Original Article

Effects of simulated microgravity by RCCS on the biological features of Candida albicans

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Abstract: During the spaceflight, a wide variety of microorganisms may be carried to the outer space by astronauts and aviation component. The yeast Candida albicans is an important opportunistic pathogen responsible for a variety of cutaneous and systemic human infections in human body, and the yeast cell itself could be affected by various stressful environmental factors including the weightless environment. We evaluated the effects of simulated microgravity on biological features of Candida albicans using the rotary cell culture system (RCCS). The growth curves of Candida albicans cultured in RCCS were recorded by spectrophotometer, the morphogenic switches were observed by optical microscope, and the viability of cells exposed to the various concentrations of fluconazole solution was assayed by flow cytometry at 7th, 14th and 21st day of experiment. The results showed that Candida albicans SC5314 under modeled microgravity were manifested as the growth curves leftward-shifted, lag phase shortened, along with logarithmic phase and stationary phase forwarded (P < 0.05). The simulated microgravity increased the growth rate and mycelia formation of Candida albicans. A statistically significant decrease in viability was detected in cells cultured for 7 d, 14 d and 21 d in group of simulated microgravity compared with the control group (P < 0.05). The increase of exposure time to simulate microgravity resulted in the decrease of viability of cells accordingly in same drug concentration compared with the control group. The study demonstrated that the three weeks’ simulated microgravity in RCCS had a noticeable affect on the growth status of mycelia and spores and the morphogenic switches of Candida albicans, meanwhile, the yeast cells under simulated microgravity showed an increased antifungal susceptibility to fluconazole.

Keywords: Simulated microgravity, Candida albicans, fluconazole, drug susceptibility

Introduction

During the spaceflight, a wide variety of microorganisms may be carried to the outer space by astronauts and aviation component [1]. Studies have showed that the microgravity has a series of adverse impacts on the microorganisms. Their mutation in spaceflight, if left uncontrolled, will seriously endanger the astronaut’s health and affect the implementation of aerospace instrument. This is certainly the urgent problem in the field of manned spaceflight [2, 3].

The yeast Candida albicans is a harmless commensally in the oral cavity, digestive tract and genital region of healthy people, which also could cause superficial infections and life-threatening systemic diseases. With the increase of immunocompromised individuals due to HIV infection, organ transplantation and application of chemotherapy and indwelling devices, invasive candidiasis has become a serious public health problem in the recent several decades [4, 5]. Being an important opportunistic pathogen, Candida albicans is responsible for a variety of cutaneous and systemic human infections in human body, and the yeast cell itself could be affected by various stressful environmental factors, including the weightless environment [6, 7].

The present study was undertaken to investigate the effects of simulated microgravity on
the biological features of *Candida albicans*. The rotary cell culture system (RCCS) was applied to mimic the environment of simulated weightlessness. This study was designed to provide a reference for the medical monitoring and support of spaceflight.

**Materials and methods**

*Experimental groups and culture of Candida albicans*

*Candida albicans* SC5314 were purchased from China General Microbiological Culture Collection Center (CGMCC), and divided into experimental group (n = 8) and control group (n = 8). RCCS (Synthecon, US) was used to culture the *Candida albicans*. The yeast was maintained in YPD/YEPD medium (Oxoid, UK). The cultured solution was mixed with YPD/YEPD medium (1:1), and the samples of 1.0 ml suspension were taken and added respectively into the RCCS of experimental group and control group, making an initial concentration of *Candida albicans* SC5314 solution at $2.5 \times 10^6$ cfu/m. The cultured solution (1.0 ml) was taken every 24 hours, and the remaining was discarded. New culture medium was added into RCCS for continuous rotating culture, and the subculture lasted for 21 days.

*Growth curves of Candida albicans SC5314 cultured in RCCS*

The growth curves of *Candida albicans* SC5314 were recorded by using spectrophotometer (Type 721, Shandong Gaomi Caihong Analytical Instruments Co., Ltd. China) on the 7th, 14th and 21st days of RCCS culture, respectively. In brief, the steps were as follows. The samples of the *Candida albicans* SC5314 solution was taken from the experimental group and the control group on the 6th, 13th and 20th days, respectively, and the concentration was adjusted to $1.0 \times 10^4$ cfu/ml, then the solution (1.0 ml) was added into RCCS for next 24 hours culture, respectively. The solution was taken every other hour, and the spectrophotometer was used to record the growth curves.
Candida albicans under simulated microgravity

used to measure the solution’s OD value, then the growth curves were recorded.

**Morphogenic switches of Candida albicans cultured in RCCS**

The samples of *Candida albicans* SC5314 solution were taken from the incubators of experimental group and control group on the 7th, 14th and 21st days of culture, respectively. After being centrifuged, smeared, fixed and stained by HE, the mycelia and spores and the morphogenic switches of *Candida albicans* were observed under optical microscope (Olympus, Japan).

**Flow cytometry assay of antifungal susceptibility of Candida albicans cultured in RCCS**

The viability of *Candida albicans* SC5314 exposed to various concentrations of fluconazole (Pfizer PGM, France) was assayed by flow

Figure 2. Simulated microgravity affected the growth status of mycelia and spores of *Candida albicans* (HE × 400). A-C. Under simulated microgravity for 7 d, 14 d and 21 d. D-F. Control groups of 7 d, 14 d and 21 d.
Candida albicans under simulated microgravity

Figure 3. A. FSC/SSC. B1. Experiment for 100% viable cells. B2. Control for 100% viable cells. C1. Experiment for 100% dead cells. C2. Control for 100% dead cells.
Candida albicans under simulated microgravity

cytometry (FACS Calibur, Becton, Dickinson and Company, US). The main steps were as follows: Firstly, the preparation of the antifungal drug solution: Fluconazole powder was dissolved in sterile distilled water, with a stock solution at 1280 μg/mL, and stored at -70°C. Secondly, the preparation of Candida albicans solution: On the 7th, 14th and 21st days of experiment, the samples (1.0 ml) of the Candida albicans SC5314 solution was taken from the experimental group and the control group, respectively, YPD/YEPD medium was used to dilute the solution, and the concentration was adjusted to 0.5 × 10^6 cfu/ml. Thirdly, the flow cytometry susceptibility testing. FSC/SSC scatter diagram was used for gating to set target yeast, and FL-2/SSC scatter diagram was used to analyze the ratio of inactivated and viable yeast. Candida albicans solution and antifungal solution were mixed, and concentration of the final solution was in the range of 64 μg/ml - 0 μg/ml. The antifungal/yeast cells mixture was cultured at 35°C for 5 hours and centrifuged at 3500 rpm for 5 minutes, then the supernatant was removed, and 200 μl PI with concentration of 50 μg/ml was added. After 15 minutes incubation at ambient temperature, 20 μl sodium deoxycholate with a concentration of 25 mmol/L was added. After another 5 minutes staining, machine detection was started to calculate the ratio of viable yeast. At the same time, 100% viable yeast and 100% inactivated yeast were set for control. 100% viable yeast was treated with 0 μg/ml antifungal solution, and 100% inactivated yeast was treated with 0 μg/ml antifungal solution at first and then treated with 75% ethanol for 10 minutes. For each isolate, the live control tube was read first, with a gating region set to include at least 90% of the yeast cell population. The minimum inhibitory concentration (MIC) was interpreted as the concentration of antifungal agent causing a substantial shift of cells (> 50%) outside of the live gate or a substantial decrease in the number of events per second. A total of 5,000 cells were counted if cells were intact. If yeast cells were disintegrated, counting was performed for approximately 3 minutes to measure the number of events per second [8].

Statistical analysis

All data were analyzed by SPSS 17.0 software and were expressed as mean ± SE. For the comparison of multiple samples, one-way ANOVA was applied, and for pair wise comparison between groups, t test was applied. P < 0.05 was considered to be statistically significant.

Results

Growth curves of Candida albicans under simulated microgravity

Compared with the control group, the Candida albicans SC5314 under modeled microgravity were manifested as the growth curves leftward-shifted, lag phase shortened, along with logarithmic phase and stationary phase forwarded (Figure 1). It was showed that simulated microgravity promoted the growth rate of Candida albicans SC5314 significantly (P < 0.05).

Yeast cells growth of Candida albicans under simulated microgravity

Candida albicans SC5314 was stained by HE and observed by optical microscope on the 7th, 14th and the 21st day of RCCS culture. The study showed that both mycelia and spores of the yeast cells in experimental group were constantly growing with an evident increasing trend, while in control group there were spores growing but rarely mycelia growing (Figure 2). The results suggested that the simulated microgravity had a noticeable affect on the growth status of mycelia and spores and the morpho- genic switches of Candida albicans.

The susceptibility to fluconazole of Candida albicans cultured in RCCS

On the 7th, 14th and 21st day of culture in RCCS, the percentages of viable cells in the experimental group and the control group were assayed respectively by the flow cytometer, which reflected the susceptibility of Candida albicans to fluconazole in different concentrations (64-0 μg/ml) (Figure 3). A statistically significant decrease of yeast cells’ viability was detected in group of simulated microgravity compared with the control group (P < 0.05). The increase of exposure time to simulated microgravity resulted in the decrease of viability of cells accordingly in same drug concentration compared with the control group (Figures 4, 5). Note: RCCS simulated microgravity increases Candida albicans’ susceptibility to fluconazole and the susceptibility take on a rising trend with prolonged culture time.
Candida albicans under simulated microgravity
Candida albicans under simulated microgravity

**Figure 4.** The viability of cells exposed to various concentrations of fluconazole under simulated microgravity compared with the control group. A1-A3. Exposed to 4 μg/ml of fluconazole under simulated microgravity. B1-B3. Exposed to 8 μg/ml of fluconazole under simulated microgravity. C1-C3. Exposed to 16 μg/ml of fluconazole under simulated microgravity. D1-D3. Exposed to 32 μg/ml of fluconazole under simulated microgravity. E1-E3. Exposed to 64 μg/ml of fluconazole under simulated microgravity. F1-F3. Exposed to 4 μg/ml of fluconazole as control. G1-G3. Exposed to 8 μg/ml of fluconazole as control. H1-H3. Exposed to 16 μg/ml of fluconazole as control. I1-I3. Exposed to 32 μg/ml of fluconazole as control. J1-J3. Exposed to 64 μg/ml of fluconazole as control.
Discussion

*Candida albicans* is a common organism that often colonizes the human body shortly after birth, and over 25% of the general population are carriers [9]. It is an important opportunistic pathogen responsible for a variety of cutaneous and systemic human infections in human body, and it could be affected by various stressful environmental factors, including ionic pressure, ultraviolet, microwave, temperature, oxygen concentration, osmotic pressure, pH, oxidative stress injury and anti-fungal medication, etc [4-6]. The weightless environment in space flight is a special stressful status. Researches confirm that during space flight the body's microbial colonies including their translocation, strain variation and pathogenicity underwent numerous alterations [2, 3]. At the same time, the body's defensive mechanism faces a serious of disturbances, and the defensive functions depressed significantly especially during the long journey in space [10]. Thus the opportunistic pathogen like *Candida albicans* may become a potential threat to the health of astronauts [8, 11]. Searles et al. explored the phenotypic responses of *Candida albicans* following exposure to the environment stress of low-shear modeled microgravity and found that upon long-term (12-day) exposure to low-shear modeled microgravity, *Candida albicans* transitioned from yeast to filamentous forms at a higher rate than observed under control conditions. In addition, cells exposed to low-shear modeled microgravity displayed phenotypic switching, observed as a near complete transition from smooth to “hyper” irregular wrinkle colony morphology [8]. Wang et al. confirmed that the pathogenicity of *Candida albicans* treated with cli-nostat was significantly increased and was related with the signal transduction pathway Gβ-Go-AC-cAMP activated by weightlessness [12]. We found in this study that the *Candida albicans* SC5314 under modeled microgravity for 21 days were manifested as the growth curves leftward-shifted, lag phase shortened, along with logarithmic phase and stationary phase forwarded. The results showed that simulated microgravity promoted the growth rate of *Candida albicans* SC5314 significantly. Recently, studies have demonstrated that the microgravity environment put impacts on *Candida albicans* through activating the multiple parallel stressful pathways, including HOG-MAPK, Cek1, Mkc1, lipid signal, Hsp, Cap1p, and other signal transduction and regulation pathways [13, 14]. Among those, the HOG-MAPK is a major stress response pathway for osmotic pressure change, oxidative stress, etc., and meanwhile it involves in the regulation of other signal transduction pathways [14]. Cek1 through proliferation and mycelial growth involves in the forming of yeast wall [15]. During space flight, also under the simulated microgravity, the dynamic process and the fluctuated functional response of microorganisms are particularly complicated, and the specific mechanisms of *Candida albicans'* accelerated growth and proliferation need further study.
The balance between filamentous and yeast forms of Candida albicans is important for virulence due to form-specific contributions to tissue invasion and dissemination, respectively [16, 17]. It was demonstrated in our study that under 21-day RCCS simulated weightlessness the growth ability of mycelia of Candida albicans SC5314 was significantly enhanced and the generation of mycelia was significantly increased along with the prolonged phase of simulated weightlessness. Most scholars believe that simulated weightlessness as a special stress stimulates Candida albicans in the change of morphologic genes, promotes its dimorphic transition and further enhances the pathogenicity [8, 17]. Altenburg et al. argue that the simulated weightlessness significantly increases the expression of specific gene HWP1 of Candida albicans mycelia and significantly decreases the expression of specific gene YWP1 of yeast [17]. Searles et al. confirm that under simulated weightlessness the expression of Candida albicans genes ALS1, ALS3, BCR1 and TEC1 is significantly increased, which promotes the growth of mycelia [8]. Notably, the switching from commensally to pathogenic phase has been widely thought to be associated with the phenotypic plasticity of Candida albicans. It can grow in several morphological forms including unicellular yeast-form, elongated hyphae and pseudohyphae. Filamentous cells are more invasive and better at tissue penetration, while yeast cells are easy to be delivered and disseminated in the bloodstream [18]. Thus, the responses of Candida albicans to the modeled microgravity environment may inform a better understanding of yeast virulence that may be potentially encountered in vivo.

The researches found that the colony morphology alterations are consistent with increased antimicrobial resistance [19, 20]. Therefore, we further explored the effects of RCCS on antifungal resistance using flow cytometry. A statistically significant decrease of yeast cells’ viability was detected in the group of simulated microgravity compared with the control group, and the increase of exposure time to simulated microgravity resulted in the decrease of viability of cells accordingly in same drug concentration compared with the control group. Our study showed that RCCS simulated microgravity increased the Candida albicans’ susceptibility to fluconazole, and the susceptibility takes on a rising trend with prolonged culture time. Interestingly, our findings were different somewhat with that reported previously by Searles et al. that the increased time of exposure to low-shear modeled microgravity resulted in enhanced resistance to amphotericin B relative to cells in the comparable control conditions [8]. We speculate that the disparity may be due to the various designs of the studies including antifungal drugs selected, different modeled microgravity environment and rapid adaptation to weightlessness stress of Candida albicans strains. The commonly used antifungal agents at present are azole, allylamine, organic acid and polyene antibiotics. We selected fluconazole which is the representative of triazole for systemic fungal infection, and its susceptibility was determined in mycelial phase of Candida albicans. It was proved that the different susceptibility to relative drugs was existed between the yeast phase and the mycelia phase of Candida albicans [20, 21]. Together, these data indicate that the understanding of Candida albicans’ virulence, the molecular mechanisms of drugs susceptibility, and antimicrobial resistance in microgravity environment could provide an informed foundation to the medical monitoring and support of spaceflight, as well as provide better understanding of the features and mechanisms involved in host-pathogen interactions.

Disclosure of conflict of interest

None.

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