Scientific Reports

https://doi.org/10.1038/s41598-026-42366-6

Article in Press

Povidone iodine demonstrates strong efficacy in reducing Candida biofilm in an in vitro fungal prosthetic infection

Received: 3 March 2025

Accepted: 25 February 2026

Published online: 11 March 2026

Cite this article as: Hong J., Moon Y.G., Choi S.K. *et al.* Povidone iodine demonstrates strong efficacy in reducing Candida biofilm in an in vitro fungal prosthetic infection. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-42366-6

Jae-Young Hong, Yong Gyun Moon, Soo Kyung Choi, Hyoung Tae Kim & Suenghwan Jo

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

ARTICLE IN PRESS

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

ARTICLE IN PRESS

Povidone Iodine demonstrates strong efficacy in reducing Candida biofilm in an in vitro fungal prosthetic infection

Jae-Young Hong[1], Yong Gyun Moon[1], Soo Kyung Choi, PhD[1], Hyoung Tae Kim, MD[2], Suenghwan Jo, MD, PhD[1,2,*]
[1]Chosun University, College of Medicine [2]Chosun University Hospital, Department of Orthopedic Surgery
*Corresponding author, jo.suenghwan@chosun.ac.kr

Abstract

Periprosthetic joint infection (PJI) caused by fungi is rare but can lead to devastating outcomes. However, the best treatment for fungal PJI has not been established. This study aims to identify the optimal surgical irrigation solution to reduce the bioburden of Candida biofilm from prosthetic surfaces. *Candida albicans* biofilm was cultured on titanium screw caps and treated for 3 minutes and 10 minutes using five clinically used irrigation solutions: Normal Saline, Fluconazole solution (2000 µg/mL), Nystatin solution (5000 IU/mL), Chlorhexidine (0.05%), Povidone Iodine (0.35%), and Povidone Iodine (10%). The biofilm on the screw caps was quantified by counting colony-forming units after sonication to assess the effectiveness of the irrigation solutions. Irrigation with normal saline resulted in $5.9 \pm 1.6 \times 10^4$ CFU/mL of *Candida albicans* per titanium screw cap. As compared to the normal saline, the most effective irrigation solution at 3 minutes was PI 10%, achieving over a 2-log reduction in CFU/ml. Other chemical irrigation solutions resulted in approximately a 1-log reduction. Extending the irrigation time to 10 minutes led to a further reduction in biofilm, with PI 0.35% and PI 10% achieving remarked removal. These findings suggest that PI 10% is the most effective irrigation solution for treating Candida-induced PJI when applied for 3 minutes. However, its efficacy is time dependent, and prolonged irrigation can achieve clinical significance even at lower concentrations.

ARTICLE IN PRESS

ARTICLE IN PRESS

Introduction

Periprosthetic joint infection (PJI) caused by fungal pathogens represent only 1% of total PJIs but can result in significant treatment challenges and devastating outcomes[1,2]. The development of biofilm by adherence of microorganisms on the surfaces of orthopaedic implant is a central feature in the pathogenesis of PJI[3]. This biofilm, through an extracellular polymeric matrix, protects the pathogens from the immune system, antibiotic therapy, and mechanical debridement, making eradication particularly difficult[4,5].

ARTICLE IN PRESS

Intraoperative irrigation solution is an important tool that surgeons use to combat contamination and lower bacterial loads during surgery. These solutions typically consist of antibiotics or diluted antiseptics that are applied to the surgical wound after mechanical debridement of damaged tissue. While antibiotics are widely relied upon, antiseptic solutions have seen renewed interest in total joint arthroplasty, with research showing they can effectively reduce planktonic bacteria and retrospective clinical studies linking their use to lower rates of PJIs with minimal side effects[6-8]. Besides preventing PJIs, antiseptic solutions are frequently used in DAIR (debridement, antibiotics, and implant retention), as well as 1-stage and 2-stage revision surgeries to remove bacterial bioburden from surfaces like titanium alloy, polymethylmethacrylate, and plastic[9,10]. However, while existing research has demonstrated the efficacy of various antiseptic solutions against bacterial biofilms, there is a notable gap in studies focusing on fungal biofilms[4,11].

Candida albicans is a key pathogen in fungal PJI, known for its ability to form complex biofilms, which contribute to its resilience against antifungal

ARTICLE IN PRESS

treatment. Although not necessarily the majority, it is one of the more frequently identified species in fungal PJI cases, making it a significant target for this study[12]. The notorious nature of *Candida albicans* stems from its ability to transit between yeast and hyphal forms, a process central to its virulence, and its association with persistent and recurrent infections, particularly in patients with implanted medical devices[13,14].

The aim of this study is to identify the optimal irrigation solution for removing fungal biofilm, and to clarify the temporal development and morphology of the fungal biofilm, focusing on *Candida albicans* that can eventually improve clinical outcomes of patients with fungal PJIs.

Materials and Methods

ARTICLE IN PRESS

Antiseptic Solutions

Five irrigation solutions and Normal saline(for control) were selected for their relevance to intraoperative use: normal saline, nystatin 5000 IU/mL, fluconazole 2000 µg/mL, chlorhexidine gluconate 0.05%, povidone iodine (PI) 0.35%, and PI 10%[15]. The description of each solution is listed in Table 1.

Table 1. Irrigation Solutions tested

Agent(final concentration)	Brand / formulation(initial concentration)	Preparation Method*
Normal Saline	JW Pharmaceutical Co., South Korea (0.9% NaCl)	Used as is, no modification
Nystatin(5,000 IU/mL; 876-1087 µg/mL)**	Pharmascience Inc., Canada (oral suspension, 100,000 IU/ml)	Diluted 1:20 with normal saline
Fluconazole(0.2%; 2,000 µg/mL)	Pfizer Inc., USA (IV solution, 2 mg/ml)	Used as is, no modification
Chlorhexidine Gluconate(0.05%; 500 µg/mL)	Green Pharma. Co., France (2% solution)	Diluted 1:40 with normal saline
Povidone Iodine (10%; 100,000 µg/mL)	Green Pharma. Co., France (10% solution)	Used as is, no modification
Povidone Iodine (0.35%; 3,500 µg/mL)	Green Pharma. Co., France (10% solution)	Diluted 1:28.6 with normal saline

*All solutions were prepared according to the manufacturers' specifications to ensure consistency across all samples. In cases where the manufacturers' guidelines were not compatible with the experimental conditions, adjustments were made based on the manufacturers' guidance.

**Nystatin activity was expressed in International Units (IU). The commercial suspension contained 100,000 IU/mL and was diluted 1:20 to obtain a final concentration of 5,000 IU/mL, which corresponds to approximately 876–1087 µg/mL based on CLSI standards(4,600-5,710 IU/mg).

ARTICLE IN PRESS

Nystatin was chosen for its specific action against fungal biofilms. It works by binding to sterols in the fungal cell membrane, which disrupts the membrane's integrity. This disruption increases the membrane's permeability, leading to cell death. Nystatin is particularly effective in breaking down the complex structures of fungal biofilms[16].
Fluconazole is commonly used to prevent or treat fungal infections. It targets the synthesis of ergosterol, an essential component of fungal cell membranes. By inhibiting ergosterol production, fluconazole compromises the cell membrane, which is crucial for the survival of fungal cells[17,18]. Fluconazole was supplied as a powdered formulation for intravenous use and was reconstituted with 50 mL of normal saline according to the manufacturer’s instructions, yielding a fully solubilized solution with a final concentration of 2,000 µg/mL.
Chlorhexidine gluconate is a cationic bisbiguanide. It binds to negatively charged bacterial cell walls, disrupting their osmotic balance. This disruption leads to cell death. Chlorhexidine has broad-spectrum antimicrobial activity, making it effective against a wide range of pathogens responsible for PJI, including fungi[18,19].
Povidone iodine(PI) is known for its broad antibacterial spectrum and high efficacy and is commonly used in the surgical field. It has no reported bacterial resistance, making it a cost-effective option for irrigation during surgical procedures. The study utilized two concentrations of PI 0.35% and 10% for fungal biofilm. The 0.35% concentration is the most common clinically used, while the 10% concentration is the undiluted form of commercially available PI[9,20,21].

ARTICLE IN PRESS

Implant materials

The orthopaedic material used in the current study to grow fungal biofilm is Ti6Al4V screw hole caps retrieved from G7 acetabular components (catalogue #010000995, G7 acetabular component, Zimmer-Biomet, Warsaw, Indiana, USA). Prior to biofilm formation, the screw caps were cleansed with 70% ethanol followed by sonication (BRANSON 1600, Emerson, St. Louis, Missouri, USA) for 30 minutes. The caps were subsequently sterilized in an autoclave at 120°C for 20 minutes[22]. A total of 18 titanium screw caps were used in this study(3 samples per each group), and all experiments were performed in 3 independent replicates.

ARTICLE IN PRESS

Fungal cultivation

Candida albicans (KCCM 11282, KisanBio), a strain known for its robust biofilm-forming capabilities and clinical relevance, was selected[23]. Lyophilized ampoules of *Candida albicans* were reconstituted according to the manufacturer's instructions[24]. A *Candida albicans* glycerol stock was prepared by adding 5 mL of Sabouraud Dextrose(SD) broth into the dry-frozen yeasts followed by mixing sterilized glycerol at a final concentration of 50% and then stored at -80°C. To determine the exponential growth phase of *Candida albicans*, 20 µL of *Candida albicans* glycerol stock was inoculated into 20 ml of SD broth and incubated in incubator at 37°C for 72 hours. Optical density (OD) at 600 nm was measured over the 72-hour period, with results indicating that *Candida albicans* reached its exponential growth phase at 24 hours. (supplementary data 1)

Minimal inhibitory concentration of irrigation solutions on planktonic fungus

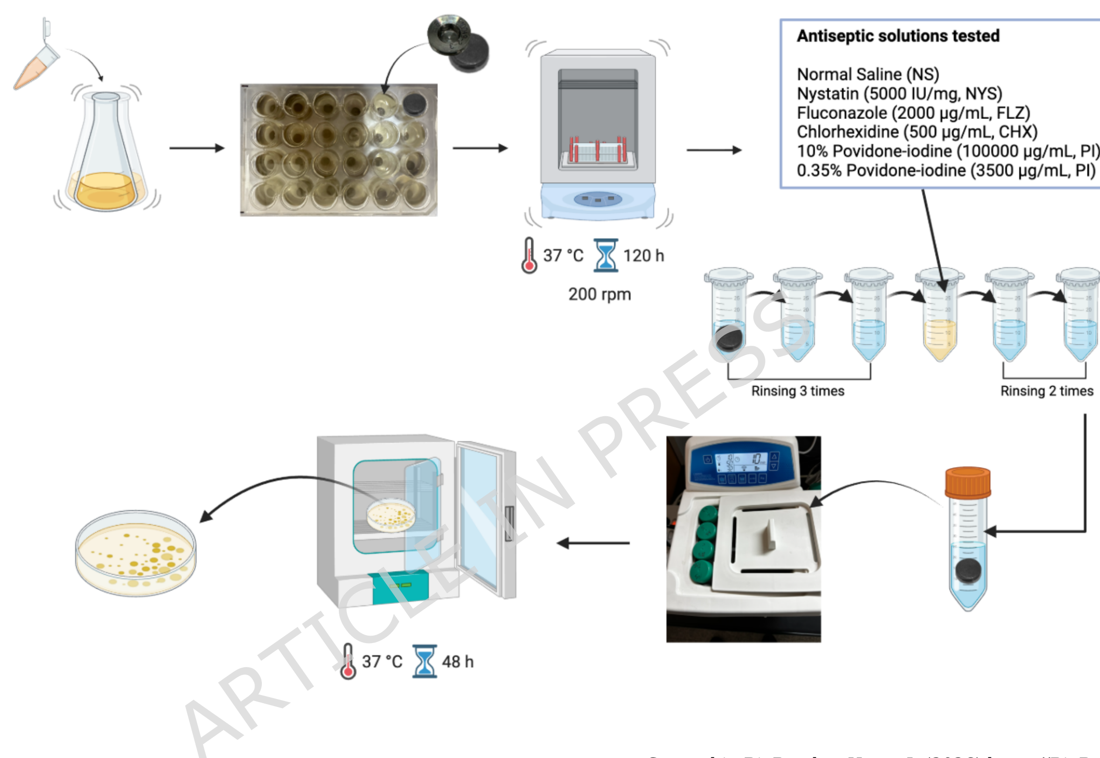
Minimum inhibitory concentration (MIC) of each antiseptic was determined against planktonic *Candida albicans* using microdilution assay. *Candida albicans* from a freshly streaked SD agar plate was suspended in 5 mL of SD broth, grown overnight in a 37°C, subcultured, and grown to a density of 4×10^{8} CFU/mL. This suspension was diluted in SD broth to 10^{4} CFU/mL. To determine the MIC, 20 µL of the diluted fungal suspension was added to each well of a 96-well plate. The wells were then filled with 100 µL of different concentrations of the antiseptic solutions, ensuring that each solution and concentration was tested in triplicate. The plate was incubated at 37°C for 24 hours. During this period, the OD at 600 nm was measured at regular intervals(4 hours) to monitor fungal growth. The MIC was identified as the lowest concentration at which no significant increase in OD was observed, indicating growth inhibition. This pattern of growth inhibition was cross-verified by plating aliquots from each well on SD agar plates and counting CFUs after overnight incubation. This dual approach ensured the accuracy of the MIC determination[25].

Biofilm formation

ARTICLE IN PRESS

ARTICLE IN PRESS

In order to grow the fungal biofilm, 1 mL aliquots of the *Candida albicans* at exponential phase were dispensed into each well of a 24-well plate, each containing a titanium screw cap facing the porous side up. The 24-well plate was then incubated at 37°C, 200 rpm in a shaking incubator for 120 hours to allow biofilm formation on the screw caps. The broth was changed every 48 hours[26]. The protocol for developing fungal biofilm is summarized in figure 1.



ARTICLE IN PRESS

Created in BioRender. Hong, J. (2026) https://BioRender.com/4l36wgl

Fig 1. Workflow for Biofilm Formation, Irrigation, and CFU counting on Titanium Screw Caps

Candida albicans was cultured to its exponential growth phase in Sabouraud Dextrose broth. The grown culture was then mixed with fresh Sabouraud Dextrose broth and inoculated into individual wells of a 24-well plate, each containing a titanium screw cap. The plates were incubated in a shaking incubator at 37°C and 200 rpm for 120 hours to facilitate biofilm formation on the screw caps. Titanium screw caps subsequently underwent treatment with various antiseptic solutions, including normal saline, fluconazole, nystatin, chlorhexidine gluconate, povidone-iodine (0.35% and 10%). The screw caps were submerged in the1 mL of irrigation solutions for either 3 minutes or 10 minutes, followed by rinsing with normal saline to remove any residual solution.

Scanning electron microscopy preparation

ARTICLE IN PRESS

The formation and the structure of the *Candida albicans* biofilm on titanium screw caps was validated with use of Scanning Electron Microscopy (SEM, FE-SEM SU8600, Tokyo, Japan). After culturing biofilm on the titanium screw caps, six samples were carefully rinsed with phosphate-buffered saline (PBS) to remove planktonic fungus. The samples were then fixed in 2.5% glutaraldehyde at 4°C for 16 hours to preserve cellular structures. Following fixation, the samples were washed three times with PBS and dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, 100%, 100%) for 10 minutes each. Critical point drying was performed to avoid the collapse of the biofilm structure due to surface tension. The dried samples were mounted on aluminium stubs and coated with a thin layer of gold-palladium to render them conductive. Imaging was carried out using a scanning electron microscope at an accelerating voltage of 5-15 kV. Images were captured at various magnifications to observe the overall biofilm architecture as well as the detailed morphology of the cells and the extracellular matrix[27]. The same preparation and imaging procedures were applied to six additional samples following irrigation with 10% povidone-iodine, allowing qualitative comparison of biofilm morphology before and after antiseptic treatment.

Effect of irrigation solutions on Fungal Biofilm

After the incubation period, the screw caps were carefully removed from the wells and were gently rinsed 3 times with PBS to remove non-adherent fungus. This process was repeated for each sample to ensure consistency. Samples were then submerged into 1mL of antiseptic solutions for 3 or 10 minutes and then rinsed 2 times with PBS. The additional 10 minutes submersion was intended to simulate extended irrigation time during surgery, with the hypothesis that increased exposure might enhance the antimicrobial efficacy. After the submersion in antiseptic solutions, an additional rinsing with PBS was performed to remove any residual irrigation solutions. To detach the fungal biofilm from the screw caps, the samples were sonicated at 40 kHz for 10 minutes. The sonicated suspension was serially diluted 10 folds in PBS and cultured on SD agar plates at 37°C for 48 hours. The number of colony-forming units (CFUs) was counted to quantify the remaining biofilm (Figure 1).

To visually confirm the biofilm-disrupting effect observed in the quantitative assay, titanium screw caps irrigated with 10% PI, which

ARTICLE IN PRESS

ARTICLE IN PRESS

demonstrated the greatest biofilm reduction, were subjected to SEM analysis.

Statistical analysis

All experiments were performed in triplicate and repeated three times to ensure the reliability and reproducibility of the results. The CFU counts of *Candida albicans* biofilms formed on titanium screw caps after irrigation with antiseptic solutions were reported as means with standard errors. The comparison among the five irrigation solutions (povidone-iodine, normal saline, chlorhexidine, nystatin, and fluconazole) was made using two-way analysis of variance (ANOVA) followed by Dunnett's post hoc test in IBM SPSS Statistics (version 29.0.1.1, IBM Corp., Armonk, NY, USA), with normal saline serving as the control. A *P*-value less than 0.05 was considered statistically significant, while mean value differences compared to normal saline exceeding 3 logs were considered clinically significant.

ARTICLE IN PRESS

Results

MIC results

Results showed that all tested concentrations of the antiseptic solutions used in the study were effective against planktonic *Candida albicans*, as indicated by the MIC values determined through OD measurements and CFU counts. However, the determination of the MIC for nystatin is challenging using optical density (OD)-based methods due to its inherent properties. In a previous study, nystatin MIC values for *Candida albicans* isolates have been observed in the range of 0.625 to 1.25 µg/mL, as measured by microdilution techniques with resazurin dye after 24–48 hours of incubation at 36°C[28]. These results confirm both the antifungal efficacy of the solutions and the appropriateness of the concentrations employed in this study(supplementary data 2, Table 2).

Table 2. Baseline minimum inhibitory concentration values for each solution against *Candida albicans*

Agent	MIC (µg/ml)

ARTICLE IN PRESS

Nystatin	0.625 - 1.25*
Fluconazole	1000 - 2000
Chlorhexidine Gluconate	1250 – 2500
Povidone Iodine	1250 - 2500

*Due to limited solubility and tendency to form precipitates often produce baseline turbidity, making OD-based MIC measurements are unreliable. Consequently, we did not determine the MIC for nystatin using the OD-based method in this study.

Effects of irrigation solutions on Fungal Biofilm

The in vitro experiments demonstrated significant variability in the efficacy of the tested antiseptic solutions against *Candida albicans* biofilm in different duration which is unspecified in fungi[29].

For the group of 3 minutes irrigation, normal saline resulted in mean 5.9×10^4 CFU/mL per screw cap. Following treatment, the antifungal agents nystatin 5000 IU/mL and fluconazole (2000 μg/mL) exhibited limited efficacy, achieving less than a 1-log reduction(90% removal). Chlorhexidine gluconate 0.05% demonstrated moderate effectiveness, providing a 1-log reduction and reducing the biofilm to 3.8×10^3 CFU/mL. Povidone-iodine 0.35% further reduced the biofilm by 1-log reduction to 2.5×10^3 CFU/mL. Notably, the most effective solution was Povidone-iodine 10%, which achieved a reduction of more than 2-log reduction($P < 0.05$ vs NS)(Figure 2).

ARTICLE IN PRESS

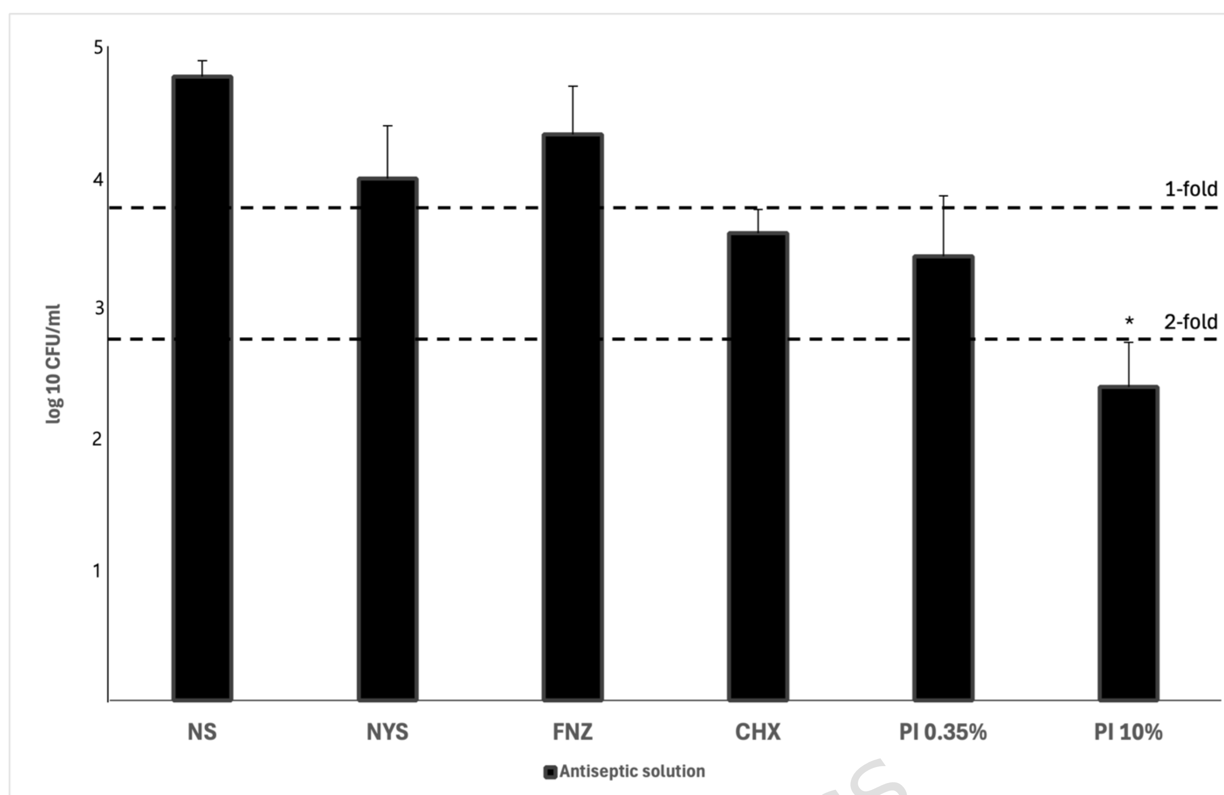


Fig 2. CFU/ml *Candida albicans* after 3 minutes of irrigation with different solutions
The solutions tested include Normal Saline(NS) (control), Nystatin(NYS) 5000 IU/mL, Fluconazole(FNZ) 2000 µg/mL, Chlorhexidine(CHX) 0.05%, Povidone Iodine(PI) 0.35%, and Povidone Iodine 10%. The dashed lines represent the thresholds for 1-log (1-fold) and 2-log (2-fold) reductions in CFU/ml compared to Normal Saline. A single asterisk (*) denotes a statistically 2-fold reduction, showing PI 10% the most effective.
Values are plotted as log10(CFU/mL); raw CFU/mL values are provided in the text.
Data are presented as mean ± SEM (n = 6)and plotted as log10(CFU/mL).

ARTICLE IN PRESS

The extended 10 minutes submersion resulted in further reductions in biofilm density across all tested solutions. Normal saline, serving as the control, resulted in a biofilm density of approximately 2.8×10^4 CFU/mL. Among the antifungal agents, nystatin 5000 IU/mL and fluconazole (2000 µg/mL) achieved similar reductions to 3 minutes submersion, indicating limited efficacy. Chlorhexidine gluconate 0.05% demonstrated improved effectiveness with a biofilm density reduction to 2.0×10^2 CFU/mL, resulting more than a 2-log reduction. PI achieved near complete removal of the biofilm in both 0.35% and 10% concentration($P < 0.05$ vs NS). This finding suggests that prolonged exposure significantly enhances the antifungal activity of the irrigation solutions, particularly for PI (Figure 3).

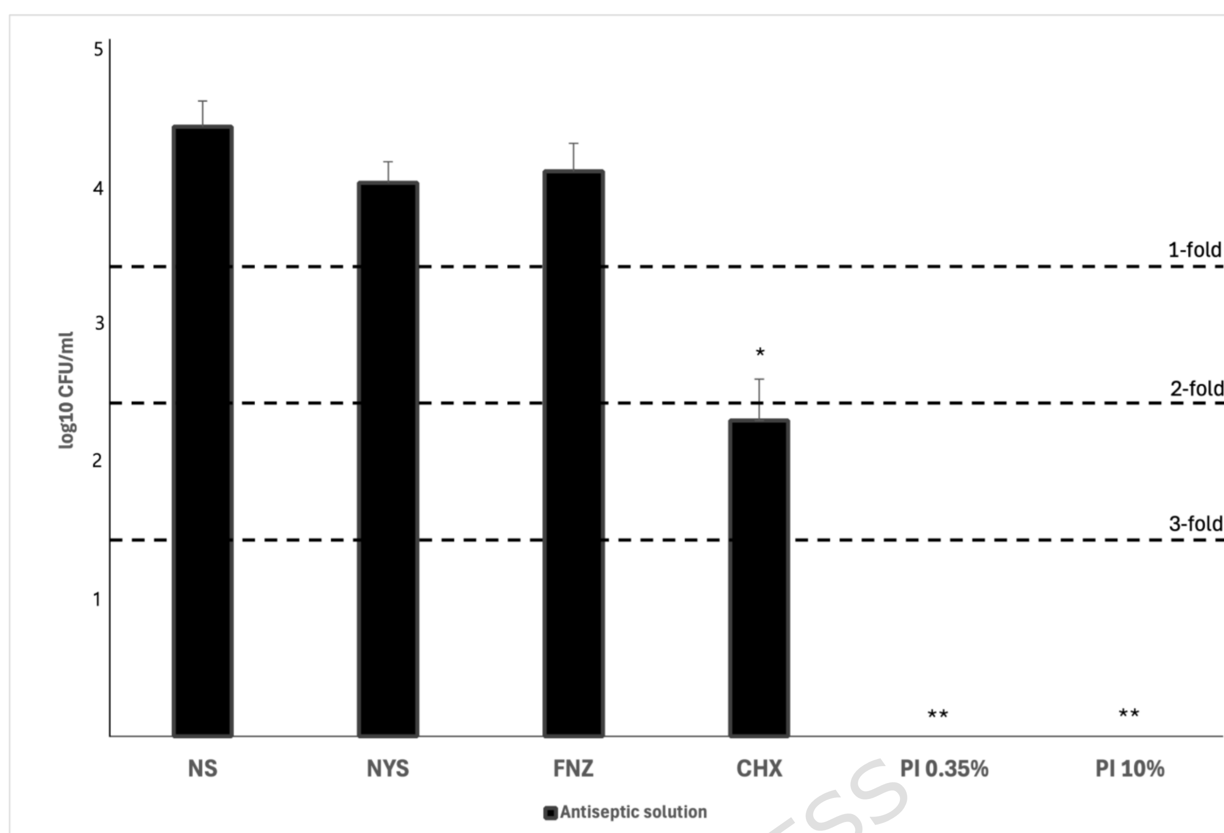


Fig 3. CFU/ml *Candida albicans* after 10 minutes of irrigation with different solutions
An extended submersion of 10 minutes revealed that antifungal agents were not significantly influenced by the prolonged exposure time. In contrast, chemical solutions exhibited a substantial effect in removing biofilm. Notably, Povidone-Iodine (PI) achieved nearly 100% biofilm removal. The dashed lines in the figure represent thresholds for 1-log, 2-log, and 3-log reductions in CFU/ml compared to Normal Saline. A single asterisk (*) indicates a statistically significant 2-log reduction, while a double asterisk (**) represents a 3-log reduction, highlighting PI's effectiveness with increased exposure time.
Values are plotted as log10(CFU/mL); raw CFU/mL values are provided in the text.
Data are presented as mean ± SEM (n = 6) and plotted as log10(CFU/mL).

ARTICLE IN PRESS

Validation of biofilms with SEM

Through the 120-hours-cultivation, SEM images revealed a dense biofilm structure composed of yeast cells and pseudohyphae embedded in an extracellular polymeric matrix. These high-resolution images highlighted that *Candida albicans* biofilm form at least in 120 hours and the complex architecture and surface adherence of the biofilm, confirming its formation on the titanium screw caps. The SEM analysis revealed extensive biofilm formation on the screw caps, particularly on the roughened surfaces, indicating the propensity of *Candida albicans* to thrive in these areas (Figure 4).

ARTICLE IN PRESS

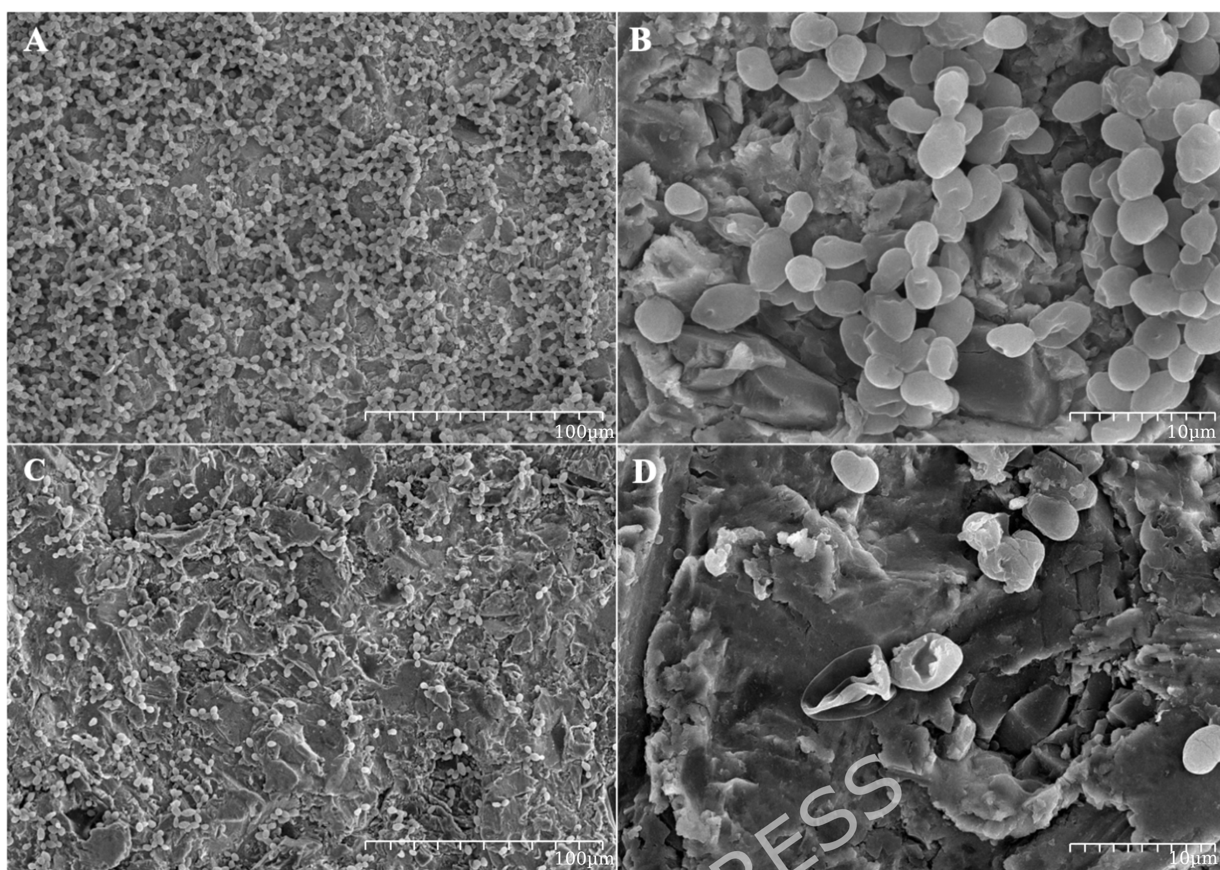


Fig 4. SEM Image of Biofilm Formation(Figure 4A,4B) & After 10 minutes irrigation of 10% Povidone-Iodine(Figure 4C,4D) on Titanium Screw Cap.

Scanning Electron Microscopy image showing the surface of a titanium screw cap after 120 hours of incubation with Candida albicans in a shaking incubator. The image reveals the formation of a dense biofilm on the screw cap porous surface(Figure 4A, 4B). In Figure 4C, 10 minutes of irrigation showed significant reduction of *Candida albicans* biofilm. Especially, in 10μm scale under SEM, *Candida albicans* appeared deformed and did not maintain its normal morphology(Figure 4D). Images were acquired at a magnification of ×500 (A, C) and ×3000 (B, D).

ARTICLE IN PRESS

Upon confirmation of fungal biofilm formation, an additional SEM image was obtained from the screw cap irrigated with 10% PI for 10 minutes, which was hypothesized to have the most potent effect. The image demonstrated a significant reduction of biofilm (Figure 4C) and *Candida albicans* with disrupted surface morphology (Figure 4D). However, some *Candida albicans* retaining normal morphology were also observed, although they appeared to have lost their bioactivity. This phenomenon has been reported in several studies, where the external structure of fungal cells remains intact while their intracellular processes and metabolic activity are significantly compromised[30].

ARTICLE IN PRESS

Discussion

This study evaluated the efficacy of antiseptic solutions against *Candida albicans* biofilm on titanium surfaces and found that PI was the most effective agent. While a 3-minute exposure resulted in only limited biofilm reduction, extending the irrigation time to 10 minutes with PI achieved near-complete removal, whereas conventional antifungal agents showed minimal efficacy. These findings underscore both the superior potency of PI and the critical role of contact time in optimizing intraoperative biofilm eradication strategies.

The ideal irrigation solution for intraoperative use in total joint arthroplasty should act rapidly, exhibit broad-spectrum bactericidal and fungicidal activity, and must not induce local or systemic toxicity[31]. As current perioperative prevention techniques are effective against planktonic bacteria, managing PJI, particularly in the presence of biofilms remains a significant challenge. Effective biofilm eradication is crucial, as incomplete removal can lead to severe complications, including limb- or life-threatening outcomes.

Our findings revealed that with a 3-minutes application, PI 10% was the most effective solution. Although it did not reach the Clinical and Laboratory Standards Institute (CLSI) definition of ≥3-log threshold for clinically significant remarked suppression of biofilm growth, PI 10% reduced biofilm density by more than a 2 log CFU. A 10-minute extended submersion significantly enhanced the antimicrobial efficacy of PI, resulting a clinically significant removal in both 0.35% and 10% concentration. In the other hand, antifungal agents such as Nystatin 5000 IU/mL and Fluconazole exhibited limited efficacy, consistent with previous studies that highlighted the inherent resistance mechanisms of fungal biofilms, including reduced drug penetration and the presence of protective extracellular matrices[13]. In this study, fluconazole was evaluated at a concentration determined by standardized preparation of the intravenous formulation for irrigation, rather than dose optimization for biofilm eradication. Therefore, the limited biofilm reduction observed should be interpreted in the context of the well-recognized tolerance of mature Candida biofilms to azole antifungals when applied as irrigation solutions. However, prolonged application of PI overcame Nystatin and Fluconazole's limited efficacy barriers, proving far more effective where

ARTICLE IN PRESS

ARTICLE IN PRESS

conventional antifungal agents failed. Notably, it is plausible that PI irrigation durations longer than 3 minutes but shorter than 10 minutes (e.g., 5–7 minutes) could provide sufficient eradication in clinical practice. Further studies are needed to explore this possibility.

These results align with previous studies demonstrating the broad-spectrum efficacy of PI against both bacterial and fungal pathogens[9,20]. The efficacy of PI can be attributed to its ability to disrupt microbial cell walls and precipitate proteins, leading to rapid microbial death. The extended exposure time further amplified this effect, and also high concentration PI 10% make it especially potent solution for Candida biofilm removal[13,32].

Our study also emphasizes the rapid formation of Candida biofilm, which can develop within 120 hours. This finding highlights the critical window for intervention and the importance of early and effective treatment strategies to prevent biofilm maturation[31].

A key novel contribution of this study lies in addressing critical gaps left by previous research. Prior studies predominantly focused on bacterial PJIs, with limited exploration of fungal biofilms. Numerous studies have tested the efficacy of antiseptic solutions for bacterial biofilm. Siddiqi et al primarily investigated the efficacy of antiseptic solutions against planktonic bacteria but did not assess their impact on fungal biofilms, particularly those caused by Candida[11]**.** In contrast, our study specifically targeted fungal PJIs, demonstrating that extended exposure to PI plays a pivotal role in maximizing its fungicidal efficacy. This discovery represents a breakthrough in the management of PJIs involving biofilms, particularly in fungal infections, which have been historically understudied. Furthermore, Premkumar et al highlighted the bactericidal efficacy of PI in eradicating bacterial biofilms, yet its efficacy against fungal biofilms was not evaluated[9]. Our findings bridge this gap by showing that proloned application of PI can effectively remove *Candida albicans* biofilm, thereby extending the clinical utility of PI beyond bacterial infections to include fungal PJIs. This evidence underscores the versatility of PI as a solution that addresses both bacterial and fungal biofilm-related PJIs. Based on current finding and also considering potential cytotoxicity of high concentration PI, we suggest applying low concentration (0.35%) PI for 10 min when fungal PJI is diagnosed. However, it should be noted that the typical irrigation time do not exceed 2 to 3 minutes during surgery and

ARTICLE IN PRESS

ARTICLE IN PRESS

therefore, if the patient's condition do not allow prolonged irrigation or when fungal PJI is suspected but is not confirmed, applying high concentration PI (10%) for short time can be considered[33].

An additional key finding is the critical role of extended submersion time in optimizing the efficacy of irrigation solutions. Prior studies, such as Christopher et al (2021)**,** evaluated shorter exposure times (15–120 seconds), which may have been insufficient for effective biofilm removal[29]. In contrast, our study demonstrated that a 10-minute submersion significantly enhances biofilm reduction, underscoring the importance of contact time in maximizing antimicrobial efficacy. This finding holds significant implications for the clinical application of antiseptic solutions during PJI treatment.

In conclusion, our study establishes that PI 10% is the most effective irrigation solution for removing *Candida albicans* biofilms at 3 minutes, but clinically effective option would be using 10-minute extended protocol with minimum 0.35% concentration PI. These results suggest that incorporating PI with preferably higher concentration into DAIR (debridement, antibiotics, irrigation, and implant retention) protocols could significantly improve the success rates of fungal PJI treatments by ensuring more thorough biofilm eradication. Further research is warranted to assess the cytotoxicity of PI of various concentration at different duration and validate these findings in in-vivo models to ensure its safe and effective application in clinical settings.

ARTICLE IN PRESS

This study has several limitations that should be acknowledged. First, the irrigation method used in this experiment involved simply submerging the titanium screw cap samples into antiseptic solutions, which does not fully replicate the complex fluid dynamics of in vivo irrigation[34]. In clinical practice, irrigation is often performed using pulsatile lavage or continuous flow, which may enhance the efficacy of antiseptic solutions by improving penetration into biofilms. Future studies should incorporate more clinically relevant irrigation techniques to better simulate real-world conditions. Second, while this study demonstrated the efficacy of povidone-iodine and chlorhexidine in reducing *Candida albicans* biofilm burden, the results may not be generalizable to other fungal species or strains. Different *Candida* strains exhibit varying biofilm-forming capacities and resistance mechanisms, which could influence the effectiveness of antiseptic solutions[35]. In addition, the biofilm culture conditions used in this study

ARTICLE IN PRESS

may not fully reflect in vivo environments, where biofilm development is influenced by dynamic shear forces, nutrient gradients, and host factors. Third, this study was conducted in vitro and, as with other in vitro experiments, may not be reproducible in vivo. Biofilms in vivo interact with host tissues, immune responses, and other microbial communities, which cannot be fully replicated in an in vitro model. Similarly, the impact of antiseptic irrigation on host tissue integrity and potential immunomodulatory effects should be evaluated to ensure that biofilm reduction does not occur at the cost of tissue toxicity or impaired healing. Based on these limitations, future experiments should include in vivo studies to validate the efficacy of antiseptic solutions in biological settings[36]. Finally, synergistic or combination antiseptic strategies should be explored. This study focused on individual antiseptic solutions, but combinations of agents (e.g., PI + CHX, antifungals + antiseptics) may enhance efficacy and reduce the risk of resistance development. Investigating such combinations could lead to more effective biofilm eradication strategies and improved clinical outcomes[20].

Despite these limitations, this study provides valuable insights into the efficacy of antiseptic irrigation solutions against *Candida albicans* biofilms on titanium surfaces. Addressing these limitations in future research will be crucial for optimizing antiseptic strategies and improving their clinical applicability.

ARTICLE IN PRESS

Data availability

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request. The raw SEM images and experimental datasets (including Excel files) supporting the findings of this study can be accessed upon request.

References

1 Gonzalez, M. R., Bedi, A. D. S., Karczewski, D. & Lozano-Calderon, S. A. Treatment and Outcomes of Fungal Prosthetic Joint Infections: A Systematic Review of 225 Cases. *The Journal of Arthroplasty* **38**, 2464-2471.e2461 (2023). https://doi.org/10.1016/j.arth.2023.05.003

2 Yilmaz, M. K., Celik, N., Tarabichi, S., Abbaszadeh, A. & Parvizi, J. Evidence-based Approach for Prevention of Surgical Site Infection. *Hip & Pelvis* **36**, 161-167 (2024). https://doi.org/10.5371/hp.2024.36.3.161

3 Tsui, C., Kong, E. F. & Jabra-Rizk, M. A. Pathogenesis of Candida albicans biofilm. *Pathogens and Disease* **74** (2016). https://doi.org/10.1093/femspd/ftw018

4 Costa-De-Oliveira, S. & Rodrigues, A. G. Candida albicans Antifungal Resistance and Tolerance in Bloodstream Infections: The Triad Yeast-Host-Antifungal. *Microorganisms* **8**, 154 (2020). https://doi.org/10.3390/microorganisms8020154

5 Paluch, E., Szperlik, J., Lamch, Ł., Wilk, K. A. & Obłąk, E. Biofilm eradication and antifungal mechanism of action against Candida albicans of cationic dicephalic surfactants with a labile linker. *Scientific Reports* **11**, 8896 (2021).

6 Ruder, J. A. & Springer, B. D. Treatment of Periprosthetic Joint Infection Using Antimicrobials: Dilute Povidone-Iodine Lavage. *J Bone Jt Infect* **2**, 10-14 (2017). https://doi.org/10.7150/jbji.16448

7 Shaffer, A. *et al.* Perioperative prophylaxis for surgical site infections in pediatric spinal surgery: a systematic review and network meta-analysis. *J Neurosurg Pediatr* **31**, 43-51 (2023). https://doi.org/10.3171/2022.9.PEDS22316

8 Iannotti, F. *et al.* Prevention of Periprosthetic Joint Infection (PJI): A Clinical Practice Protocol in High-Risk Patients. *Trop Med Infect Dis* **5** (2020). https://doi.org/10.3390/tropicalmed5040186

9 Premkumar, A., Nishtala, S., Nguyen, J., Bostrom, M. & Carli, A. Comparing the efficacy of irrigation solutions on staphylococcal biofilm formed on arthroplasty surfaces. *J Arthroplasty* **36**, S26-S32 (2021).

10 Chalmers, B. P. *et al.* Accuracy of Predictive Algorithms in Total Hip and Knee Arthroplasty Acute Periprosthetic Joint Infections Treated With Debridement, Antibiotics, and Implant Retention (DAIR). *The Journal of Arthroplasty* **36**, 2558-2566 (2021). https://doi.org/10.1016/j.arth.2021.02.039

11 Siddiqi, A. *et al.* What Is the Optimal Irrigation Solution in the Management of Periprosthetic Hip and Knee Joint Infections? *The Journal of Arthroplasty* **36**, 3570-3583 (2021). https://doi.org/https://doi.org/10.1016/j.arth.2021.05.032

12 Brown, T. S. *et al.* Periprosthetic Joint Infection With Fungal Pathogens. *The Journal of Arthroplasty* **33**, 2605-2612 (2018). https://doi.org/10.1016/j.arth.2018.03.003

13 Shang, G., Zhao, S., Yang, S. & Li, J. The heavy burden and treatment challenges of fungal periprosthetic joint infection: a

ARTICLE IN PRESS

ARTICLE IN PRESS

systematic review of 489 joints. *BMC Musculoskeletal Disorders* **25** (2024). https://doi.org/10.1186/s12891-024-07616-6

14 O'Donnell, J. A. *et al.* Efficacy of common antiseptic solutions against clinically relevant microorganisms in biofilm. *The Bone & Joint Journal* **103-B**, 908-915 (2021). https://doi.org/10.1302/0301-620x.103b5.Bjj-2020-1245.R2

15 Christopher, Z., Deckey, D., Pollock, J. & Spangehl, M. Antiseptic Irrigation Solutions Used in Total Joint Arthroplasty: A Critical Analysis Review. *JBJS Reviews* **10** (2022). https://doi.org/10.2106/JBJS.RVW.21.00225

16 Giro dos Santos, A. *et al.* The molecular mechanism of Nystatin action is dependent on membrane biophysical properties and lipid composition. *Phys. Chem. Chem. Phys.* **19** (2017). https://doi.org/10.1039/C7CP05353C

17 Suchodolski, J., Muraszko, J., Bernat, P. & Krasowska, A. Lactate Like Fluconazole Reduces Ergosterol Content in the Plasma Membrane and Synergistically Kills Candida albicans. *Int J Mol Sci* **22** (2021). https://doi.org/10.3390/ijms22105219

18 Zhang, H. *et al.* Synergistic effect of chlorhexidine and azoles on candida biofilm on titanium surface. *Journal of Medical Mycology* **33**, 101417 (2023). https://doi.org/https://doi.org/10.1016/j.mycmed.2023.101417

19 George, J., Klika, A. K. & Higuera, C. A. Use of Chlorhexidine Preparations in Total Joint Arthroplasty. *J. Bone Joint Infect.* **2**, 15-22 (2017). https://doi.org/10.7150/jbji.16934

20 Roscetto, E. *et al.* Antiseptics' Concentration, Combination, and Exposure Time on Bacterial and Fungal Biofilm Eradication. *Arthroplasty Today* **28**, 101468 (2024). https://doi.org/https://doi.org/10.1016/j.artd.2024.101468

21 Lepelletier, D., Maillard, J. Y., Pozzetto, B. & Simon, A. Povidone Iodine: Properties, Mechanisms of Action, and Role in Infection Control and <i>Staphylococcus aureus</i> Decolonization. *Antimicrobial Agents and Chemotherapy* **64**, 10.1128/aac.00682-00620 (2020). https://doi.org/doi:10.1128/aac.00682-20

22 Liu, J. *et al.* A Comparison of <i>In-vitro</i> <i>Staphylococcus aureus</i> Growth on Rough and Smooth Titanium Surfaces. *Hip & Pelvis* **37**, 197-204 (2025). https://doi.org/10.5371/hp.2025.37.3.197

23 Zore, G., Abdulghani, M., Kazi, R., Shelar, A. & Patil, R. Proteomic dataset of Candida albicans (ATCC 10231) Biofilm. *BMC Research Notes* **16** (2023). https://doi.org/10.1186/s13104-023-06436-6

24 ATCC. *Mycology culture guide*, <https://www.atcc.org/resources/culture-guides/mycology-culture-guide> (2022).

25 Pierce, C. G. *et al.* A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. *Nat Protoc* **3**, 1494-1500 (2008). https://doi.org/10.1038/nport.2008.141

ARTICLE IN PRESS

ARTICLE IN PRESS

26 Chandra, J., Mukherjee, P. K. & Ghannoum, M. A. In vitro growth and analysis of Candida biofilms. *Nat Protoc* **3**, 1909-1924 (2008). https://doi.org/10.1038/nprot.2008.192
27 Relucenti, M. *et al.* Microscopy Methods for Biofilm Imaging: Focus on SEM and VP-SEM Pros and Cons. *Biology (Basel)* **10** (2021). https://doi.org/10.3390/biology10010051
28 Nenoff, P., Krüger, C., Neumeister, C., Schwantes, U. & Koch, D. In vitro susceptibility testing of yeasts to nystatin – low minimum inhibitory concentrations suggest no indication of in vitro resistance of Candida albicans, Candida species or non-Candida yeast species to nystatin. *Clinical and Medical Investigations* **1** (2016). https://doi.org/10.15761/cmi.1000116
29 Christopher, Z., Tran, C., Vernon, B. & Spangehl, M. What Is the Duration of Irrigation? An In Vitro Study of the Minimum Exposure Time to Eradicate Bacteria With Irrigation Solutions. *The Journal of Arthroplasty* **37** (2021). https://doi.org/10.1016/j.arth.2021.10.013
30 Kim, K. S. *et al.* Quantitative and qualitative analyses of the cell death process in Candida albicans treated by antifungal agents. *PLoS One* **6**, e28176 (2011).
31 Jo, S. *et al.* The Infected Polypropylene Mesh: When Does Biofilm Form and Which Antiseptic Solution Most Effectively Removes It? *The Journal of Arthroplasty* **39**, S294-S299 (2024). https://doi.org/10.1016/j.arth.2024.04.081
32 Siddiqi, A., Abdo, Z. E., Springer, B. D. & Chen, A. F. Pursuit of the ideal antiseptic irrigation solution in the management of periprosthetic joint infections. *J. Bone Joint Infect.* **6**, 189-198 (2021). https://doi.org/10.5194/jbji-6-189-2021
33 Charoenwisetsin, S., Jiranantarat, V., Hirunyachoke, P. & Udomkiat, P. Effect of intraoperative cold solution irrigation to reduce postoperative pain in knee osteoarthritis patients who underwent unilateral primary total knee arthroplasty: a double-blinded randomized controlled trial. *BMC Musculoskeletal Disorders* **25** (2024). https://doi.org/10.1186/s12891-024-07732-3
34 Poilvache, H. *et al.* Synergistic Effects of Pulsed Lavage and Antimicrobial Therapy Against Staphylococcus aureus Biofilms in an in-vitro Model. *Frontiers in Medicine* **7** (2020). https://doi.org/10.3389/fmed.2020.00527
35 Marzucco, A. *et al.* Evaluation of Biofilm Production and Antifungal Susceptibility to Fluconazole in Clinical Isolates of Candida spp. in Both Planktonic and Biofilm Form. *Microorganisms* **12**, 153 (2024). https://doi.org/10.3390/microorganisms12010153
36 Liu, J. X., Werner, J., Kirsch, T., Zuckerman, J. D. & Virk, M. S. Cytotoxicity evaluation of chlorhexidine gluconate on human fibroblasts, myoblasts, and osteoblasts. *Journal of Bone and Joint Infection* **3**, 165-172 (2018). https://doi.org/10.7150/jbji.26355

ARTICLE IN PRESS