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# Study of Candida albicans Biofilm Inhibition on Coated Medical Devices

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# Independent Study Class for Investigating Coated Materials affecting *Candida species* Growth

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## Abstract

Many human infections resulting from medical devices or implants contamination are associated with microbial aggregation and biofilm formation. Mechanical Engineering and Biology Department students collaborate under an independent study class dedicated to applied surface coatings. They explore different methods directed to inhibit the biofilm growth and proliferation by coating the material surfaces with antimicrobial metallic thin films. The interdisciplinary aspect of the work helps students from engineering understand the importance of medical and biological perspectives, and reciprocal, biology students understand the engineering approach. Students form a team searching for viable solutions to real-life problems. They investigate the development of different *Candida spp. (Candida species)* biofilms on pristine and metallic coated fibrous substrates. The biocompatible substrates (fibrous filters and membranes) are coated with copper and silver nanoparticles in the form of thin films with varying thicknesses. The coating process takes place at room temperature and high vacuum, in a physical vapor deposition equipment. The team examines the development and possible inhibition of different *Candida spp.* on coated materials by following standardized protocols; students work on the engineering and biology aspects of the research and cooperate to collect, analyze and assemble the results.

## Keywords

Independent study, antimicrobial coatings, silver and copper thin films, *Candida species* biofilm, biofilm inhibition

## Introduction

The use of metallic or ceramic nanoparticles as coatings applied on biocompatible substrates is recently of great interest due to their successful results in inhibiting growth and/ or killing microbes. Nanoparticles in the form of coatings are less susceptible to enable development of microbial resistance.[1-4] Students collect the most recent information in the field from both engineering and biology perspectives using the latest journal publications (Outcome 7, ABET). Advisers guide the literature search directions as students learn about the most advanced research in engineering and biology-related areas. The physical vapor deposition methods under consideration for developing metallic coatings on surfaces without affecting the substrates' morphology and quality are: thermal evaporation, magnetron sputtering, and electron beam depositions.[5,6]

Previous studies on thin films applied on fibrous materials indicated adequate coverage of the substrates with nanoparticles and good antibacterial behavior of the coatings.[7,8] Current directions of interest are inhibition and possible annihilation of *Candida spp. (Candia albicans* and *Candida auris)* fungus developed on fibrous substrates. Microbial biofilm formation is a significant cause of human and animal infections due to medical device contaminations;[9,10] therefore, the treatment of these infections is becoming a research priority.[11,12]

The independent study class is addressing the Outcome 2 (ABET) as students "apply engineering design to produce solutions that meet specified needs with consideration for public health, safety and welfare". One specific feature of *Candida spp*. pathogenicity is its ability to form biofilms. This feature has been observed in a diverse set of environments that include biotic surroundings (e.g. aquatic environments, plant tissues, and mammalian tissues) as well as abiotic surroundings (e.g. catheters, prosthetic devices, and biomaterials).[13] Since biofilm formation plays an essential role in developing candidemia (bloodstream infections), antimicrobial therapies and antibiofilm strategies are aiming to prevent biomaterial-associated *Candida spp.* infections.[11] The development of candidemia driven in patients with implanted biomaterials (such as catheters, dental implants, and prosthetic devices) motivates the development of preventive strategies to control the infection.[13-15] Current investigation uses silver and copper nanoparticles in the form of thin films coated on fibrous substrates and explores their effects on annihilating *Candida spp.* Students participate both in the coating process and biology-related testing and investigation. The independent study class aims to develop students ability to "function effectively on a team whose members establish goals, plan tasks, meet deadlines, provide leadership" in agreement to Outcome 5 (ABET).

## Methods

Microbial growth and biofilm formation are investigated for two types of substrates: fibrous filters (30 microns porosity) and fibrous membranes (2 microns porosity). Thin films of silver and copper are deposited on the fibrous substrates by physical vapor deposition method using direct current (DC) magnetron sputtering equipment. Depositions are performed close to room temperatures (25 to 40 $^{0}$C) and low pressures ($10^{-3}$ torr). The metallic targets in use for coatings' development are high purity metals (silver and copper 99.5%). In order to observe and precisely monitor the coating thickness during deposition, the magnetron sputtering equipment is using an internal crystal quartz microbalance. After performing the coatings, the thickness is measured ex-situ using a profiler (KLA Tencor). All substrate materials (pristine and coated) are handled with nitrile gloves in order to avoid contaminations and are placed in plastic bags soon after performing the coatings. Optical and Keyence digital microscopy are employed to observe the substrate materials before and after performing the coatings and to investigate the development of *Candida spp.* colonies on substrates. Several strains of *Candida spp.* (e.g. *C. albicans* sand *C. auris* strain #0381 and #0383) are tested in order to characterize the formation and growth of the biofilm. The overnight cells are prepared by inoculating a single colony cell from yeast peptone dextrose (YPD) agar plates into a 5ml YPD liquid media followed by an incubation at 30 ℃ in a shaking incubator. Overnight cultured *Candida* broth is diluted to $10^{5}$ cells/mL concentration using the RPMI medium (a growth medium used for cell culture). Figure 1 is representing the methodological workflow for *Candida spp*. biofilm assay. The samples coated with silver and copper are washed three times with PBS (phosphate buffered saline) and exposed to UV light for 5 minutes on each side (front and back) to decontaminate their surfaces prior to experiments. The procedure is performed using a biosafety cabinet.

Four coating thicknesses are selected for the initial study: 100 nm, 300 nm copper thickness and 100 nm, 300 nm silver thickness, along with control samples (uncoated substrates). Each material is then placed in a 24-well plate and it is fully submerged in 1 mL of *Candida spp.* suspension. The plate is later incubated at 37 ℃ for three hours to allow cells attachment to the samples' surface. 100 µL from each well is collected and placed into a 96-well plate to measure the optical density (OD) at time zero, and then a 12-well plate is used and placed into a 37 ℃ shaker (90 rotations per minute) for three hours. After three hours of incubation, the cells and media collected from each well and 100 $\mu$L with proper dilution are plated on YPD media and left in the 37 ℃ incubator for 24 hours to count the colony forming unit (CFU). 0.1% PBS is used to wash each material before transferring it into a new 24-well plate containing a fresh RPMI media and kept at 37 ℃ for an additional five hours. After five hours of incubation, the cells collected from the media and surface of each sample and 100 $\mu$L with proper dilution were plated on YPD media and left in the 37 ℃ incubator for 24 hours to count the CFU.

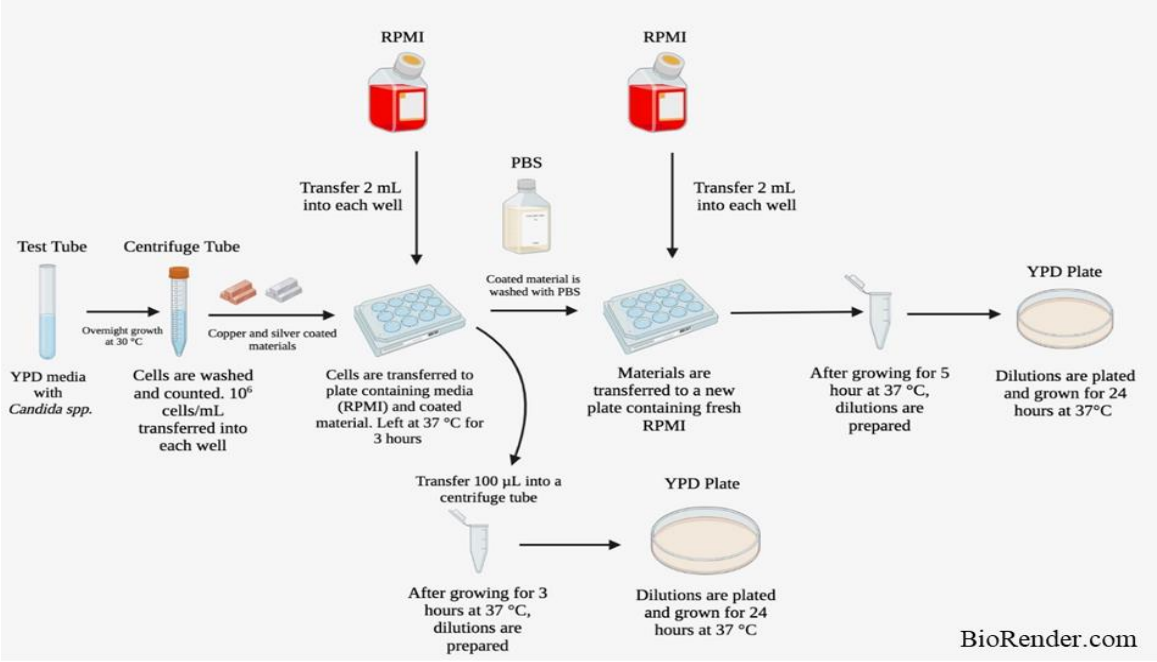



Figure 1: Methodological workflow for *Candida spp*. biofilm assay: schematic representation of a 24-well-plate–based optical method for the quantitative and qualitative measurements of *Candida spp.* biofilm formation (figure created with BioRender.com).

## Results and Discussion

Two different copper and silver coating thicknesses (100 and 300 nm) are under consideration for evaluating the influence of the coating thickness on *Candida spp.* biofilm formation. In order to establish the correct time required for performing a specific coating thickness, students are performing in-situ measurements using crystal quartz/ piezoelectric monitoring balance. The measurements are compared to ex-situ measurements using the KLA Tencor profiler. An example of

coating thickness (in nanometers) progression with time (in minutes) for magnetron sputtered copper is presented in Figure 2, where it is observed that coating thickness increases linearly with time. Similar graphs are obtained for other types of metallic coatings, including silver (not represented). Students learn about the specifics of physical vapor deposition methods and DC magnetron sputtering equipment. They perform high vacuum, room temperatures thin films depositions. The independent study class encourages students to acquire "an ability to conduct appropriate experimentation, analyze and interpret data, and use engineering judgement to draw conclusions" in conformity to Outcome 6 (ABET). Students are analyzing and establishing the required working parameters (e.g. pressure during deposition, working potential, coating times) for the coating processes. After performing the coatings, the substrates with selected copper and silver thicknesses are placed in clean, closed plastic bags in order to avoid further contamination and are transferred to biology investigation.

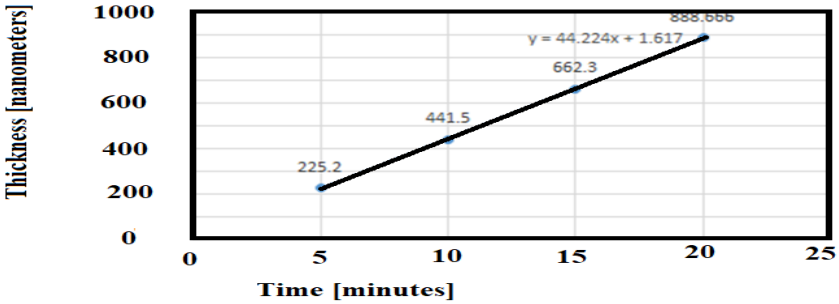
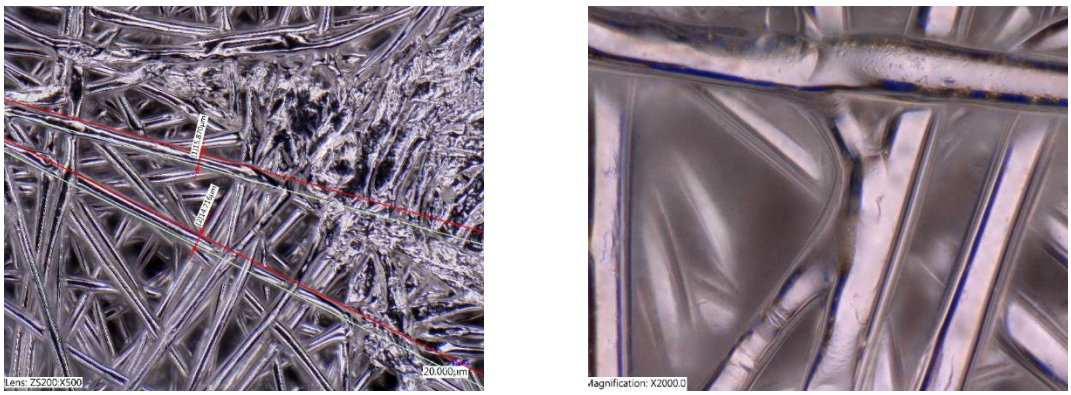



Figure 2. Evaluation of thickness (nanometer) versus time (minutes) for magnetron sputtered copper

Figure 3 is the digital microscopy image representing 300 nm thickness silver coated fibrous substrates using 500X magnification (a) and using 2000X magnification (b). The image (a) is showing the fibrous substrate with a specific pattern that remained unchanged after performing the coatings.

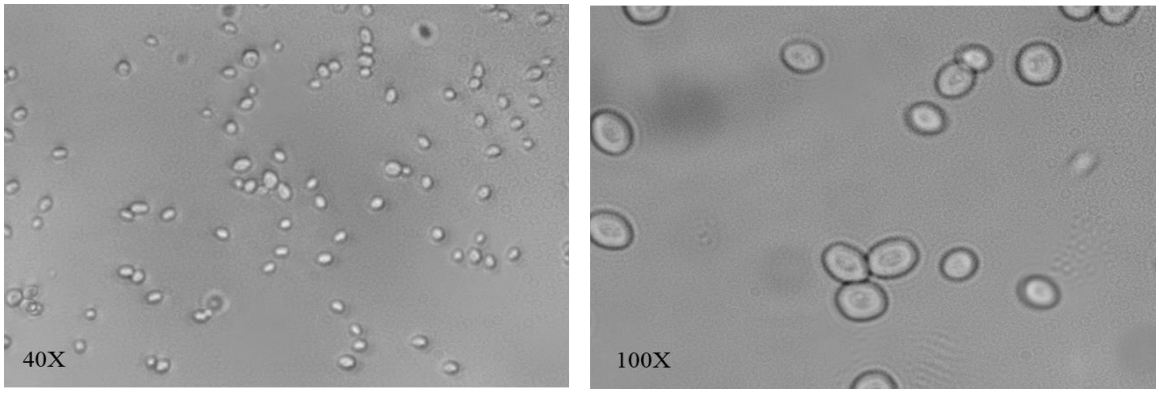


(a) (b)

Figure 3. Digital microscopy of 300 nm silver coated fibrous filter (a) 500X, and (b) 2000X magnification

The image (b) is representing magnified coated fibers displaying that metallic thin films offered appropriate coverage of the fiber substrates. Coatings are also applied to membrane substrates (not represented).

Representations of *Candida spp.* (e.g. *C. albicans*) development used for the biology testing and evaluations are captured in Figure 4. Students are using optical microscopy to acquire images that are showing the *C. albicans* (40X magnification (a) and 100X magnification (b)).




(a) (b)

Figure 4. Optical microscopy of *C. albicans* in yeast form: (a) 40X, and (b) 100X magnification

The fibrous filters and membrane metallic coated substrates are further exposed to different *Candida* strains. Figure 5 represents the general experimental setting containing the antimicrobial testing results for pristine (uncoated), copper and silver coated fibrous filter papers.

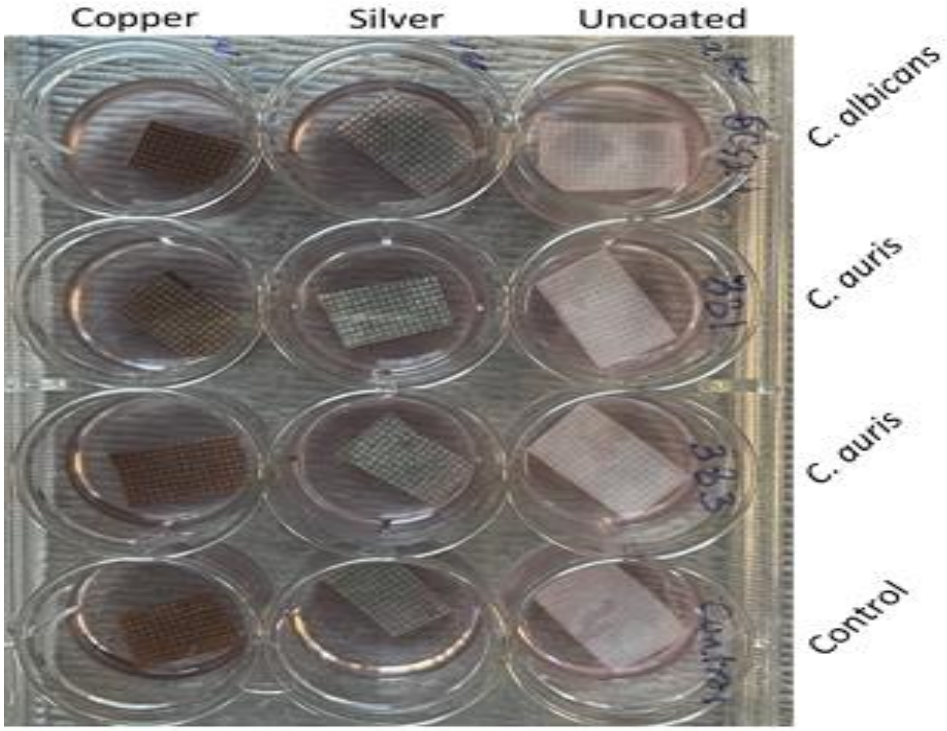



Figure 5. Experimental setting for antimicrobial testing

Biology students under the professor supervision and advisement are establishing the experimental setting. A similar experimental setting is used for testing the membrane substrates (not represented). The copper and silver-coated fibrous filters and membranes are evaluated further by colony counting.

Figure 6 is a general example of the experiment that represents the evolution of *C. albicans* after three hours (a) and after five hours (b), respectively. The figure shows that number of *C. albicans* colonies is initially higher on the silver coated and uncoated (control) materials compared to the copper coated materials. This suggests that the copper coating could be more effective in inhibiting the growth of *C. albicans* cells than silver after three hours of incubation. However, after washing the samples and incubating them for an additional five hours with fresh media, the number of colonies of *C. albicans* cells is lower on the silver coated substrates compared to the uncoated (control) materials (b). This suggests that silver may effectively inhibit the attachment of *C. albicans* on the surface of silver coated fibrous materials. No *C. albicans* cell growth is observed after five hours of incubation on the fibrous materials coated with copper, indicating that cells were killed after the initial, three hours, Figure 6 (a). The results suggest that copper coated materials could kill *C. albicans* cells while materials coated with silver could inhibit the attachment and further biofilm formation, as observed in Figure 6 (b).

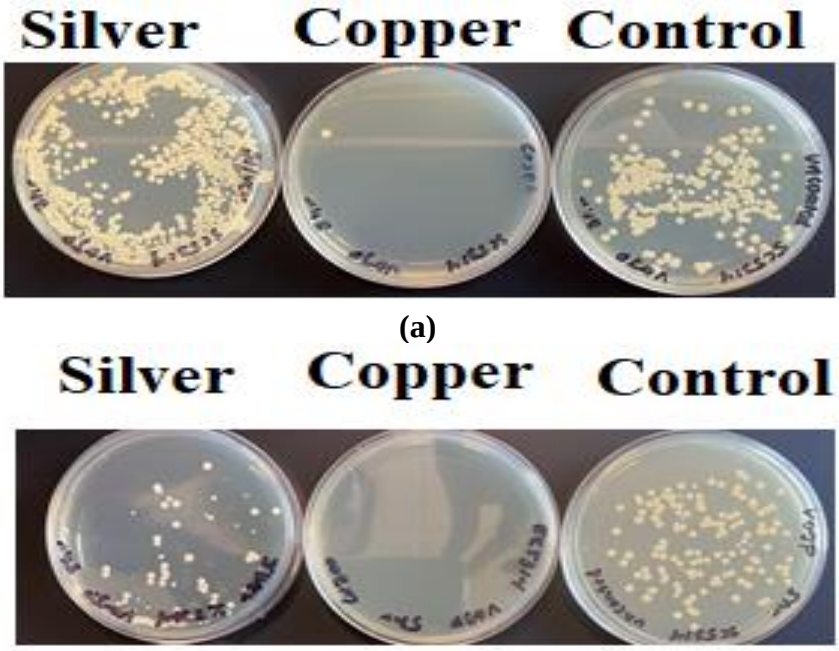



Figure 6. Evolution of *C. albicans* exposed to pristine, silver, and copper coated fibrous filters after 3 hours (a) and 5 hours (b) of exposure

Similar results are obtained for other two *C. auris* strains*:* 0381 and 0383 (not represented).

Future investigations are necessary to observe the influence of different coating thicknesses on the biofilm formation and the effects of the selected protocol (e.g. temperature, humidity, pH, shaking velocity, incubation time). The ongoing independent study class is enabling students' participation to interdisciplinary, concrete problems. Students are collecting data and working on the graphical representations of results in preparation for future presentations. They develop the "ability to communicate effectively with a range of audiences" in conformity to Outcome 3 (ABET).

## Conclusions

Mechanical engineering and biology students are working in collaboration for an independent study class to find solutions to the invasive growth of *Candida spp.* fungus on surfaces (e.g. fibrous surfaces). The team is bringing the knowledge and expertise for setting up techniques to coat the surface of substrates with silver and copper nanoparticles. Students are following the established protocols for biology testing. The initial results show that copper and silver coatings are efficient to inhibit the growth of *Candida Spp*. strains. Students collaborate to collect the results and to improve further on the testing procedures. The independent study class offers broad experience and exposure to students bringing real life problems to experimental labs while working together to solve the problems. The concept of this independent study class and student related involvement addresses the ABET Outcomes 2, 3, 5, 6 and 7.

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