Treatment of Antifungal Resistant Candida Biofilms And Clinical Dermatosis Using A Direct-Current, Atmospheric-Pressure Cold Plasma Micro-Jet

Peng Sun, Shuang Yu, Haiyan Wu, Jue Zhang, Jing Fang

Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China

Yi Sun, Wei Liu

Department of Dermatology and Venereology, Peking University First Hospital, Beijing, China

WeiDong Zhu

Saint Peter’s College, Jersey City, New Jersey, USA

Abstract: In this paper, a direct-current, atmospheric-pressure, He/O 2 (2%) cold plasma microjet (PMJ) was applied to Trichophyton rubrum (the most frequent dermatophyte), Candida spp (which causes thrush and vaginal candidiasis) and three other types of fungi. Effective inactivation was achieved both in air and in water within 5 min of plasma treatment [1]. Ten dermatosis biofilms (C. glabrata, C. albicans, C. krusei) were also performed, effective inactivation was also observed. The inactivation was verified by a XTT test. Severely deformation of the biofilms was observed after PMJ treatment through SEM. The existence of ·OH, ·O 2-, 1O2 was verified by Electron Spin Resonance (ESR) spectroscopy. Optical emission spectroscopy also showed strong atomic oxygen emissions in air. The sessile minimal inhibitory concentrations (SMICs) of three antifungal medicines against the Candida spp. biofilms were decreased to 2-6 fold dilutions in PMJ treated group in comparison with untreated controls. This novel approach holds potential in clinical treatment of dermatosis.

Keywords: Antifungal; Candida biofilm; Electron Spin Resonance; Non-thermal plasma jet; Minimal inhibitory concentrations

1. Introduction

Candidiasis, caused by Candida species, is the most common fungal infection in humans [2, 3]. Beside invasive disease including candidemia and candidiasis in deep-seated organ, mucocutaneous disorders such as oral candidiasis, vaginal and vulvovaginal candidiasis, have become the major clinical problem [4, 5]. Although Candida species are the microorganism exhibiting planktonic unicellular form, filamentous growth or complex multicellular structure is observed mainly in the infected tissues [6]. The structured microbial communities attached to surfaces and encased within a matrix of exopolymeric materials is now defined as biofilms [6, 7], which can form on various implanted medical devices such as vascular and urinary catheters, joint prostheses, cardiac valves, artificial vascular bypass devices, and those being topically used including contact lens and dentures[7-10]. The biofilm with the complex structure is resistant to both host defense and commonly used antifungal drugs [7, 11, 12]. The cells of Candida species, dropped constantly from the structured microbial communities, can spread and further cause antifungal treatment failure, devices failure or persistent infections [9]. And the contribution of biofilm to the increasing of candidiasis has been now confirmed [13]. Thereby, to develop novel approaches inactivating candidal biofilm has great significance in treating candidiasis, especially those related to Candida biofilm.

Compared with traditional therapeutic methods, non-thermal plasma as a physical method could provide a more economic and effective method. In addition, the simple generation system of plasma and its gaseous form provide the possibility to penetrate in inhomogeneous surfaces, cavities and fissures down to the micrometer scale, and allow combining this technique with minimally invasive surgery or with antimicrobial chemotherapy. In addition to several
papers reporting the plasma fungicidal ability against *Candida albicans* [14, 15], our group [1] have shown that non-thermal plasmas can effectively inactive the strains of *Candida* species including fluconazole-resistant *C. albicans*, *Candida glabrata* and *Candida krusei*. We also found that the antifungal susceptibility of *Candida* species strains to common antifungal drug is enhanced after they were treated with plasma. These results indicated that non-thermal plasma could be a potential treatment method or supplementary treatment method for candidiasis. In the present study, we further investigated the fungicidal effect of non-thermal plasma on *Candida* biofilms (which are considered more difficult to inactive than their planktonic counterparts), and its effect on antifungal susceptibility of *Candida* biofilms to common antifungal drug.

2. Materials and Methods

Ten strains of *Candida* species used in this study were isolated and stored in our laboratory as shown in Tab 1. RPMI-1640 medium was used to re-suspend the pellet and adjust the final density to $1.0 \times 10^6$ cells/ml for all strains. One hundred microliter of prepared suspension was pipetted into selected wells of 96-well microtiter plates and every replication was separated by an empty well. After incubating statically for 24 hours at 37°C, the biofilm was formed.

3. Results

**Fungal elements embedded in Candida biofilm were severely damaged after treatment with plasma**

From SEM, healthy yeast cell and pseudohyphae with smooth surfaces were observed in biofilm treated with He/O$_2$ gas flow (without PMJ), as shown in Fig 1-a. Samples treated with PMJ for 10s show crude shape of sessile cells and some fractured cells (Fig 1-b). When the treatment time was extended to 20s, deformed and ruptured cells were observed (Fig 1-c). Noticeably, the majority of components were fragile, fissile yeast cells and pseudohpyaeha after 30s PMJ treatment (Fig 1-d). When the treatment time reached to 1min (Fig 1-e), the biofilms lost their original morphologic characteristics and were degraded to clusters of cell fragments, which included rupture, distortion and shrinking of the outer layer. This degradation led to the leakage of cell inclusion. Above morphological changes of cell wall are considered detrimental for the survival of the fungi, which were not seen in the negative control samples where only He/O$_2$ flow was introduced. In summary, highly structured community of *Candida* biofilm was destroyed by PMJ gradually.

![Figure 1](image1.png)

**Figure 1.** A picture of plasma microjet (PMJ)

*Enhancement of fungicidal effect of common antifungal drugs on plasma-treated Candida biofilm*

The minimal inhibitory concentrations (MICs) of amphotericin B, fluconazole, and caspofungin against the planktonic cells of above-mentioned 10 strains were performed.

The SMICs value of fluconazole, amphotericin B, and caspofungin for the groups treated with PMJ (treatment with plasma for 10s, 20s and 30s) showed a significant reduction, compared with those for the untreated candidal biofilm. After being treated with plasma for 20s or 30s, small part of the Candida species cells in the Candida biofilm were survived while most of them were inactivated, as shown by incubation of the plasma-treated biofilm in RPMI1640 medium for 24h at 37°C. Since being completely inactivated within 1 min, the antifungal
activity of the common antifungal drugs against those Candida biofilm treated with plasma for 60s was not tested. Detailed information about the antifungal results will be discussed on the conference.

**Free radicals were generated by He/O₂ (2%) non-thermal plasma**

Since we showed in the previous study [1] that He/O₂ (2%) cold plasma inactivated the plantonic cells of Candida species in both air and water via partly producing reactive oxygen species (ROS), we also observed that [16] the oxidative stress pathway of Eukaryotic cells Saccharomyces cerevisiae could lead to their hypersensitivity to plasma treatment. It is supposed that ROS generated by plasma in the present study contribute partly to the damage of candidal biofilms. Further ESR assaying showed ·OH, ·O₂⁻, and Ö₂ were detected in He/O₂ (2%) cold plasma treatment, confirming above inference. The ROS including ·OH, ·O₂⁻, and Ö₂ were proven to cause lipids and proteins of cytomembrane suffering from oxidative damage which lead to impediment of ion transition. Furthermore, the oxidative molecular could combine with DNA causing additional cell damage [17].

**Funding**

This research is supported by National Natural Science Foundation of China (30970131) and Beijing Natural Science Foundation (7102149) to Dr. Wei Liu, and is also sponsored by Bioelectrics Inc. (U.S.A.), MST Program of International Science and Technology Cooperation (under Grant # 2009DFB30370: “Cold Plasma induced biological effect and its clinical application studies”) and National Basic Research Program (No. 2007CB935602)

**References**


Figure. 2. SEM results of SC5314 biofilm before and after plasma treatment, from left to right, the magnificent scale increases from ×2000 to ×22000: (a), a negative control treated with He/O2 flow; (b), that treated with PMJ for 10 seconds; (c), that treated with PMJ for 20 seconds; (d), that treated with PMJ for 30 seconds; (e), that treated with PMJ for 60 second

<table>
<thead>
<tr>
<th>Isolate</th>
<th>BMU02971</th>
<th>BMU03213</th>
<th>BMU04801</th>
<th>SC5314</th>
<th>ATCC6258</th>
<th>BMU05102</th>
<th>BMU05137</th>
<th>BMU00271</th>
<th>BMU01689</th>
<th>BMU04388</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Pharynx</td>
<td>Oral mucosa</td>
<td>Oral mucosa</td>
<td>Blood</td>
<td>Sputum</td>
<td>Oral mucosa</td>
<td>Oral mucosa</td>
<td>Blood</td>
<td>Knee</td>
<td>Intraperitoneal fluid</td>
</tr>
<tr>
<td>Species</td>
<td><em>C.albicans</em></td>
<td><em>C.albicans</em></td>
<td><em>C.albicans</em></td>
<td><em>C.krusei</em></td>
<td><em>C.krusei</em></td>
<td><em>C.glabrata</em></td>
<td><em>C.glabrata</em></td>
<td><em>C.glabrata</em></td>
<td><em>C.glabrata</em></td>
<td><em>C.glabrata</em></td>
</tr>
</tbody>
</table>

Tab 1. Ten strains of Candida species used in this study