carried out with a commercial microwave oven with a 1 kW, 2.45 GHz magnetron to determine the sterilization effect of microwave-only treatment. The effectiveness of microwaves for the sterilization of microorganisms has been well established by numerous studies over the previous decades [11, 22, 24], although much more exposure time, one or more minutes, was required for sterilization in these studies than in the current study. In this study, the microwave-only treatment of up to 1 min demonstrated no sterilization of fungi, as shown in Fig. 4. This result indicated that the sterilization effects of microwave-induced argon plasma were due to the plasma, but not the microwave.

In conclusion, this study confirmed that the fungal sterilization effects were caused by generated free radicals, UV light, and the etching process in plasma, and the microwave plasma system used in this study required much less exposure time than that described for most published systems [5, 12, 14, 20], because of the high plasma density, the large number of free radicals, and the strong intensity of the UV light.

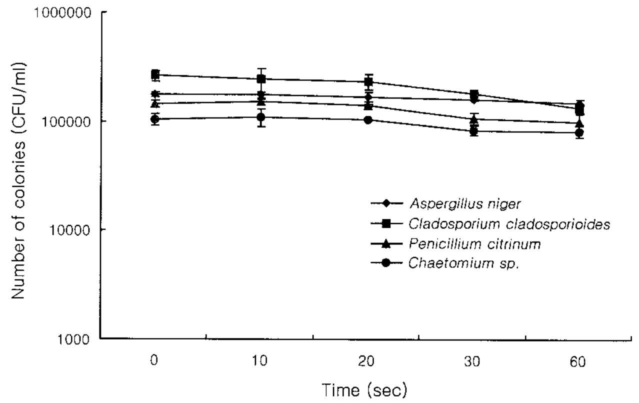

Fig. 4. Sterilization effect on fungi by a commercial microwave oven.

These results indicate that this sterilization method for paper materials contaminated with fungi is easy to use, requires significantly less exposure time than traditional methods and other plasma sterilization methods, and is also nontoxic. Obviously, more tests and in-depth studies of this process are needed. Nevertheless, this study represents a first step in showing that microwave-induced argon plasma at atmospheric pressure is a powerful sterilization tool for use on paper materials.

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