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Supporting information

Facile single-step bioconjugation of the antifungal agent caspofungin onto materials surfaces via an epoxide plasma polymer interlayer

Thomas D. Michl [a, ‡], Carla Giles [a, ‡], Alasdair T. Cross [b], Hans J. Griesser [a], Bryan R. Coad [a,c,±]

[a] Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia

[b] The University of Bath, Claverton Down, Bath BA2 7AY, UK

[c] School of Agriculture, Food and Wine, University of Adelaide, Adelaide SA 5005, Australia

‡ These authors contributed equally

± Corresponding author: bryan.coad@adelaide.edu.au

Experimental details

Materials and Methods

Materials

Water was purified using a Synergy Millipore system to 18.2 mΩ*cm. Allyl glycidyl ether (AGE), Bovine Serum Albumin (BSA) and Phosphate buffered saline (PBS) tablets were purchased from Sigma Aldrich, Australia. Acetone, ethanol and sodium dodecyl sulphate were supplied by Chem Supply Australia. 12-well Nunclon plates and RBS were obtained from Thermo Fisher Scientific Australia. Caspofungin Acetate acquired from Jomar Life Research, Australia. Silicon wafers were purchased from Micro Materials and Research Consumables Pty Ltd. 18 mm glass coverslips supplied by ProSciTech Australia. Spider broth was prepared to the following composition: 20g Nutrient broth (Oxoid, Australia), 20g Mannitol (Sigma-Aldrich, Australia), 4 g K_2HPO_4 (Sigma-Aldrich, Australia) dissolved to 1 L in distilled water then pH adjusted to 7.2 with NaOH.

Epoxide plasma polymerization

Deposition of allyl glycidyl ether (“epoxide”) plasma polymer (AGEpp) was performed using RF plasma in continuous wave, followed by pulsed duty cycle using a custom-built plasma reactor.[1,2] The vacuum pump (Edwards RV5) was turned on, with the ballast valve opened, and was left running for 10 minutes to allow it to warm up to improve pumping and purge impurities. While the pump was warming up, the base of the reactor was attached to the system and the copper electrode was placed in position. Grease was applied to the joint at the top of the glass cold trap (Apiezon Products, M

Grease) which was then attached to the system, which ensured that the grease was evenly spread to allow for a good seal. The cold trap was placed into the Dewar which was then carefully filled with liquid nitrogen. After ten minutes the ballast valve on the pump was closed, the isolation valve further down the system was shut and the Pirani gauge was switched on. The valve on the pump was opened to start evacuating air in the system and the pressure was left to decrease to $3x10^{-2}$ Torr to check if any leaks were present in the system. Samples to be coated were placed on the copper electrode and the reaction chamber and brass electrode were put into position. All of the valves on the reaction chamber were closed and the isolation valve was opened, evacuating the reaction chamber. Once the pressure had decreased to $3x10^{-2}$ Torr, the flask containing AGE was attached to the system and evacuated so that only monomer vapour was present. Once the pressure of the system had decreased to $3x10^{-2}$ Torr, the monomer valve was opened and the pressure was set to $2x10^{-1}$ Torr. The power leads from the power generator (Advanced Energy, Model number 5002-005-C) were connected to the brass electrode and the power generator was switched on. The RF was turned on and power was set to 25W, with any adjustments to reflected power being made using the matching box (Advanced Energy, Model number 5022-000-E). The continuous plasma was left to run for 1 minute and then the pulse generator (20 ms period, 1 ms on, RS Components Ltd., Stock Number 610-629, Serial Number 007715) was connected and the pulsed plasma was left to run for a further 2 minutes. The power generator was then turned off and the AGE was left to flow over the samples for three minutes to ensure no unreacted radicals were left on the surface. The monomer valve was then closed and once pressure had decreased to $4x10^{-1}$ Torr the isolation valve was closed. The air valve on the reaction chamber was then carefully opened to ensure the samples were not disrupted and once the reaction chamber pressure had equalised the samples were removed from the chamber.

Binding of Caspofungin to epoxide surfaces

800 µL of 0.1 mg/mL caspofungin solution was added to each well of a 12 well-plate containing a sample. The samples were left to incubate at room temperature overnight.

Analytical methods

Ellipsometry

Ellipsometry was carried out to determine the thickness of the deposited epoxide layers on the surface of silicon wafer pieces. This was performed using a variable angle spectroscopic ellipsometer (VASE, J.A. Woollam Co. Inc. NE, USA), with measurements carried out at 65˚, 70˚ and 75˚ and wavelengths of light from 250 nm to 1100 nm in 10 nm intervals. The amplitude ratio, ψ, and the phase difference Δ were measured at each wavelength and angle. A Cauchy model was used to fit the data (the two equations below were used for that purpose). Equation 1 is the Cauchy equation and is a mathematical

representation of the refractive index of the layer being measured. Equation 2 is a mathematic representation of the extinction coefficient of the layer.

$$n(\lambda) = B + \frac{C}{\lambda^2} + \frac{D}{\lambda^4} \quad (1)$$

$$k(\lambda) = \alpha e^{\beta(1.24\mu m\left(\frac{1}{\lambda} - \frac{1}{\gamma}\right)} \quad (2)$$

The optical constants within the model were adjusted to produce the best fit between the measured data and the model. Residual differences between the model and measured data were represented by the mean square error (MSE). The data were fitted to minimise the MSE.

Epoxide stability testing

The stability of the epoxide layers was tested by washing them using multiple solutions and methods. Samples were sonicated for 5 minutes in water, acetone or ethanol and let soak for additional 10 minutes. One additional sample was washed with PBS buffer once and then incubated in 0.01 M SDS solution at 70 ˚C for 15 minutes; followed by rinsing five times with PBS and three times with deionized water. The samples were then dried under nitrogen.

Stability of Surface-Grafted Caspofungin

Three glass coverslips were coated on one side using the epoxide plasma method previously described. One coverslip had no further treatment done to it and was used as a control. One sample was treated with caspofungin by covering the sample in 0.1 mg/mL caspofungin solution and then being left overnight to bind to the surface. The sample was then washed using the full SDS washing procedure previously described. The final sample was first washed using the full SDS washing procedure and then the caspofungin was applied following the same method used for the other sample and finally washed five times with PBS and three times with water. All samples were dried under N_2 and then analysed by XPS to determine the nitrogen content on the surfaces, which is indicative of the amount of caspofungin bound to the surface.

X-Ray Photoelectron Spectroscopy (XPS)

Surface analysis of the surfaces was performed using a Kratos Axis Ultra DLD unit (Kratos Analytical, Manchester, UK) equipped with a monochromated Al Kα X-ray (1486.6eV) source operating at 225 W. Photoelectrons were collected over a binding energy range of -10 to 1110 eV with a step size of 0.5 eV at a dwell time of 55 ms. An electron take-off angle of 90˚ to the sample within an analysis area of 0.3 mm by 0.7 mm was used to collect the data. A pass energy of 160 eV was used to acquire the survey spectra and the software package CasaXPS (version 2.3.16 PR 16) was used to interpret the spectra with Shirley baseline correction.

Static Biofilm Assay

The biomass quantification method used here was previously described.[3] *Candida albicans* ATCC 90028 and *Candida glabrata* ATCC 90030 were maintained at -80 ^{0}C. Prior to use, *C. albicans* and *C. glabrata* were sub-cultured onto Sabouraud-Anti agar plates (Thermo Fisher Scientific, Australia). Treated glass coverslips as described above were placed into a 12 well plate (Nunc, Australia). Then to each well 1 mL of a bovine serum albumin solution (BSA) in PBS (0.1 mg/mL) was added and incubated at 37 ˚C under agitation of 100 rpm overnight. Following this, each well was aspirated and washed two times with 1 mL of phosphate buffered saline (PBS pH 7.4) (Sigma-Aldrich, Australia). A suspension of 1 x10^6 *C. albicans* or *C. glabrata* was prepared in Spider broth (1L in distilled water: 20 g Nutrient broth (Oxoid, Australia), 20 g Mannitol (Sigma-Aldrich, Australia), 4 g K_2HPO4 (Sigma-Aldrich, Australia), pH adjusted to 7.2 with NaOH) [4]). To each well 1 mL of the prepared *Candida spp.* suspension was added and then the plate was incubated for 90 min at 37 ˚C under agitation of 100 rpm to allow for the *Candida* adherence.

Following adherence step each well was aspirated, with the aspirate collected (90 min time point). Next each well was again gently washed three times with PBS and 1 mL of room temperature spider broth was added to each well and further incubated at 37 ^{0}C at 100 rpm for 24 hr to allow the biofilm to develop. Following incubation the supernatant was again collected (24 hr time point) and the wells were again washed gently three times and the coverslips were transferred to a tube containing 10 mL of PBS and were sonicated 43 kHz for 1 min and vortexed for 1 min for three cycles. The disrupted biofilm cells were serially diluted 10 fold and 100 µl plated in duplicate onto Sabouraud-Anti agar plates (Thermo Fisher Scientific, Australia) and incubated at 37 ^{0}C for 24 hr. Colonies were counted and biofilm mass enumerated ((CFU/mL x 10 mL)/Surface Area cm^2). All experiments were performed in triplicate (minimum n=9). Statistical evaluation was performed using GraphPad Prism 6, using the students paired t test.

Presence of *C. albicans* in the supernatant

The collected supernatant from the 90 min adherence phase the and the 24 hr biofilm formation phase the supernatant was serially diluted 10 fold and plated onto Sabouraud-Anti agar plates and incubated at 37 ^{0}C for 24 hr counted and CFU/mL enumerated.

Microscopy

Live dead images were processed according to the live/dead Baclight viability staining kit (Invitrogen, Australia).

1 mL of 1x10^5 CFU/mL of *C. albicans* ATCC 90028 in spider media was added to wells containing both AGEpp and AGEpp-caspofungin coated coverslips. Each well was incubated for its designated

time 2 hr, 4 hr, 8 hr and 24 hr. After the designated incubation time, the *C. albicans* suspension was aspirated and discarded, the coverslip was transferred to a clean plate containing 600 µl of physiological saline (0.9 % w/v NaCl) containing 1 µl of stain prepared according to the manufacturer's instructions and incubated for 15 min in the dark at 37 ^{0}C stain. After incubation the wells were washed very gently twice with 600 µl of saline. Then the coverslip was mounted onto a glass slide and imaged on an Olympus microscope (Ix53 inverted microscope), with three sites imaged per surface. Images were overlayed using the FIJI image processing software. Live and dead cells were counted and percentage of live vs dead calculated for each surface and time point.

Microbiology results

C. albicans growth in the supernatant and on surfaces

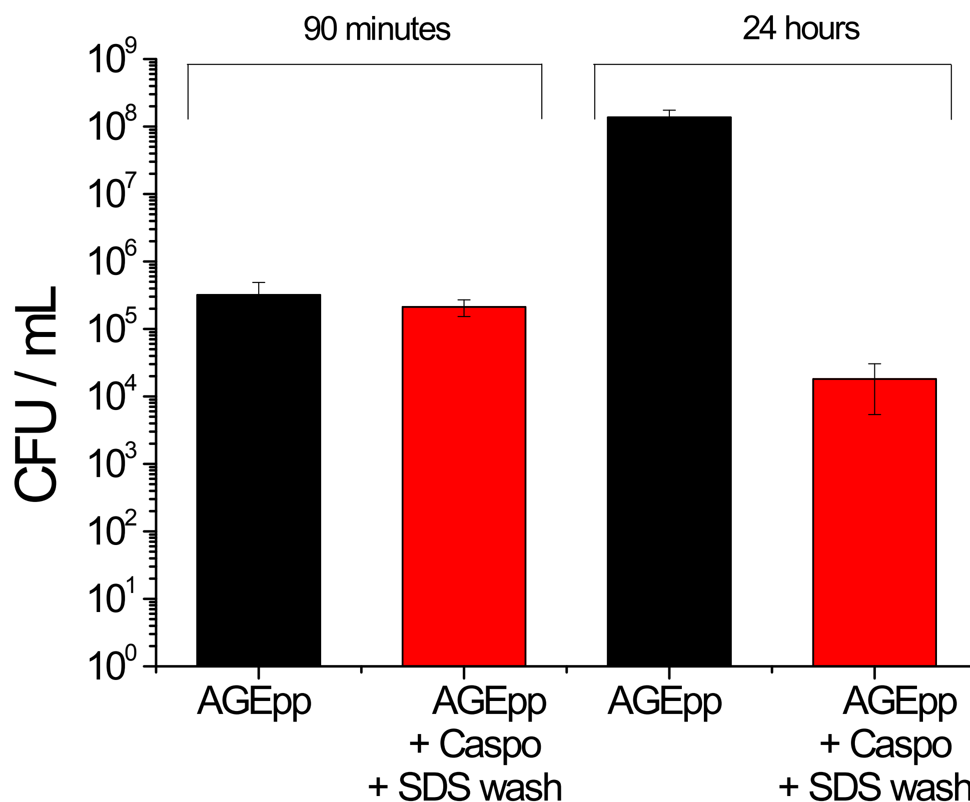

Figure S1: CFU of *C. albicans* in the supernatant after 90 minutes adhesion time

Live/Dead microscopy

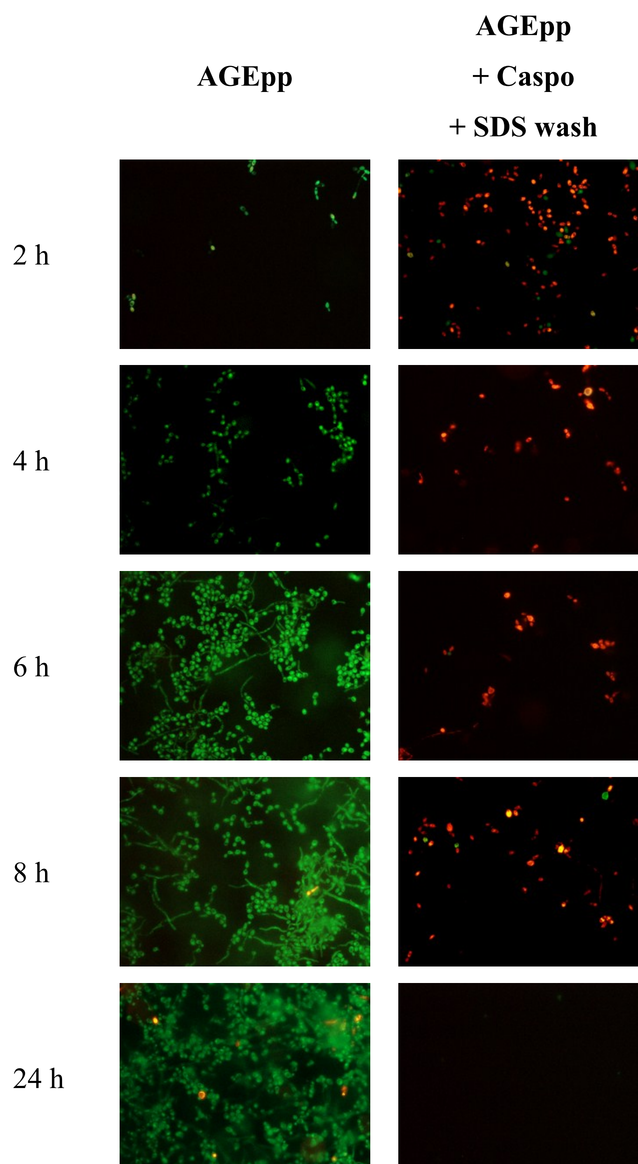

Figure S2: Micrographs (40X) of live-dead stained *C. albicans* after various times of contact with coatings

All images at 40X lens Scale 20 µm. Live cells green, dead cells are red. Completed using a Baclight Live/Dead cell viability kit. Microscope Olympus inverted. Here each treatment and time is one representative image of the 3 images that were counted. Below graphs are the combined counts from the individual images.

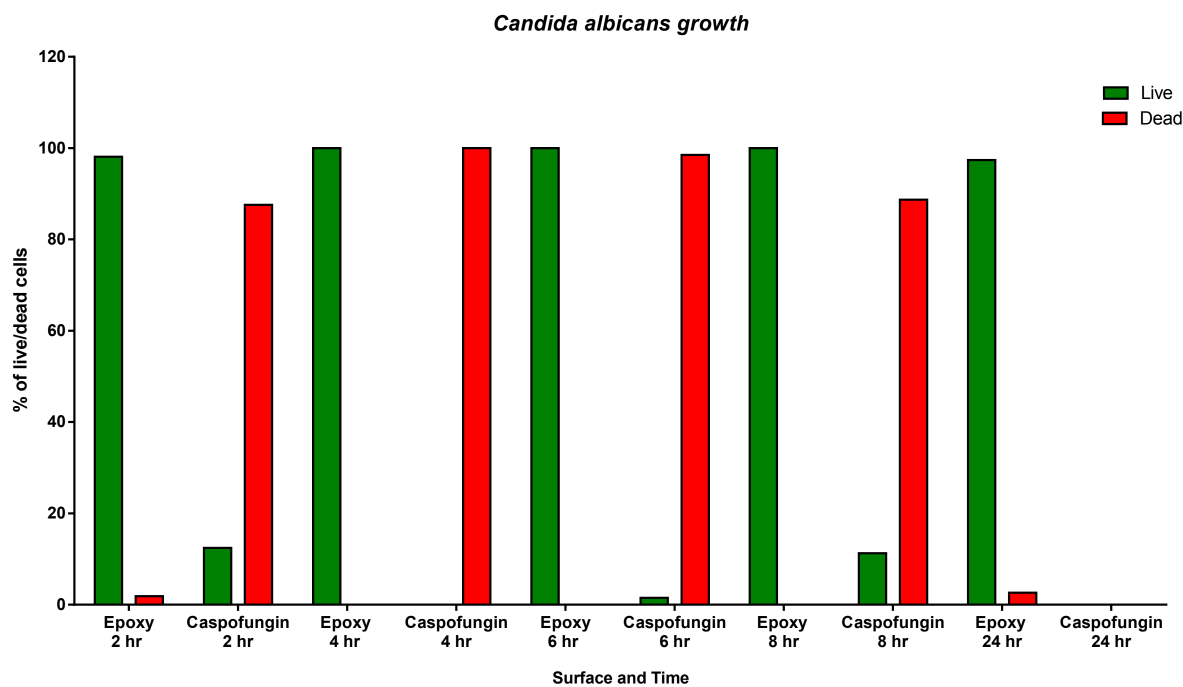

Figure S3: Micrograph counts of *C.albicans* after various times of contact with coatings

References

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