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Candida parapsilosis isolates from burn wounds can penetrate an acellular dermal matrix

Erika Okuno[a,b], Isabele Carrilho Jarros[a], Patricia Souza Bonfim-Mendonça[a], Glória Vicente de Rezende[b], Melyssa Negri[a], Terezinha Estivalet Svidzinski[a,*]

[a] Laboratory of Medical Mycology, State University of Maringá, Brazil
[b] Burn Treatment Center of the North Paraná University Hospital, State University of Londrina, Brazil

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ABSTRACT

We isolated and identified yeasts from burn wounds and evaluated the ability of *Candida parapsilosis* isolates from burn wounds to penetrate an acellular dermal matrix (ADM). A prospective study was conducted with patients from the burn treatment center of North Paraná University Hospital in Londrina, Brazil from February 2015 to January 2016. Yeast cultures were obtained from the tissue of burn wounds that had been debrided and cleansed with 2% chlorhexidine. After identification and confirmation of the purity of the culture, the yeasts were placed on ADM fragments and incubated for three or seven days. During the study period, 273 patients were treated, and 36 of these patients fulfilled the inclusion criteria and provided samples for culture. Yeasts were isolated in 19.44% (n = 7) of the cultures, and the following species were identified: *C. parapsilosis* (57.1%), *C. albicans* (28.6%), and *C. glabrata* (14.3%). *C. parapsilosis*, the most frequent species, was chosen for the ADM tests. We demonstrated active penetration of the ADM by the yeast isolates from burn wounds. *C. parapsilosis* grew on ADM and penetrated the matrix, indicating that this yeast, which is common in skin and cutaneous wounds, has the potential to colonize and pass through ADM, a medical device that is frequently used to dress and regenerate burn wounds.

1. Introduction

Thermal burns cause an estimated 265,000 deaths per year. In addition, burns also have physical and psychological consequences, leading to a heavy global public health burden [1]. Although the prognoses for burn patients have greatly improved over the last decade, owing to progress in dressing and care guidelines, such as early debridement and broad antibiotic therapy, there is still considerable morbidity and mortality among burn patients, mainly due to complications from infections [2,3]. In recent years, the incidence of bacterial infections in burn patients has decreased, while that of fungal infections has gradually increased. This increase is due to risk factors, including the use of central venous catheters, urinary catheters, mechanical ventilation, broad-spectrum antibiotics, hemodialysis, and total parenteral nutrition [3,4]. Fungi account for 20–25% of the infections in burn wounds [5–8], and fungal infections significantly increase the seriousness of burn injuries [5,6].

Candida spp. is the most frequent genus of fungi colonizing burn wounds, and colonization by *Candida* yeasts is considered a risk factor for the development of candidemia, which has an incidence rate of approximately 5% [9,10] and a mortality rate of up to 15% [6]. Although *Candida albicans* is the most common species in patients at burn treatment center intensive care units (ICU), in recent decades, the number of infections caused by *Candida* non-*C. albicans* (CNCA) has increased [11]. One of these CNCA species, *C. parapsilosis*, currently stands out; however, its etiopathogenesis has yet to be fully clarified.

Burn wound infections can be established following one of two pathways. The first pathway is endogenous, and it involves atrophy of the gastrointestinal mucosa, which allows for translocation of *Candida* spp. from the intestine to the wound site. The other exogenous pathway appears to be more common, especially for *C. parapsilosis* infections. This species colonizes either the skin, aided by the formation of biofilms in wounds or medical devices such as catheters. Association of biofilm formation ability by the microorganism, added to patient immunosuppression, promotes fungal infection [12,13].

Burns disrupt the skin barrier; therefore, it is important to cover burn wounds as quickly as possible. Autologous grafting is ideal, but usually impossible; therefore wounds often remain uncovered, making

* Corresponding author. Division of Medical Mycology, Teaching and Research Laboratory in Clinical Analysis, Department of Clinical Analysis of State University of Maringá, Paraná, Brazil, Av. Colombo, 5790, CEP: 87020-900, Maringá, PR, Brazil.
E-mail addresses: tiesvidzinski@uem.br, terezinha.svidzinski@gmail.com (T.E. Svidzinski).

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the patient more susceptible to often fatal infections. Cadaveric skin transplantation has helped decrease mortality among burn patients, but this procedure is not always available. Thus, a new treatment technology has been developed that uses an acellular dermal matrix (ADM) derived from bovine or porcine tissue. ADM is suitable for covering wounds and has considerable treatment potential. Although it is not a substitute for autologous tissue, it can be used to cover deep burn areas, providing time to reverse immunosuppression and epithelialize donor areas [14].

The use of ADM for early coverage of burn wounds has countless benefits, such as reduced fluid loss, accelerated and more aesthetic healing, greater elasticity, and decreased contracture. In addition, ADM provides adequate mobility and function of affected joints. ADM also offers improved quality of life and decreased mortality [14]. Despite these benefits, the use of this material on wounds can facilitate infections, since it provides an environment conducive to the growth of microorganisms.

Little is known about the risk of fungal infections in burn patients treated with ADM, and there are few studies of fungi, especially emerging ones, such as *C. parapsilosis*. Recently, we developed a model for evaluating the interactions between skin-colonizing yeasts and ADM [15]. We found that all evaluated yeasts species, including *C. parapsilosis*, have the capacity to colonize and migrate through ADM, which therefore may increase the potential risk of infection in burn patients. The objective of this study was to evaluate the capacity of *C. parapsilosis* isolates from burn wounds to permeate ADM *in vitro*.

2. Materials and methods

We first investigated the frequency of *Candida* yeast on burn wounds and evaluated the capacity of *C. parapsilosis*, a common species isolated from burn wounds, to penetrate an ADM.

2.1. Study type and population

A prospective, observational, descriptive study was conducted from February 2015 to January 2016. This study was previously approved by the Ethical Committee for Research Involving Humans of the State University of Maringá, Brazil/Plataforma Brasil (CAAE: 33218214.40000.0104). The study sought a greater understanding of colonization by yeasts in patients at the burn treatment center of the Hospital Universitário Norte do Paraná (North Paraná University Hospital), Londrina, Brazil, in collaboration with the Research and Clinical Analysis Laboratory, Division of Medical Mycology, Universidade Estadual de Maringá (State University of Maringá), Brazil.

2.2. Admission of burn patients

All burn patients in this study were evaluated during hospitalization. Under sedation and analgesia, their wounds were cleaned, and the blisters were removed using a toothbrush and chlorhexidine digluconate (2%). The wounds were then covered with 1% silver sulfadiazine cream and bandaged with three layers.

2.3. Inclusion criteria

All patients who received care during the study period were included, provided they met the following criteria: a burn wound classified as large or medium according to the Lund and Browder chart, aged 18–65 years, hospitalized within 72 h of the burn, and provided written informed consent.

2.4. Exclusion criteria

The exclusion criteria were as follows: small burn; burn limited to airway; electric burn; pregnancy; immunocompromising conditions, including diabetes mellitus, autoimmune diseases, degenerative diseases, or benign or malignant tumors; renal or hepatic impairment prior to burn; receiving topical, oral, or parenteral antifungals during the days preceding the burn.

2.5. Collection of biological materials and yeast isolation

Following routine cleaning, sample material was collected from the burns with a sterile swab (5 cm × 5 cm), distal to the hip area, passing through the center of the wound. This swab was discharged into a petri dish containing Sabouraud dextrose agar (SDA; Merck, Munich, Germany) supplemented with polymyxin and chloramphenicol. The plates were incubated at 25 °C for up to five days, and yeast growth was observed. Positive cultures were transported to the laboratory, according to Brazilian regulatory standards (NR-15), where the yeasts were identified using classical methods, including micromorphological analysis and biochemical tests [15,16], the identifications were confirmed by VITEK® MS (BioMérieux, Marcy l'Étoile, France).

2.6. Acellular dermal matrix

In the study, we used PELNAC® Artificial Dermis (fenestrated type, size: 120 mm × 240 mm; Gunze Limited, Kyoto, Japan) to restore the dermis of severe burns. For the experiment, the ADM was stored dry at room temperature and was aseptically trimmed with a scalpel to 1 cm^2. The fragments were hydrated with sterile physiological saline (0.9% NaCl) for 15 min at room temperature to generate a gelatinous substrate, according to the manufacturer's instructions.

2.7. In vitro assessment of ADM-yeast interaction

All *C. parapsilosis sensu stricto* isolates obtained from the burn wounds were selected for the *in vitro* study. Pilot tests were performed to determine the best yeast to suspension volume for the ADM assay. Prior to the assay, 5 ml of SDA with chloramphenicol was dispensed into each well of a six-well microplate (TPP, Switzerland). Next, wetted ADM fragments were aseptically transferred to the media using tweezers. Then, 20 µl of a 0.9% NaCl suspension containing 1×10^7 yeast per ml, was added to the center of each fragment. For each *C. parapsilosis* isolate, the tests were performed in triplicate in two independent assays. A positive control was included in which the yeast suspension was added directly to the culture medium (without the matrix), and a negative control was included that contained matrix without culture medium, to confirm the sterility of the ADM. The microplates were incubated at 35 °C in an oven for three or seven days.

After each the incubation period (three or seven days), quantitative and qualitative analyses were performed to assess the capacity of *C. parapsilosis* to colonize and penetrate the ADM.

2.7.1. Quantitative study

For the quantitative analysis, the ADM fragments were removed, and the agar underneath the ADM was scraped with an inoculation loop and discharged into Eppendorf tubes containing 1 ml of 0.9% NaCl solution. This solution was serially diluted, and a sample of the dilutions was plated on SDA. These plate cultures were incubated at 25 °C for 48 h to determine the colony-forming units (CFU).

2.7.2. Qualitative study (print)

For the qualitative analysis, the ADM fragments were removed from the surface of the culture medium (microplate) after the 7 day incubation period. One fragment was used to obtain a panoramic image through a digital microscope at 800 × magnification (Color CMOS Sensor, High Speed DSP). For the imaging, the contaminated ADM was carefully removed and fixed in 100% methanol for 15 min.

The other fragments were used to "print" the ADM to verify the possible passage of yeast through the matrix. Using several microscope