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—DR. MICHEL KOO

UNRAVELING BIOFILMS

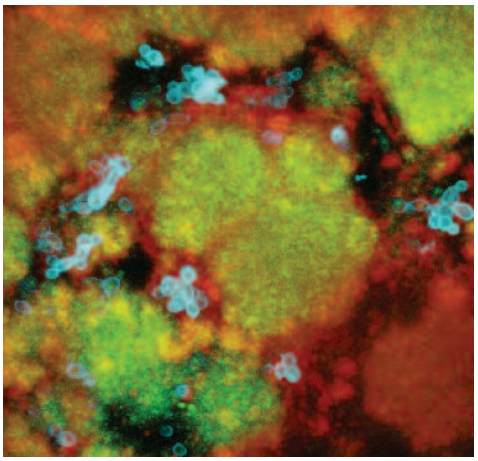
DR. MICHEL KOO EMPLOYING UNIQUE APPROACHES TO TARGET BIOFILMS AND TOOTH DECAY

OPPOSITE: Hyun (Michel) Koo, DDS, PhD, joined Penn Dental Medicine last September as Professor, Department of Orthodontics. In his lab, Dr. Koo focuses on understanding how pathogenic biofilms cause oral infectious diseases, particularly dental caries (or tooth decay). He uses advanced molecular and imaging technologies to unveil the principles of biofilm assembly on tooth surfaces. At the same time, he is developing new therapeutic approaches to target the biofilm at its core—the extracellular matrix-scaffold—using naturally-occurring and synthetic agents.

OF KOREAN DESCENT, Dr. Hyun (Michel) Koo was born in Germany, raised in Brazil, and has worked in the United States for a decade and a half. As a result, he has mastered English, Korean, and Portuguese. But that tally does not include the fact that he is also fluent in the complementary but distinct languages of biochemistry, microbiology, and clinical dentistry.

Now a professor in the Department of Orthodontics at Penn Dental Medicine, Dr. Koo's comfort in many realms has led him to clinically relevant discoveries in unexpected places, from the Amazon rainforest, to exotic fruits and plants, to humble beehives and cranberries.

The driving force linking these diverse discoveries is Dr. Koo's desire to understand and find treatments against disease-causing biofilms, the sticky mix of microbes, glue-like polymers and other materials that affixes itself to many surfaces. His focus is on the biofilm known as plaque that accumulates on the tooth's surface, leading to breakdown of enamel and onset of the disease dental caries, commonly called tooth decay or cavities.



"Our main purpose is to find out how microbes build up biofilms and cause diseases and then hopefully find a better way to prevent them," he says.

A UNIQUE APPROACH

Dr. Koo's interest in research emerged as a dental student in Brazil. While most students went home for summer vacation, he spent the breaks in the lab doing research that stoked his interest in biochemistry and microbiology. After graduating, he began to

practice dentistry but maintained a fervent curiosity that eventually drove him back to the classroom. But instead of seeking further dental-specific training, he entered a master's program in food science and biochemistry at the State University of Campinas (UNICAMP), Brazil—the first dental student ever to do so.

"I thought, the oral cavity is the first point of entry for everything we consume," he says. "I was excited to learn from the fascinating world of food science if there was anything to be discovered about preventing oral disease or promoting oral health."

Working with a mentor (Dr. Yong Park), Dr. Koo began a search for new substances that could provide oral health benefits using biotechnology in the form of microbial enzymes and plant-food chemistry.

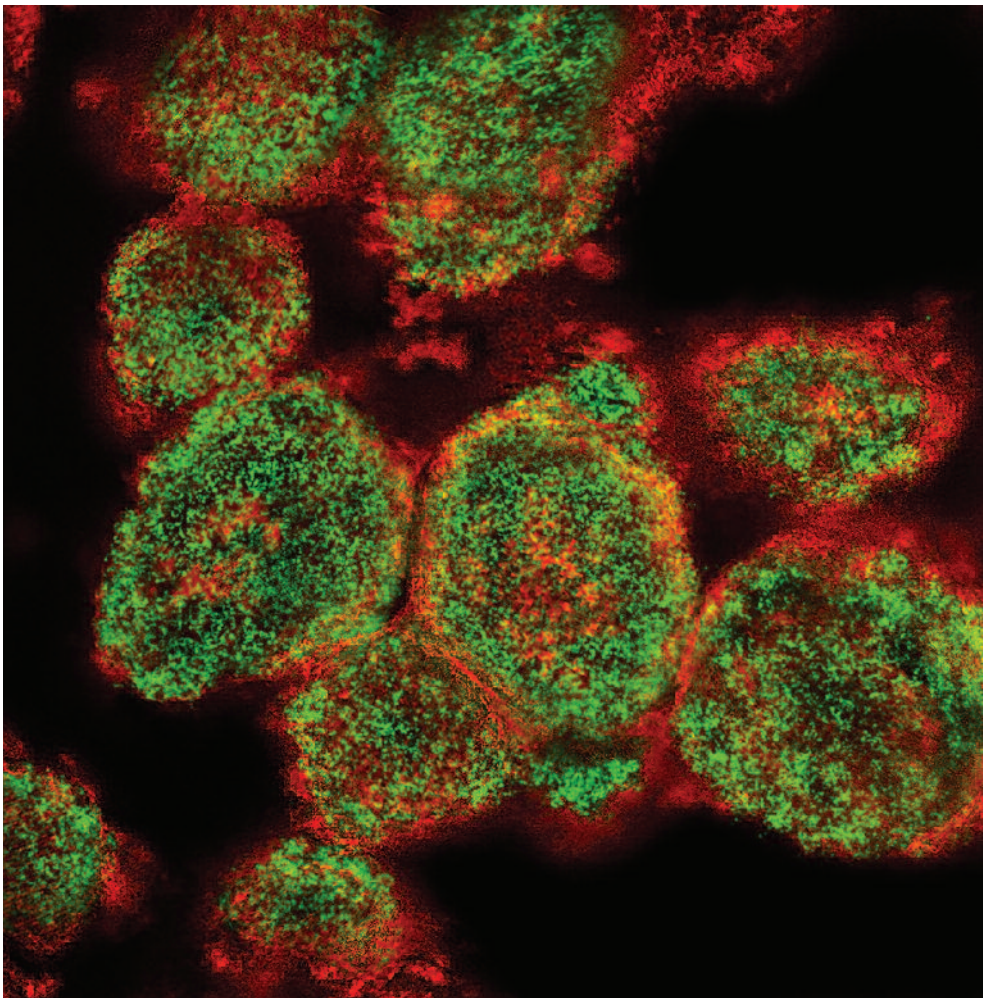
"Sugar is the arch criminal of dental caries as it fuels harmful bacteria to build up plaque and make acids that dissolve teeth," says Dr. Koo. "We wanted to find specific microbes in nature that could use this sugar to produce new types of sugar molecules that have similar sweetening properties but couldn't be metabolized in the oral cavity by bacteria."

To find these novel sugar-metabolizing microbes, Dr. Koo collected samples in dozens of different ecosystems.

"We looked in places from the cleanest, purest ecosystems like the Amazon rainforest to rotting food and insects in São Paulo open markets," he says.

He amassed an array of more than 3,000 samples and identified a few new microbes that could produce alternative sweetening yet non-cariogenic and non-caloric sugars; some of which are now being developed by the food industry.

A pull toward unlikely sources for discovery continued in another project Dr. Koo developed, this time looking at honeybee hives. Bees seal and protect their hives with a substance called propolis, which they produce from resins they collect as they visit a variety of plants. Dr. Koo and colleagues found that a number of small molecules isolated from propolis have anti-biofilm properties. By targeting an enzyme produced by the main culprit behind dental caries, the bacterium *Streptococcus mutans,* the researchers demonstrated that propolis-derived compounds could inhibit the formation of biofilms and the development of dental caries in rodents.



"Starting with food science and compounds found in nature, we now have a better understanding of oral diseases, and the basis of products that could prevent those diseases," he says.

BIOFILM ARCHITECTURE IN 3D

Dr. Koo continued his scientific inquiries in Brazil while earning a Ph.D. in oral biology (Dr. Jaime Cury, mentor) at FOP-UNICAMP in a joint program with the University of Rochester, where he subsequently joined the faculty. "At Rochester, I had an incredible opportunity for academic growth, particularly working with Dr. William Bowen, a world-authority in dental caries research," says Koo. While continuing to look for tooth decay therapies, he also delved more deeply into understanding how pathogenic bacteria, such as *S. mutans,* assemble plaque biofilms.

Using advanced imaging technology that allows him to reconstruct a three-dimensional picture of a biofilm, Dr. Koo has found that the bacteria are able to cluster tightly together because they are enmeshed in a matrix comprised of glue-like polymer molecules and other extracellular materials.

"Biofilms are highly organized microbial communities, forming a structure almost like a tissue," says Dr. Koo. "We show how the 'scaffolding' of the matrix creates a highly compartmentalized architecture, while making the biofilms very sticky on the surface."

In 2012, Dr. Koo and colleagues published a paper in *PLoS Pathogens* demonstrating that not only does the matrix provide a scaffold and make the biofilm gooey and sticky, but it also helps to create acidic microenvironments. By devising a novel three-dimensional pH-mapping technique, Dr. Koo showed highly localized, matrix delineated acidic compartments throughout the biofilm. These niches of low pH affects everything from the balance of microorganisms that can thrive inside of them, to the behavior of *S. mutans,* to the severity of damage to tooth enamel that results in cavities.

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"Our data offer new avenues for further elucidation of how cell-matrix interactions govern the formation of pathogenic biofilms, while introducing new tools and methods for biofilm research," Dr. Koo says.

Dr. Koo's probing of the matrix structure and biofilm microenvironment has led to new ways of thinking about how to prevent or treat plaque build-up on the teeth. Instead of solely targeting the bacteria themselves, Dr. Koo and his team are looking for ways to degrade or stop matrix production. This strategy has the added benefit of avoiding treatment with antibiotics, which some bacteria can eventually learn to evade.

DEVELOPING NOVEL THERAPIES

Much like his previous explorations in the Amazon and other ecosystems, Dr. Koo has continued to look in some unlikely places for new anti-biofilm compounds to target the matrix. His investigations have revealed potentially useful compounds in cranberries and in the waste product from the wine-making industry—the leftover grape skin, pulp and seeds called pomace. Dr. Koo's research has shown that molecules found in cranberries called proanthocyanidins can reduce the synthesis of polysaccharides in the matrix.

In parallel, Dr. Koo has methodically examined the bioactivity of each of the food-derived compounds discovered in his lab to help design an effective therapy against cariogenic biofilms. He has looked at how combinations of various molecules could synergize, enhancing the overall therapeutic effect.

In one line of research, Dr. Koo and colleagues examined the possibility of pairing the proven standby, fluoride, with newly discovered anti-biofilm agents. The rationale was simple yet promising: Fluoride helps to prevent mineral loss and rebuild tooth mineral during acid attack, but has limited effects against bacteria and biofilm formation.

"We thought that including agents that impair acid production, a terpenoid, and biofilm matrix build-up, a flavonoid, could complement and enhance the effectiveness of fluoride," Dr. Koo says.

He discovered that the therapeutic effect of this combination therapy was superior to that of the potent antimicrobial chlorhexidine as well as fluoride, effectively reducing the development of caries disease in an animal model. Recently, Dr. Koo and colleagues published a paper in *Antimicrobial Agents and Chemotherapy* revealing the mechanisms by which these food-derived compounds, together with fluoride, disrupt the assembly of the biofilm matrix, and enhance the overall anti-caries activity.

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Studying these microbial communities enmeshed in a polymeric matrix could pave the way for relevant findings beyond the mouth—ranging from barnacles on a ship hull to plaque on heart valves and medical devices—as biofilms are often associated with many diseases in humans as well as industrial and naval issues.

"We always have the idea in mind of how our research in the oral cavity might have broader applicability," Dr. Koo says.

ABOVE: (top) Dr. Koo's research has shown that molecules found in cranberries, called proanthocyanidins, could disrupt the assembly of the biofilm matrix and be used to develop new therapies against cariogenic biofilms. (bottom) Prospective anti-biofilm agents are initially tested in the laboratory using biofilms formed on hydroxyapatite (a tooth enamel-like material).

OPPOSITE: Biofilm structure is highly complex. Bacterial 'islets' or microcolonies (green) are enmeshed and surrounded by an intricate extracellular 'scaffold,' known as matrix (red), which is comprised of polysaccharides and other materials.

DISCOVERING A CROSS-KINGDOM PARTNERSHIP

This knowledge of biofilm architecture and assembly extends to an important public health concern. Dr. Koo has a special interest in a disease that affects children early in life, called early childhood caries. It involves a highly destructive and painful form of tooth decay that affects toddlers, particularly those from backgrounds of poverty.

"It's a costly and terrible disease and very psychologically damaging to kids because they can't even smile," he says. "The tooth decay can become so severe that treatment often requires surgery in the operating room."

Some of Dr. Koo's latest work, to be published in the May issue of *Infection and Immunity,* aims to identify the factors that make these infections so virulent in children, with an eye toward preventing or treating the disease.

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Though researchers had long known *S. mutans* was a primary culprit in the disease, Dr. Koo and collaborators, as well as other scientists, probed dental plaque from children with the disease and almost always found the fungus *Candida albicans* together with high levels of *S. mutans*. The discovery piqued Dr. Koo's interest, because although *C. albicans* sticks to the cheek and tongue, it was not believed to be a common resident in dental plaque formed on teeth.

"We were puzzled," Dr. Koo says. "*Candida* usually does not associate with *S. mutans,* nor does it colonize teeth very effectively."

The investigators discovered that an enzyme secreted by *S. mutans,* which the bacterium uses to produce extracellular polysaccharides from sugar, also binds to and enables *Candida* to produce a glue-like polymer in the presence of sugar. *Candida* then uses this same polymer to adhere to teeth and to bind *S. mutans,* two abilities it otherwise lacks.

"The combination of the two organisms led to a greatly enhanced production of the biofilm matrix," Dr. Koo says, "drastically boosting the ability of the bacterium and the fungus to colonize the teeth, increasing the bulk of the biofilms and the density of the infection."

And because of the biofilm's compartments of low pH, this accumulation led to greater levels of acid next to the teeth that can dissolve enamel, leading to cavity formation. The investigators showed that infection by *S. mutans* and *C. albicans* together doubled the number of cavities and boosted their severity several fold in rats.

"It is an intriguing interaction where a fungus is converted into a fierce stimulator of cariogenic biofilm formation," says Dr. Koo

"Our data will certainly open the way to test agents to prevent this disease," Dr. Koo says, "and, even more intriguing, the possibility of preventing children from acquiring this infection."

NEW HORIZONS

The novelty and merit of Dr. Koo's work has been recognized through several awards, including the IADR Distinguished Scientist Award and IADR/GSK Innovation in Oral Care Award, as well as funding from the National Institutes of Health, the U.S. Department of Agriculture, and industry. Having just come aboard Penn Dental Medicine's faculty last September, he says he is honored to be part of what he sees as "a world-class winning team for research," with ample opportunities for cross-disciplinary collaboration within the School and across the campus to make new discoveries and to bring them into clinical use. For example, Dr. Koo is starting to collaborate with Dr. Henry Daniell, Professor, Departments of Biochemistry and Pathology, on a project to investigate the potential of using his antimicrobial peptides to control cariogenic biofilms.

Dr. Koo's recent sabbatical leave in Dr. Ken Yamada's lab (a leading scientist in cell/matrix biology) at NIDCR/NIH brought new ideas to study biofilms. He's particularly interested in taking advantage of the nanotechnology and engineering expertise at Penn, perhaps utilizing the new Singh Center for Nanotechnology, located just at the other end of Penn's campus from Penn Dental Medicine. He plans to build on previous work, in which he has explored strategies for drug delivery using nanoparticles.

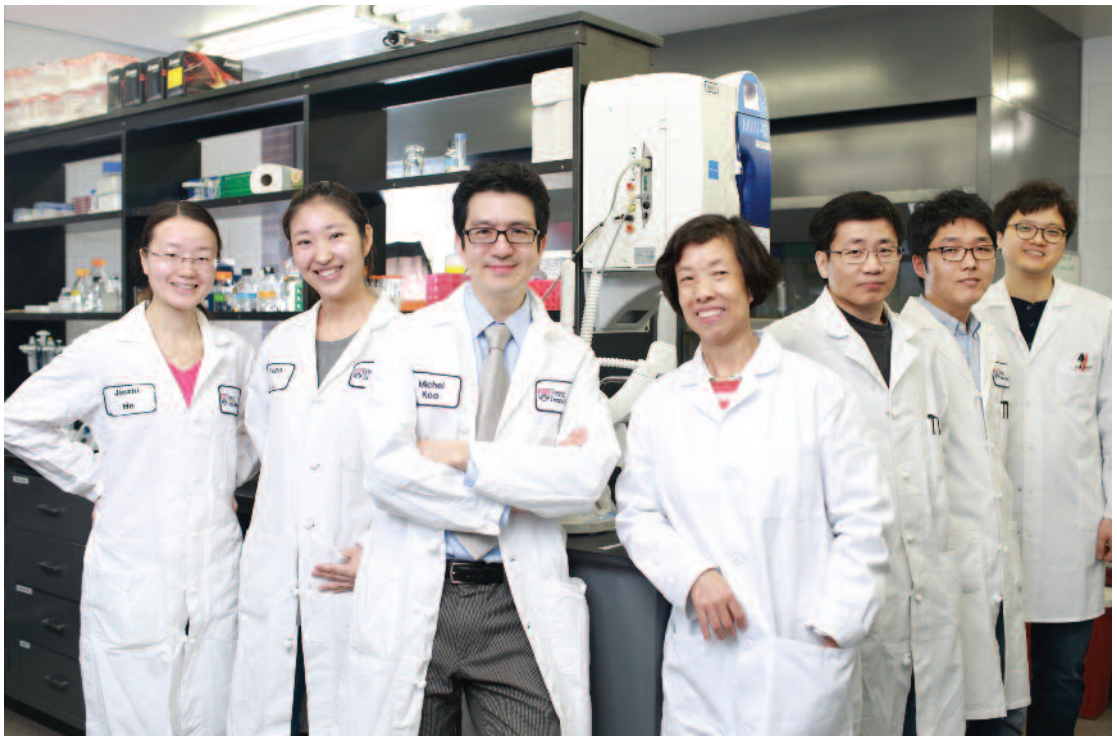
"One of the major challenges of topically-delivered compounds is rapid clearance of the agents in the mouth before they have time to exert their full therapeutic effect," he notes.

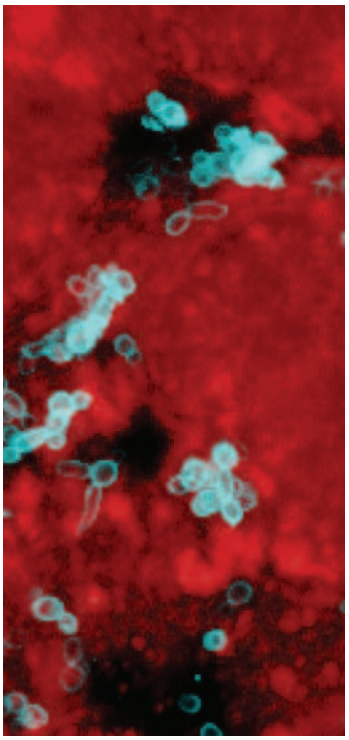
By engineering low-cost and high-adhesive nano-carriers, the therapeutics can be retained for longer periods of time, increasing drug efficacy. He hopes these technologies will lead to development of more-effective therapies against biofilm-associated oral diseases.

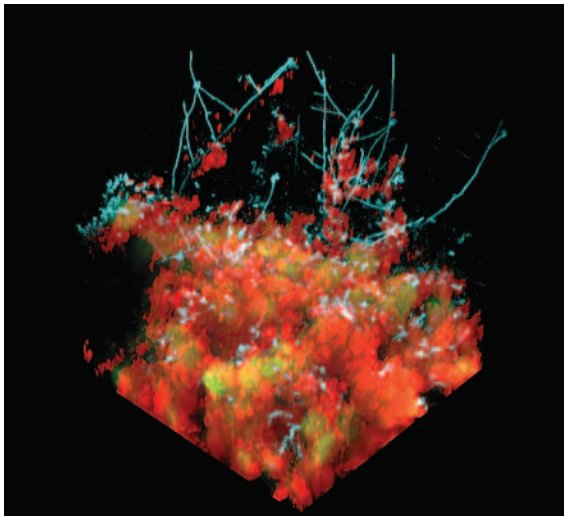
At Penn Dental Medicine, Dr. Koo sees himself able to fully realize the potential of these and other clinically relevant technologies.

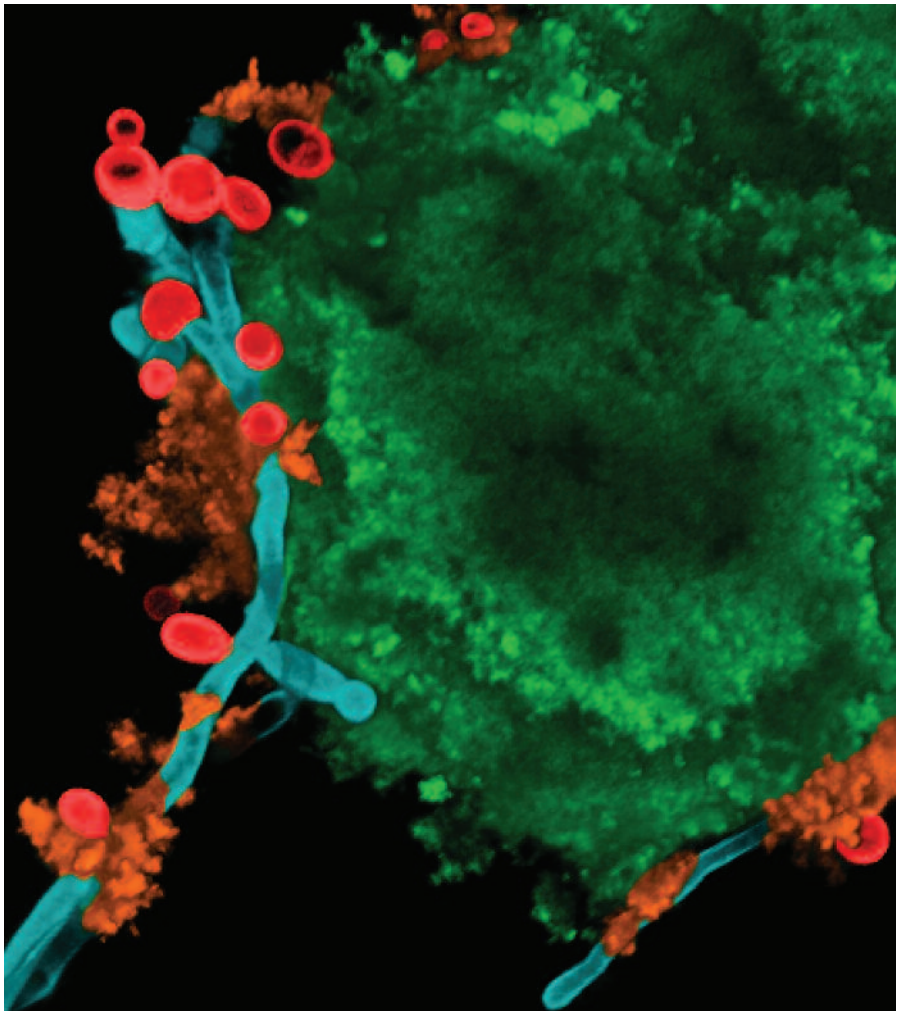
"There are so many ways different disciplines can help us, from biomedical sciences to nanotechnology to engineering approaches and Penn's philosophy is centered in promoting the integration of knowledge," he says. "I'm in the right environment with all the necessary support to further advance our mission of conducting innovative research and developing new therapies to make a difference." ■

—By Katherine Unger Baillie









TOP: Members of the Koo Lab, left to right: Jinzhi He, Dr. Yuan Liu, Dr. Michel Koo, Yong Li, Dr. Lizeng Gao, Dr. Geelsu Hwang, Dr. Dongyeop Kim.

ABOVE: The 3D architecture of a fungal-bacterial biofilm.

RIGHT: This close-up image of the biofilm illustrates the spatial relationship between *Candida albicans* cells and a *Streptococcus mutans* microcolony. Yeast (red) and hyphal forms of *C. albicans* (blue) are found associated in the periphery of the microcolony structure (green); specific areas where fungal cells are associated with the microcolony are highlighted in orange.