Faculdade de Engenharia
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e.Biofilm description

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Solutions to the big questions in science and engineering requires a diversity of thought that originates when the questions are ruminated upon and explored via multiple perspectives and disciplines. Biofilm research exemplifies the benefit of an interdisciplinary approach to research. Biofilms are self-organized, cooperative communities of microbes that are embedded in biological matrix. Biofilm impacts multiple industries including oil and gas production, cooling towers, paper mills, food processing plants, and consumer product production plants. Biofilm is a challenge in the built environment in terms of surface disinfection, infection control and the degradation of building materials. In the clinic, biofilm is linked to infected indwelling medical devices such as urinary catheters and orthopedic devices, and chronic infections such as cystic fibrosis and wounds. The goal in the above examples is to find a product or process that prevents, kills, controls, or removes biofilm.

Multiple steps must be accomplished for a strategy to be effective against biofilm, including understanding how the biofilm is growing and responding to stimuli in the environment of interest, being able to recreate these growth patterns in the laboratory, testing the efficacy of proposed treatment strategies, and finally validating that the proposed strategy will work in application area of interest. Depending upon the application, the proposed strategy needs to be approved either by a regulatory body (for medical or human health contexts), or by good practice standards recognized by industry, as in the case of biocides used to control biofilm in the oil and gas industry. Interestingly, many of the applications mentioned above still rely on viable plate counts, presence absence assays or ATP to make decisions as to whether a strategy works. A few application areas use secondary indicators. For instance, in the clinic, if a person presents with no infection, then it is assumed that the treatment was effective. In the case of the built environment, if the end results are aesthetically appealing, then the strategy is deemed successful. In terms of large industrial systems, if the system is functioning as designed in terms of heat, mass, or energy transfer, then the treatment strategy is deemed effective. In all these examples, knowing the numbers of viable cells is not necessary to make an informed decision. What if we understood the physiology of the cells within the biofilm, and how they were spatially arranging themselves in relationship to other microbes and the matrix constituents? Our hypothesis is that mapping the physiology and 3D spatial geography of biofilm *in situ* will enable us to grow more reproducible biofilms that may be used for informed decision making by regulators, industry, and clinicians on strategies to control and exploit biofilm in industrial and clinical applications.

e.BIOFILM has four focus areas of study that correspond to four available PhD positions. All four focus areas will collaborate and build upon each other's work, applying an interdisciplinary approach to investigate how to generate a more reproducible biofilm from multiple perspectives.

Focus area one will use FISH and targeted transcriptomic imaging along with matrix stains to map an industrial multispecies biofilm that includes the pathogen *Legionella*. The research will

apply probes developed by the FISH Group, FEUP, U of Porto research team to the multispecies biofilm grown in a biological reactor model to understand how the biofilm responds to changes in stimuli and if this response is predictable and can be controlled.

Focus area two will apply the transcriptomics approach developed at the Centre of Biological Engineering, U of Minho to study the interspecies interaction and/or cooperation through a gene expression profile analysis and using an in vitro multispecies chronic wound biofilm model, developed at the FEUP, U of Porto. The student will investigate the role of time as a critical factor influencing cell activity as it relates to oxygen depletion, and the species numbers involved in the influence of gene patterns, spatial arrangement, and treatment response. The student will also apply FISH probes to map the bacteria within the biofilm.

Focus area three will focus on 3D image analysis and assessing repeatability, reproducibility, responsiveness, and ruggedness of imaging outcomes. The utility of available 3D mapping tools will be explored to determine how their library codes may be applied or modified to answer the research questions being asked in focus areas one and two. Fundamentally, this research will help to set guidelines for what defines reproducibility for imaging outcomes and transcriptomics. This student will also assist in developing a database for the data collected by focus areas one, two and four.

Focus area four will focus on developing a computation model that describes how the 3D spatial geography of cells changes over time when different factors are introduced, such as when one protein is expressed vs another. The model will be validated using the database developed by focus area three that was collected by focus areas one and two.

The results from focus areas one and two will be compared and contrasted from the perspective of developing a standard method with the goal of optimizing the method for growing a reproducible biofilm with the most relevant characteristics. Key to this comparison will be the image analysis tools and guidelines for assessing reproducibility developed by focus area three. Focus area four will also apply image analysis tools to the computer generated biofilm.

In addition to research, the students in all 4 focus areas will participate in training schools that focus on analytical methods for the study of biofilm control (instructor Dr. Phil Stewart), imaging biofilms (instructor Dr. Heidi Smith), and statistics, modelling, and data management for biofilms (instructor Dr. Al Parker). The students will be expected to participate in academic seminars and meetings with regulatory and industrial partners. The program will focus on career development, including project management, networking, career pathways in industry and academia, ethics and equality, communication skills and community outreach. The students will be given opportunities to travel and collaborate with researchers at biofilm institutions and laboratories located internationally