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ORIGINAL ARTICLE

Investigating Effects of Nano- to Micro-Ampere Alternating Current Stimulation on *Trichophyton rubrum* Growth

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Background: Fungi are eukaryotic microorganisms including yeast and molds. Many studies have focused on modifying bacterial growth, but few on fungal growth. Microcurrent electricity may stimulate fungal growth. **Objective:** This study aims to investigate effects of microcurrent electric stimulation on *Trichophyton rubrum* growth. **Methods:** Standard-sized inoculums of *T. rubrum* derived from a spore suspension were applied to potato dextrose cornmeal agar (PDACC) plates, gently withdrawn with a sterile pipette, and were applied to twelve PDACC plates with a sterile spreader. Twelve Petri dishes were divided into four groups. The given amperage of electric current was 500 nA, 2 μA, and 4 μA in groups A, B, and C, respectively. No electric current was given in group D. **Results:** In the first 48 hours, colonies only appeared in groups A and B (500 nA and 2 μA exposure). Colonies in group A (500 nA) were denser. Group C (4 μA) plates showed a barely visible film of fungus after 96 hours of incubation. Fungal growth became visible after 144 hours in the control group. **Conclusion:** Lower intensities of electric current caused faster fungal growth within the amperage range used in this study. Based on these results, further studies with a larger sample size, various fungal species, and various intensities of electric stimulation should be conducted.
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-Keywords-
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INTRODUCTION

Fungi are eukaryotic microorganisms including yeast and molds[1]. The study of fungi holds great scholastic value in application to biotechnology as starter cultures and in treatment of fungal infection[2]. *Trichophyton rubrum* is the most common fungal pathogen that causes tinea pedis and onychomycosis[3,4]. It recurrently infects humans and usually persists for very long time, causing public health concerns. Potassium hydroxide test has been widely used to diagnose fungal infections, but the sensitivity and specificity of this test are relatively low[5]. The best diagnostic method is through culture study by incubating the specimen on a specific culture medium. However, this measure is time consuming and often delays diagnosis. Stimulating fungal growth without fungicidal effects could reduce the diagnostic time.

There have been a lot of research on modifying bacterial growth, but little on modifying fungal growth[6]. Although high intensities of electric current could induce tissue damage and cause antifungal effects, some authors thought that microcurrents could stimulate fungal growth.

In the present *in vitro* study, we sought to investigate effects of microcurrent electric stimulation on the growth of *T. rubrum*.

MATERIALS AND METHODS

Materials

1) Inoculum

The medium used to culture the fungus was composed of

potato dextrose cornmeal agar (PDACC) with peptone, Tween 80 and antibiotics (chloramphenicol 500 mg L^{-1} and cycloheximide 500 mg L^{-1}). Standard sized inoculums of *T. rubrum* derived from a spore suspension were applied to PDACC plates (Catholic Skin Clinic, Daegu, Korea). The spore suspension was prepared by applying 5 ml of distilled water to a 3-week-old *T. rubrum* culture that was later gently withdrawn with a sterile pipette and was applied to twelve PDACC plates with a sterile spreader. Twelve Petri dishes were divided into four groups. The given amperage of electric current was 500 nA, 2 μA, 4 μA in group A, B, C, respectively, and no electric current was given in group D. The electric current was applied after drying dishes for 15 minutes at room temperature.

2) Electrical apparatus

The electrical circuit was turned on to activate the system (alternating current [AC], intensity changeable, frequency: 8 Hz, Granthe®; Cosmic Co., Seoul, Korea) which is small (90 mm [H]×52 mm [W]×19 mm [T], weight 49 g) (Fig. 1).

Methods

All procedures were conducted aseptically to prevent contamination by bacteria or other fungi. Two pieces of stainless steel electrodes (1 mm in diameter and 2.5 cm long) were inserted through the top portion of a sterile Petri plate 2.5 cm apart. The electrodes were placed in the agar for 30 minutes at room temperature with or without electric current application using the electric stimulation apparatus as previously described. An AC of 500 nA, 2 μA, or 4 μA was applied to three different groups for 30 minutes at room temperature. All amperages were confirmed with an independent ammeter. After electric stimulation, each Petri plate was incubated at 25°C, under natural light and humidity provided by the culture medium. The cultures were periodically analyzed after 48, 72, 96, 120, 144, and 192 hours.

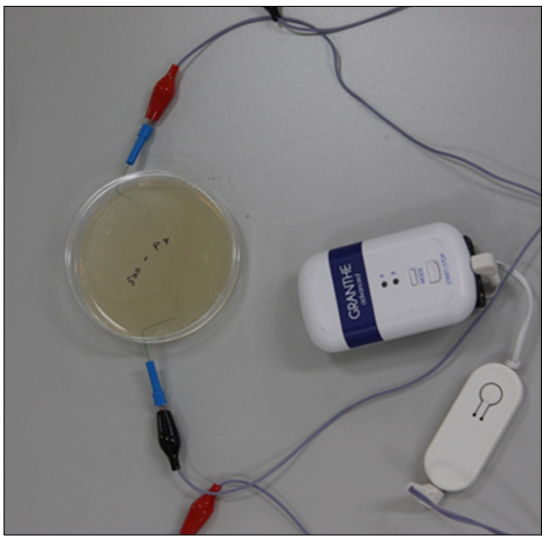

Fig. 1. Diagram of the electric apparatus.

RESULTS

During the first 48 hours, we observed that fungi in groups A and B grew faster than those in the other two groups. Two of the fungal plates exposed to a 500 nA electric current produced more and larger colonies, with fewer colonies grown in the plates exposed to a 2 μA current. Meanwhile there was no visible fungal growth in the plates exposed to a 4 μA current or the unexposed plates. After 96 hours of incubation, all plates in groups A and B showed more explicit growth, while a barely visible film of fungal growth was seen in plates group C. No fungal growths were observed in the control group.

After an incubation period of 144 hours, we observed the development of compact colonies spread across the whole surface of the culture medium in all but one of the plates in group A. The colonies exposed to the lower electric current were denser compared to the colonies exposed to the higher current. Group D developed visible fungal growth at this time. Unlike other reported cases, production of gas bubbles, discoloration, liquefaction, and depression around the cathode was not observed in this study. The results are summarized in Fig. 2.

DISCUSSION

In this study, we sought to determine whether microcurrent electric stimulation could stimulate fungal growth. The results of our study clearly indicate that the duration of incubation time for *T. rubrum* growth is decreased significantly after the application of microcurrent electric stimulation. Lower intensities of electric current resulted in faster fungal growth in the amperage range used in this study.

The mechanism of stimulation of fungal growth by electric current is difficult to surmise. However, Cheng et al.[7] reported that electric currents could stimulate cell growth. Stimulatory effects of electric current began at 10 μA and inhibitory effects began at 750 μA. In this study, only 500 nA to 4 μA currents stimulated fungal growth. The mechanism for how electric currents can stimulate cell growth can be proposed in different manners. Adenosine-5′-triphosphate (ATP) generation can play an important role in stimulating cell growth. During electrostimulation, elec-

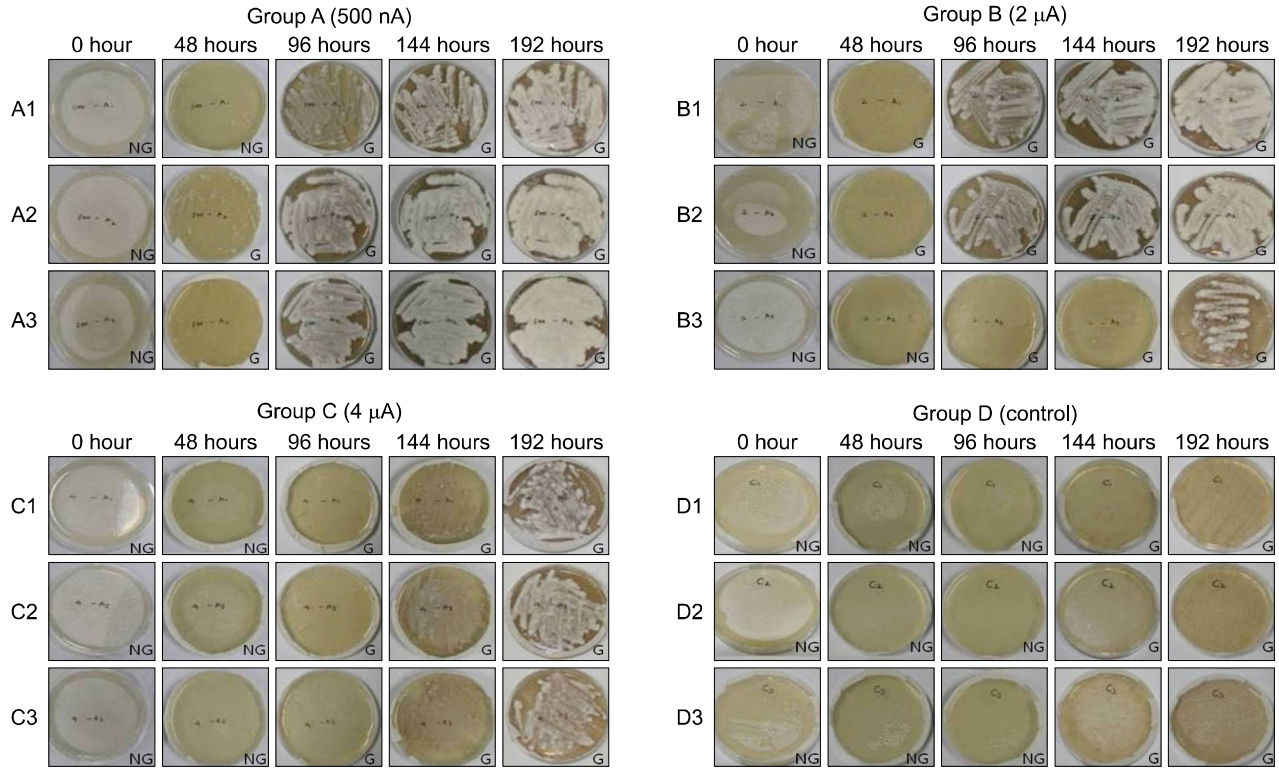

Fig. 2. Petri slides with *Trichophyton rubrum* fungal culture in group A (A1～A3), B (B1～B3), C (C1～C3), and D (D1～D3) after 0, 48, 96, 144, and 192 hours of incubation arranged chronologically. G: growth, NG: no growth.

trons react with water on the cathode side of the electrode to produce hydroxyl ions and on the anode side of the electrode to produce protons. Thus, between the anode and cathode interface, a proton gradient across the tissue and the medium is created. Hence, protons under the influence of the electric field and the concentration gradient should move from anode to cathode. As migrating protons reach the mitochondrial membrane-bound H+-ATPase, ATP will be formed[8,9]. With increased ATP levels, more proteins could be synthesized. The increased ATP generation is partially responsible for the increased protein synthesis[10,11]. Another mechanism of stimulating protein synthesis is by changing amino acid availability, which is equally increased because of stimulated amino acid transport[12]. Thus, the electricity induces ATP generation and increases amino acid availability to cause more protein synthesis and consequently, faster cell growth. An important factor here is that the electric current does not affect DNA metabolism, suggesting that the stimulatory and inhibitory effects of microcurrents on protein synthesis activity occur independently[12].

We have found that the intensity of the microcurrent is important in stimulating fungal growth. In contrast to our study, it has previously been suggested that electrical currents have antifungal effects. Kalinowski et al.[13] suggested that low-voltage direct current electrostimulation acts as a fungicide in a dose-dependent manner in *T. rubrum*. Low-voltage direct current electric stimulation in the range of 500 μA to 3 mA was applied *in vivo* to *T. rubrum*. The results of this study clearly demonstrated the fungicidal effect of electric stimulations in this current range. The authors proposed that fungal cell death was caused by damage or denaturation of key cellular enzyme, damage to DNA, damage or disruption of cell membranes, or damage or destruction of key cellular transport systems. The applied current was more than a hundred to a thousand times higher than the electric stimulations used in our study. These high current electric stimulations could have damaged the fungal cells. Thus, we conclude that electric currents can stimulate fungal growth at very low amperages (500 nA to 4 μA) and can inhibit fungal growth at higher amperages (500 μA to 3 mA).

This study was designed as a pilot study to investigate the effect of alternating microcurrent electric stimulation on fungal growth. There are several limitations to our study. First, we examined relatively few fungal plates and studied only *T. rubrum*. We plan to include larger sample sizes and various fungal species in future studies. Second, an investigation of the underlying molecular biology could not be conducted in this study; thus, we were unable to pro-

vide any mechanistic insight into how electric stimulation affects fungal growth.

Despite these limitations, we conclude that the results of our study can be used to design further controlled trials of large studies. Based on the results of this pilot study, microcurrent electric stimulation can be applied to fungal cultures to provide a number of benefits. First, researchers could obtain more fungal colonies in a shorter period with microcurrent electric stimulation, enabling basic studies of fungi. Second, it could be applied clinically by using microcurrent electric stimulation in fungal incubators. The diagnostic time would be considerably shortened by using culture methods of fungal infections. Lastly, it could be helpful in incubating useful commercial fungi, such as mushrooms, because microcurrent electric stimulation does not affect DNA metabolism[14].

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