Mechanical Engineering - Biology Interdisciplinary Independent Study Class for Controlling the Biofilm Formation

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Abstract:

Students from Mechanical Engineering and Biology Department are collaborating under an independent study class for investigating and controlling the fungi adhesion and biofilm formation on medical device surfaces. The class is aiming to apply engineering perspectives and recommend solutions to a current biological problem. Students are initially performing literature search and gathering specialized knowledge in order to investigate the evolution and possible inhibition of *Candida spp*, one of the most common sources of fungal infections in humans. A promising approach aiming to inhibit the initial planktonic cells attachment on surfaces is by using coatings with antimicrobial and antibiofilm agents. In order to accomplish their goals, students are designing the experiments in mechanical and biology areas. They are working on depositing thin films by using direct current high vacuum dual magnetron sputtering system. Students are learning to develop and monitor the formation of the coatings and to investigate the resulted coated materials. They are further collaborating for the biological investigations and related data assessments. *Candida* strains behavior is analyzed on pristine surfaces and on copper and silver thin films and MXene coated surfaces.

Keywords

Candida spp., Biofilm, Copper thin films, Silver thin films, Antimicrobial coatings

Introduction

The interdisciplinary independent study class is creating an environment where students have the opportunity to collaborate for investigating solutions to real world problems such as microbial acquired resistance. Students are exposed to engineering and biology advanced knowledge and learn to effectively communicate and work together. The focus of the class is to emphasize the engineer professional importance and evidence how engineering methods can make an impact by searching and proposing solutions to contemporary biological related problems (Outcome 2, ABET). Students are investigating different biology related topics, such as the biofilm formation - a complex three-dimensional structure consisting of groups of microorganisms. For example, fungi adhering to living or inorganic materials are producing the biofilm in the form of a sticky, extracellular polymeric substance (Figure 1A).[1,2] In order to understand the problem complexity, students are exposed to literature search and further discussions to acquire specialized knowledge from reputable published sources. The biofilm formation on abiotic surfaces is a current problem of high importance in healthcare-associated infections, especially those related to prosthetic and indwelling medical devices, such as urinary catheters and orthopedic implants.[2–4] The problem occurs for all classes of material substrates: metals, ceramic, polymers or composites. Despite efforts to maintain sterility, implantable and prosthetic medical devices can quickly become contaminated, leading to device failure, chronic infections, and high mortality rates.[1–4] It is recognized that inhibition of fungi adhesion and biofilm formation on the surface of medical devices could reduce the risk of surgical site infection. *Candida albicans* and *Candida auris* are the most common sources of fungal infections in humans and can be fatal in immunocompromised patients by causing systemic bloodstream infections.[5,6] Recently, the rate of *Candida* infections has increased rapidly due to the growing number of transplant recipients, cancer and HIV patients.[6, 7] In recent years, the availability and use of various antifungal agents have increased drug resistance in *Candida* species. *C. auris* is a worldwide hospital-acquired fungal pathogen that causes a broad spectrum of infections and commonly colonizes medical devices such as central venous catheters.[8] The purpose of the independent study class is to propose solutions aiming to inhibit the initial adhesion of the opportunistic pathogen *C. albicans* and *C. auris* on medical devices by modifying the surface of the device (Figure 1). The study is using control surfaces (uncoated porous surfaces) and their efficacy in resisting the growth of *Candida* species are compared to similar porous surfaces coated with copper, silver thin films and MXene coatings (two-dimensional transition metal carbides, carbonitrides, and nitrides).

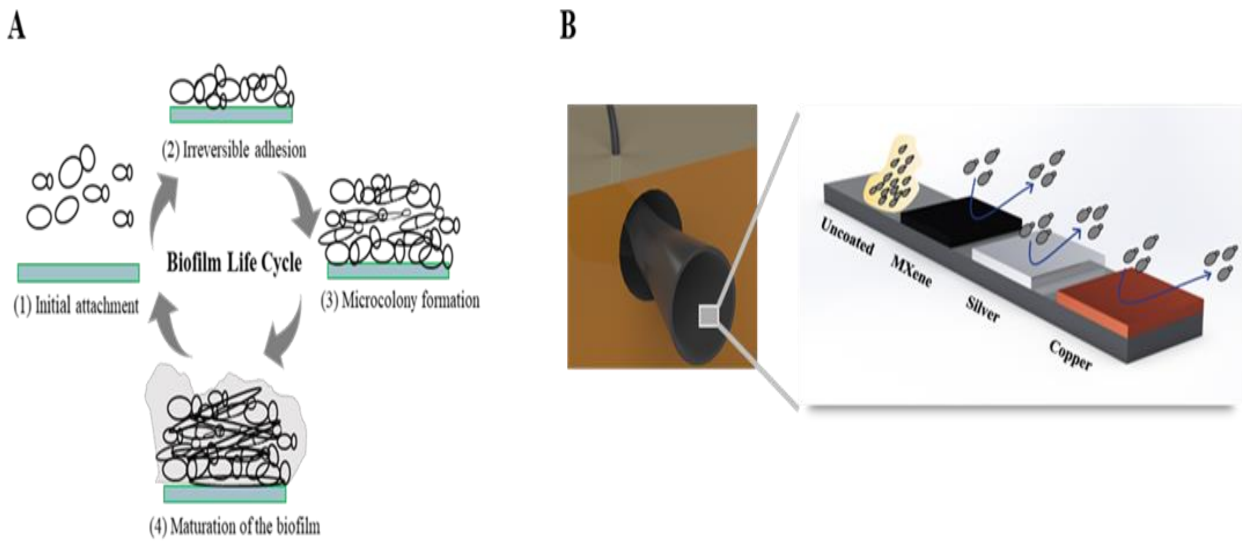

Figure 1: A) Dynamic biofilm life cycle on a medical device: (1) initial attachment of microbes, (2) irreversible adhesion or attachment, (3) microcolony formation, and (4) maturation of the Biofilm. B) Management of *Candida* biofilms: A model illustrating different surface modifications as strategies for inhibiting fungal adhesion and/or decreasing bacterial viability.

The current independent study class is designed as a continuing class, extended over few semesters. Students are developing their abilities to design parts of the experiments and jointly conduct the experiments in the mechanical engineering and biology laboratories.

Strains, media, and growth conditions

Two strains of *C. auris* (named 0833 and 0831) and one strain of *C. albicans* (SC5314) were acquired from the Centers for Disease Control and Prevention (CDC). All strains were stored in 25% (vol/vol) glycerol solution at -80°C to prevent chromosome instability induction. *C. auris* strains were grown in YPD medium (20 g/L peptones, 10 g/L yeast extract, 20 g/L glucose; for solid medium, 20 g/L agar was added), and RPMI (Sigma, Saint Quentin Fallavier, France). Students are actively learning about different strains of fungi in the acquisition context, about the importance of proper storage of biological materials and the correct methods of handling the microbiological cultures during experiments. For the current study, cells were inoculated on agar plates, stored at -4°C, then inoculated in liquid YPD, and incubated overnight at 30°C with shaking at 200 rpm. Then, cells were centrifuged at 5000 rpm for 5 minutes, resuspended, and washed three times with a wash buffer. Once the strains were washed, the ODs were measured to determine ratios for working solutions.

Surface Coatings with Silver, Copper thin films

Direct current high vacuum dual magnetron sputtering system (Kurt J. Lesker) is used for coating commercially available micron size porous materials at low temperatures (room temperature). The base pressure inside the equipment is 10^{-3} Pa, and it is achieved using a dry roughing pump and a turbomolecular high vacuum pump where the vacuum level is monitored using an ion gauge. Students have the opportunity to acquire knowledge about high vacuum systems and thin film depositions and investigations. They are working with state-of-the-art equipment for coating depositions and related investigations. The microporous material substrates are handled with pristine nitrile gloves throughout all required steps for the coating processes and during the antimicrobial testing procedures in order to limit their possible contamination with other types of microbes. The Keyence Digital Microscope and Scanning Electron Microscope (SEM) are used to investigate the pristine and metallic coated substrates surface morphology. Figure 2 is showing an example of the Scanning electron microscopy of the porous materials after performing the coating.

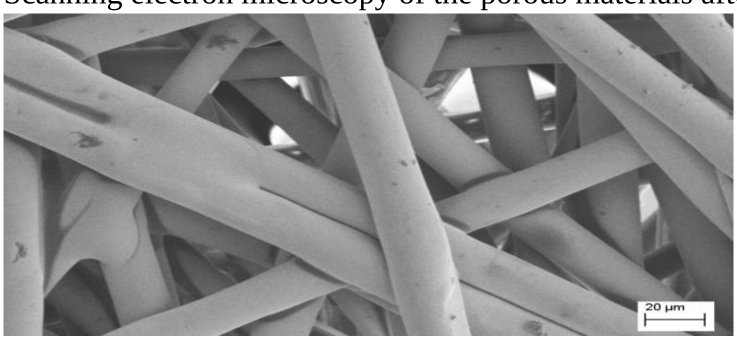

Figure 2. Scanning electron microscopy of filter paper fibers after coating with 1 µm thickness silver

Measurement of contact killing

For the growth curve experiment, 10^5 cells/ml (OD_{600nm}=0.1) are mixed with 1ml of RPMI media in flat-bottom 12-well plates in the presence of the coated and uncoated filter at 37°C. Copper or silver pieces are put under ultraviolet (UV) light for 5 minutes on the front and back sides to eliminate contamination. Then each filter is fully submerged into the RPMI media, and the plate is stored at 37°C shaker for 2 hrs. After two hours of incubation, the plate is removed from the shaker and washed: each filter three times with 0.1 x phosphate-buffered saline (PBS), and the optical density at 600 nm is measured every two hours to calculate the doubling time.

At the end of the experiments, students are gathering results and are learning how to professionally organize their data. They also learn to communicate their research effectively graphical and oral for future students or professional conference presentations (Outcome 3, ABET).

Conclusions

Two teams of students from Mechanical Engineering and Biology Department are working together under the professors' guidance to apply engineering and biology knowledge and offer solutions that are important for public health, safety, and welfare. They are working towards finding viable solutions for inhibiting the biofilm formation of *Candida albicans* and *Candida auris*, the most common sources of fungal infections in humans. Students are responsible for designing the experiments, collecting and preparing data. The metallization of surfaces by depositions of silver and copper thin films and MXene coatings are investigated in order to protect surfaces from biofilm formation. Students are exposed during the class to "hands-on" activities in the mechanical engineering, physical vapor deposition, biology area and use applied knowledge to solve biology-related problems. They are communicating their results and build up reports for future conference participations and publications.

References

(1) Festas, A.; Ramos, A.; Davim, J. Medical Devices Biomaterials – A Review. *Proc. Inst. Mech. Eng. Part J. Mater. Des. Appl.* **2019**, *234* (1), 218–228. https://doi.org/10.1177/1464420719882458.

(2) Khatoon, Z.; McTiernan, C. D.; Suuronen, E. J.; Mah, T.-F.; Alarcon, E. I. Bacterial Biofilm Formation on Implantable Devices and Approaches to Its Treatment and Prevention. *Heliyon* **2018**, *4* (12), e01067–e01067. https://doi.org/10.1016/j.heliyon.2018.e01067.

(3) Zheng, Y.; He, L.; Asiamah, T. K.; Otto, M. Colonization of Medical Devices by Staphylococci. *Environ. Microbiol.* **2018**, *20* (9), 3141–3153. https://doi.org/10.1111/1462-2920.14129.

(4) Josephs-Spaulding, J.; Singh, O. V. Medical Device Sterilization and Reprocessing in the Era of Multidrug-Resistant (MDR) Bacteria: Issues and Regulatory Concepts. *Front. Med. Technol.* **2021**, *2*, 16. https://doi.org/10.3389/fmedt.2020.587352.

(5) Horton, M. V.; Nett, J. E. Candida Auris Infection and Biofilm Formation: Going Beyond the Surface. *Curr. Clin. Microbiol. Rep.* **2020**, *7* (3), 51–56. https://doi.org/10.1007/s40588-020-00143-7.

(6) Fungal Diseases and Conditions - Candida; Reports from Panjab University Advance Knowledge in Candida (Biofilm Formation by Candida Auris Isolated from Colonising Sites and Candidemia Cases). *Health & Medicine Week*. Atlanta June 28, 2019, p 4911.

(7) Lanternier, F.; Cypowyj, S.; Picard, C.; Bustamante, J.; Lortholary, O.; Casanova, J.-L.; Puel, A. Primary Immunodeficiencies Underlying Fungal Infections. *Curr. Opin. Pediatr.* **2013**, *25* (6), 736–747. https://doi.org/10.1097/MOP.0000000000000031.

(8) Li, J.; Hirota, K.; Goto, T.; Yumoto, H.; Miyake, Y.; Ichikawa, T. Biofilm Formation of Candida Albicans on Implant Overdenture Materials and Its Removal. *J. Dent.* **2012**, *40* (8), 686–692. https://doi.org/10.1016/j.jdent.2012.04.026.

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Sahar Hasim has been an Assistant Professor at the Department of Biology, Mercer University, since 2020. She received her Ph.D. in Microbiology and Molecular Biology and her B.S. in Biology from the University of Nebraska-Lincoln. Before joining Mercer University, she was a lecturer in Biology at Columbus State University. As a postdoctoral research associate, she has four years of research experience delivering scientific solutions at the University of Tennessee Knoxville and Oak Ridge National Laboratory. At Mercer University, her main research emphasis focused on host-pathogen interaction, biophysics properties of *C. albicans* cell wall, and inhibiting *C. auris* biofilm formation on medical devices.

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