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A second-order kinetic model on the survival profile of *Candida albicans* in biofilms

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Abstract

The prevalent use of indwelling medical devices has almost paralleled the increasing frequency of fungal infections commonly found in clinical practice. The present study examined *Candida albicans* biofilms on the surfaces of polyvinyl chloride (PVC) endotracheal tube, silicone urinary catheter, and silicone nasogastric tube. The viable *C. albicans* in biofilms was quantified using standard plating procedure. Several kinetic rate equations were employed to describe the survival profile of the viable population. *Candida albicans* exhibited remarkably heterogeneous growth patterns on the different medical devices reflecting variations on its adhesion potential and biofilm formation. A significant difference in the viability of *C. albicans* in biofilms on the surfaces of the medical devices was observed among monitoring points. Survival profile of *C. albicans* in biofilms followed a second-order kinetic model. Quantitative descriptions regarding growth patterns and kinetic profile of the fungus were obtained on these model biofilms. These findings can provide additional information to better understand the complex biology of *C. albicans* and to possibly explain the resistance patterns of fungal biofilms with the existing available antifungal drugs.

Key words – Fungal infections – Medical Devices – Polyvinyl chloride polymer – Silicone polymer – Survival analysis

Introduction

The prevalence of infections due to pathogenic microorganisms including *Candida albicans* has been increasing over the years (Pfaller & Diekema 2007). *Candida albicans* has been observed to thrive in the skin, oral cavity and gastrointestinal tract, respiratory and genitourinary tracts particularly the vulvovaginal and perianal areas (Pfaller & Diekema 2007). However, this fungus has been implicated in various infections particularly candidiasis which can progress from superficial to a more deleterious systemic infection (Rekha & Visyasagar 2013). Increasing cases of candidiasis

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have been observed (Ghannoum & Rice 1999) which was attributed to the use of various indwelling and implant devices (Richards et al. 1999, Shin et al. 2002, Taff et al. 2012). The surfaces of these clinically relevant devices provide substrates for cell adhesion and subsequent biofilm formation (Shin et al. 2002, Mohamed & Al-Ahmadey 2013) resulting in nosocomial infections (Douglas 2003, El-Azizi et al. 2015). Currently, there has been no research conducted which assessed the viability profile of *C. albicans* in biofilms on the surfaces of medical devices. This study quantitatively characterized the kinetic relationship between the viable sessile fungal cells and time. These assessments provide a comparative analysis on the population dynamics of *C. albicans* on medical devices with different chemical compositions. Hence, the present study quantified the viable *C. albicans* population in the biofilms on PVC endotracheal tube, silicone urinary catheter, and silicone nasogastric tube surfaces using standard plating procedure. Furthermore, several kinetic rate equations were employed to describe the growth profile of the viable population. Quantitative descriptions including growth patterns and kinetic profile of *C. albicans* were obtained on these model biofilms on the surfaces of different clinically important medical devices. Findings of this study can provide additional information for a better understanding of the complex biology of *C. albicans* and some possible explanations on the resistance patterns of fungal biofilms with the existing available antimycotics.

Materials & Methods

Biofilm formation of *Candida albicans* on medical devices

An isolate of *Candida albicans* (ATCC 14053) was given by the University of the Philippines Manila (Department of Medical Microbiology, College of Public Health). Subsequently, the organism was grown on Sabouraud dextrose agar (SDA) plates (48 h incubation at 37°C) prior to infection. The inoculum was prepared following the protocol of Andes et al. (2004) with slight modifications. Three distinct colonies were suspended into 10 mL sterile distilled water. A serial dilution technique was applied to determine the density of the inoculum and its viable fungal count was confirmed using SDA.

Modified methods of Hawser & Douglas (1994) and Chandra et al. (2001) were employed for growing biofilms. Disks (0.16 cm^2: 0.2 cm x 0.8 cm) from the PVC endotracheal tube (ID: 7.5 mm, OD: 11.1 mm, Sacett, Portex), silicone urinary catheter (Fr18, Surgitech+, Fujian Bestway Medical Polymer Corp.), and silicone nasogastric tube (Fr5, Medline®, NeoMed, Inc.) were prepared. The prepared disks were sterilized, oven-dried, and individually placed in 96-well microtiter plates. The disks were charged with inoculum (100 μL) and 50 mM glucose (200 μL) and then incubated at 37°C for a period of 72 h to allow biofilm formation.

Viability and kinetic profile of *Candida albicans* in biofilms

Since fungal biofilm formation has been observed as early as 4 h (Sumalapao et al. 2018), serial monitoring (4-72 h) of the viable population of *C. albicans* in biofilm was done. Microscopic examination of the biofilm formation was performed. For the viability evaluation (Sumalapao et al. 2018), the disks were washed with 0.15 M PBS (5 mL). To remove adherent sessile cells, disks were immersed into a 10 mL sterile distilled water, vortexed for 15 s. To quantify the viable sessile fungal count, the suspension was plated on SDA and incubated at 37°C for 24 h. Each test was performed in triplicates. The results were reported as mean ± standard deviation (log_{10} CFU/mL).

Numerical calculations and statistical analysis

Viable fungal counts in biofilms were compared using analysis of variance and Bonferroni test. To elucidate the growth rate profile of *C. albicans*, several kinetic equations (Sumalapao et al. 2017) including zero-order, first-order, and second-order models were examined (Table 1). All statistical assessments were calculated using STATA© V12.0 at 5% significance level.

Table 1 Survival kinetic model equations.

Order	Rate Equation	Linearized Form
Zero	$\frac{dP_t}{dt} = c_o$	$P_t = c_0 t + P_o$
First	$\frac{dP_t}{dt} = c_1 P_t$	$\log P_t = \log P_o + \frac{c_1}{2.303} t$
Second	$\frac{dP_t}{dt} = c_2 {P_t}^2$	$\frac{-1}{P_t} = c_2 t + \left(\frac{-1}{P_0}\right)$

P_t ($\log_{10}$ CFU mL^{-1}): population count at time t (h); c_o ($\log_{10}$ CFU mL^{-1} h^{-1}): zero-order rate constant; c_1 ($\log_{10}$ CFU mL^{-1} h^{-1}): first-order rate constant; c_2 (mL $\log_{10}$ CFU^{-1} h^{-1}): second-order rate constant; CFU: colony forming unit

Results

Microscopic examination of *C. albicans* biofilms on the surfaces of medical devices was performed (Fig. 1). The fungus exhibited remarkable heterogeneous growth patterns on the different medical devices reflecting variations on its adhesion potential and biofilm formation. The viable *C. albicans* in biofilms was quantified using standard plating procedure (Fig. 2). Results on the viable count monitoring of *C. albicans* are presented in Table 2. This study has identified that the fungal population increased as the biofilms formed over time. In PVC endotracheal tube, the mean viable population counts on the 24, 36, 48, 60, and 72 h significantly varied from the 4 and 8 h population counts (p<0.05). Moreover, viability counts on the 24-h biofilm significantly differed when compared with 48, 60, and 72 h biofilms (p<0.05), and the viable population counts on 48 h also significantly differed from the 60 and 72 h biofilm formation (p<0.05). For the silicone urinary catheter, a significant change in the viable fungal counts was observed from 36-h biofilm formation when compared with the 4 and 8 h population counts (p<0.05). The viable population counts from 36 h did not significantly differ when compared with the viable counts in the subsequent monitoring points. Viable *C. albicans* population counts on the surface of silicone nasogastric tube significantly differed every 12-h interval from 12 to 60 h biofilm formation, but the population counts did not differ on the 60 and 72 h biofilm formation (Table 2).

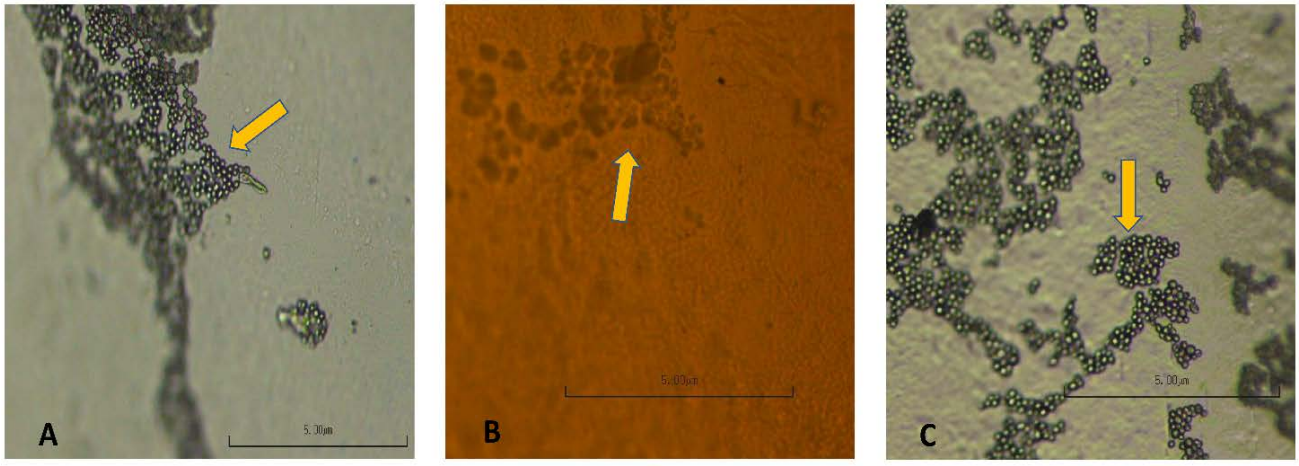

Fig. 1 – Micrographs of *Candida albicans* biofilms on (A) polyvinyl chloride endotracheal tube, (B) silicone urinary catheter, and (C) silicone nasogastric tube surfaces. Structures in yellow arrows indicate 24-h biofilm formation viewed using light microscopy at high power objective.

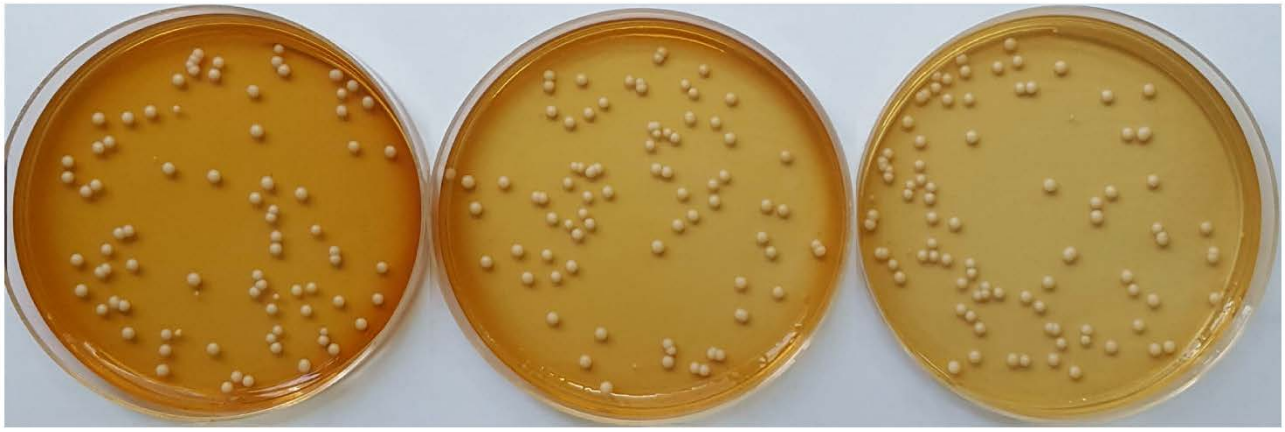

Fig. 2 – Morphological appearance of *Candida albicans* colonies in Sabouraud dextrose agar plates, 24 h incubation at 37°C.

Table 2 Viable *Candida albicans* cell count in biofilms on the surfaces of different medical devices at different monitoring intervals.

	Viable population count (mean ± standard deviation, log_{10} CFU/mL)		
Time (h)	**Polyvinyl chloride endotracheal tube**	**Silicone urinary catheter**	**Silicone nasogastric tube**
4	2.985 ± 0.009^{a}	3.780 ± 0.032^{a}	3.139 ± 0.008^{a}
8	3.028 ± 0.024^{a}	3.787 ± 0.029^{a}	3.156 ± 0.009^{ab}
12	3.048 ± 0.011^{ab}	3.806 ± 0.027^{ab}	3.168 ± 0.007^{b}
24	3.132 ± 0.049^{bc}	3.832 ± 0.026^{ab}	3.351 ± 0.010^{c}
36	3.226 ± 0.020^{cd}	3.893 ± 0.036^{bc}	3.395 ± 0.005^{d}
48	3.304 ± 0.016^{d}	3.964 ± 0.045^{c}	3.430 ± 0.003^{e}
60	3.799 ± 0.054^{e}	3.968 ± 0.034^{c}	3.849 ± 0.007^{f}
72	3.812 ± 0.040^{e}	3.967 ± 0.038^{c}	3.849 ± 0.013^{f}

Values containing similar superscript letters in a given column do not differ at 5% significance level. CFU: colony forming units

When the kinetic survival of *C. albicans* biofilm on the surfaces was assessed using the zero-order rate equation, the viable fungal counts on the surface of silicone urinary catheter behaved in accordance to this model (R^2=0.9347, Table 3). The doubling time of the fungal population in biofilm is approximately 102 h (4.25 d) under the zero-order kinetic model. Further examination on the survival profile of fungal populations, the first-order kinetic model generated a better fit of the experimental data as justified by higher coefficients of determination and smaller error values (Table 3). Fungal biofilm populations are expected to have doubling time of 1 d for PVC endotracheal tube, 4 d for silicone urinary catheter, and 11 d for silicone nasogastric tube under the first-order kinetic rate equation. However, when the viability of *C. albicans* in biofilms was examined using the second-order kinetic model, estimates of the parameters showed coefficients of determination (R^2) closest to unity: 0.9266, 0.9354, and 0.9359 for PVC endotracheal tube, silicone urinary catheter, and silicone nasogastric tube, respectively (Table 3). These findings suggest that the survival of *C. albicans* in biofilms on medical devices behaved in accordance with the second-order kinetic model. These results are further justified by the smallest sum of squares of the error measures and lowest *p*-values (Table 3).

Table 3 Estimates of the parameters describing the survival of *Candida albicans* in biofilms on the surface of medical devices.

Kinetic Model	Parameter	Polyvinyl chloride endotracheal tube	Silicone urinary catheter	Silicone nasogastric tube
Zero-order	c_0	82.6051	56.3283	86.6085
	P_o	-95.5516	5.77e3	3.71e2
	R^2	0.7809	0.9347	0.8179
	SSE	8.56e6	9.91e5	7.47e6
	P	0.0036	0.0001	0.0020
First-order	c_1	0.0289	0.0074	0.0025
	P_o	7.56e2	5.88e3	1.34e3
	R^2	0.8981	0.9357	0.9185
	SSE	0.0799	0.0032	0.0476
	P	0.0003	0.0001	0.0002
Second-order	c_2	-0.0011	-0.0002	-0.0009
	P_o	2.9181	3.7711	3.0823
	R^2	0.9266	0.9354	0.9359
	SSE	4.31e-4	1.40e-5	2.54e-4
	p	0.0001	0.0001	0.0001

c_o: zero-order constant; c_1: first-order constant; c_2: second-order constant; *SSE*: sum of squares of the error; *p*: p-value; R^2: coefficient of determination; P_0: initial population.

Discussion and Conclusion

Candida is the most common genus of fungi that colonizes medical devices by forming resilient biofilm (Desai et al. 2014). The increasing use of implanted medical devices enhances the device-associated infections, including fungal biofilm formation. With more than one million reported cases of nosocomial infections resulting from the prevalent use of urinary catheters (Nett et al. 2014), *Candida* spp. were identified as the causative agents of at least 25% of these urinary infections (Fisher et al. 2011). In intensive care unit patients, the most common infective nosocomial cause of mortality is ventilator-associated pneumonia (Mariyaselvam et al. 2017), and the typical fungus found on the surface of the PVC endotracheal tubes used in ventilation is *C. albicans* (Machado & Webster 2017). *Candida albicans* adheres on the surface of medical devices including silicone nasogastric tubes which are used for patients with complications such as poor voluntary intake or gut dysfunction to facilitate nutrient uptake (Blumenstein et al. 2014). In this study, we have presented that the growth of *C. albicans* in biofilms on the surfaces of different medical devices has a survival kinetic profile in accordance with the second-order rate equation. The organism exhibited faster adhesion and subsequent proliferation on the surface of silicone urinary catheter when compared to that of PVC endotracheal tube and silicone nasogastric tube. In general, the growth profile exhibited a linear pattern until 24 h after inoculation of *C. albicans* and increased exponentially from 24 to 48 h. These quantitative assessments provide a comparative analysis on the population dynamics of *C. albicans* on medical devices with different chemical compositions. *Candida albicans* has been shown to survive in the biofilms on the surfaces of medical devices and their counts increased slightly over time. Our study has identified the increasing viable fungal population which behaved in a nonlinear manner simulating an exponential growth under a second-order kinetic equation.

In the assessment of the viability of the fungal population on these biofilms, our study employed the standard plate method. In most researches, plate counting is used frequently in assessing the viability of microorganisms (Sumalapao et al. 2017). However, with the cell viability complex nature, whether cells are culturable or not in the chosen growth medium and specified conditions (Sumalapao et al. 2017), fungal cells that are not culturable *in vitro* but are active, can still have some possible detrimental health effects. Hence, there is a need for a more advanced and appropriate method to explore. Due to the complex effects of diversed fungal cell populations, an

advanced method can provide more accurate viability quantification, generate better parameter estimates, and have more defined descriptions of the fungal population dynamics.

In conclusion, *C. albicans* biofilms on the surfaces of different medical devices have a complex architecture and heterogeneous structure. A significant difference in the viability of *C. albicans* in biofilms on the surfaces of the medical devices was observed among monitoring points. Growth profile on the survival of *C. albicans* followed a second-order kinetic model. The fungus exhibited remarkable heterogeneous growth patterns when monitored in varying medical devices reflecting its variability on adhesion potential, biofilm morphology, and survival profile. Furthermore, this study on biofilms can provide additional information for a better understanding of *C. albicans* complex biology and some plausible explanations on its resistance patterns with the existing available antifungal drugs.

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