CLAY FOR THE CURE

(Above) Antibacterial clays can be created by combining laboratory synthesized smectite and pyrite. This powder, shown here at the bottom of a test tube, grows significantly when hydrated and can be applied topically to wounds. (Right) To test the antibacterial efficacy of the synthetic clays, Keith Morrison must work in a Risk Group 2 biosafety laboratory.

EACH year, nearly five million people die from illnesses in which antibiotic resistance played a role. Over one million of these deaths could have been prevented if there was a successful treatment for the bacterial infection. Antibiotic-resistant infections have caused more annual deaths than the HIV/AIDS epidemic, and the number is expected to rise to 10 million by 2050, outpacing the rate of cancer deaths. Addressing the rapidly growing inability to treat life-threatening infections requires novel approaches. One such approach involves clay, a type of fine-grained soil with minerals such as kaolinite.

Keith Morrison, a researcher at Lawrence Livermore, says, “Throughout history, clays have been used for their healing properties—clay baths and clay masks are just two examples. The use of clay has been passed down from generation to generation, but no studies exist to explain what makes a clay antibacterial.” Interest in clay as a potential solution to antibiotic-resistant infections entered the mainstream in the early 2000s when French philanthropist Line Brunet de Courssou used a topical clay application to treat Buruli ulcers—flesh-destroying bacterial infections that cause permanent disabilities, often requiring amputation—with great success.

Without rigorous scientific studies, the evidence for different clays’ antibacterial properties is anecdotal. Understanding of the mechanisms has been limited by insufficient records to indicate the sources of clays used for antibacterial applications and whether the clays were chemically treated. Moreover, the antibacterial effectiveness of natural clays can vary significantly from shovelful to shovelful, even within the same deposit of clay. Research to understand how clays enable processes or interactions with bacterial cells, and how these processes depend on specific characteristics present in clays, is key to unlocking how to use clays in clinical settings.

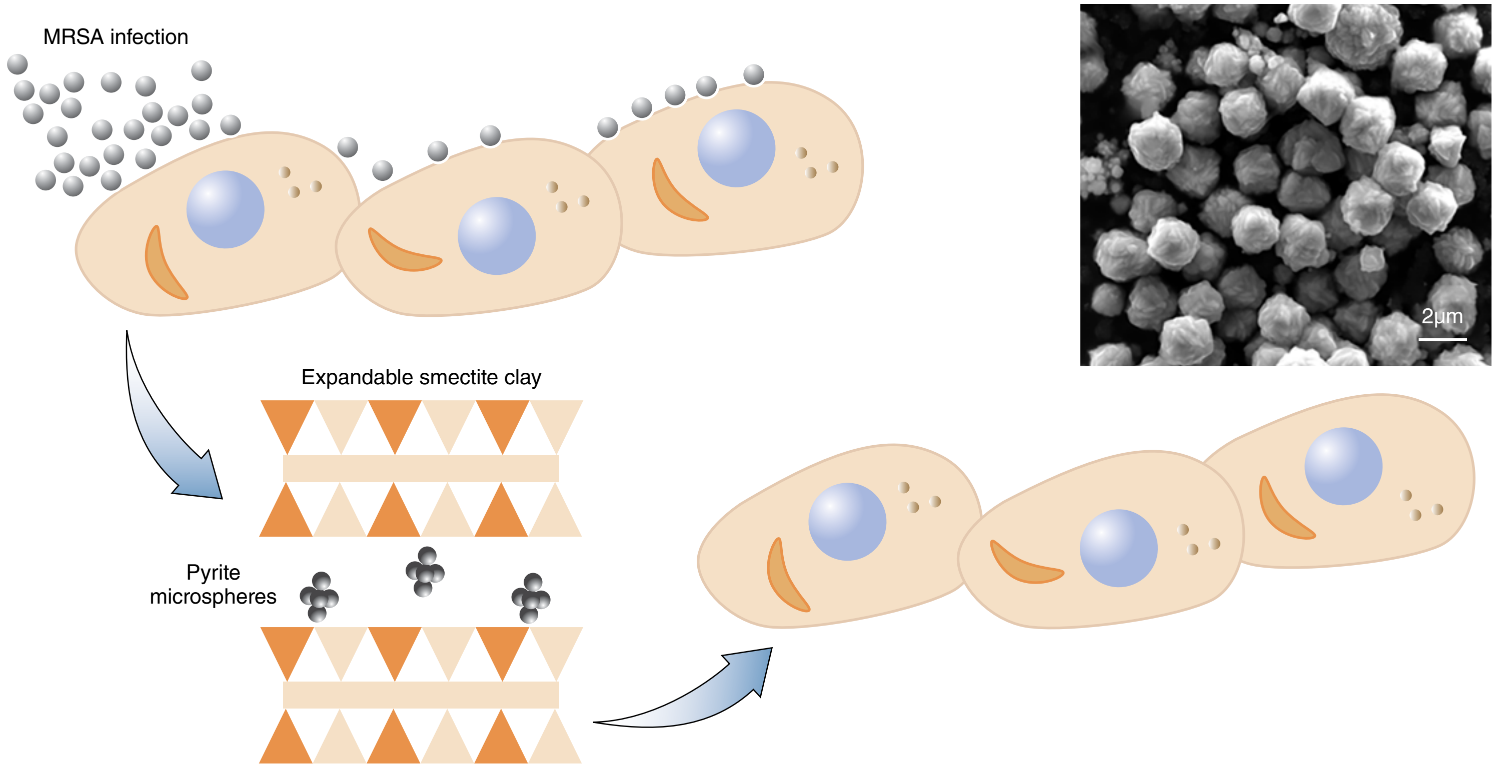
Mimicking Geology

To achieve clinical applications for antibiotic-resistant infections, the ability to first understand and then control clays’ geochemical processes (chemical processes that affect the amount, distribution, or structure of geological systems, such as clays) is important. Morrison began studying which properties—chemical and geological, among others—make certain clays antibacterial, and which properties do not, during his graduate studies at Arizona State University. Several factors make up a clay’s inhibition zone—its ability to act as an antimicrobial agent and inhibit microorganism growth—leading to bactericide. Morrison sought to isolate these factors and control them in a laboratory setting. “We realized we had to start from scratch and that if we were going to find the materials with the characteristics leading to bactericide in nature, we have to find and map the clays’ sources and study the geology, the zones in the clay that kill the bacteria, and how those zones formed,” says Morrison.

Morrison and his team found multiple factors that contribute to a clay’s ability to fight pathogens. Within the samples, every clay with antibacterial properties came from hydrothermally altered volcanic deposits, and each clay was a specific type called smectite—an extremely expandable class of clay that can adsorb water and exchange cations. “When hydrated with water, one heaping tablespoon of this clay can have the effective surface area of an entire football field,” says Morrison. Additionally, the smectite samples all contained pyrite, commonly known as fool’s gold, which contains iron and plays a role in strengthening the clays’ antibacterial mechanisms.

At Lawrence Livermore, Morrison received Laboratory Directed Research and Development funding to apply the findings on antibacterial clays’ specific properties to mimic the natural process of their formation in a laboratory setting. The team’s effort was the first-ever attempt at synthetically recreating a geochemical process for combating antibiotic resistance.

Morrison’s team creates both the smectite and the pyrite from each material’s initial components in the laboratory. Smectites, a mixture of silica, magnesium, and lithium building blocks



A topical application of synthetic antibacterial clay to a MRSA-infected wound, depicted by cells and their components (tan, blue, and brown figures) leads to the interaction of smectite and pyrite providing the wound with an abundance of ferrous iron (not depicted here), reacting with the bacteria, and restricting the infection's progression. A scanning electron microscope image (top right) depicts the structure of iron sulfide particles in synthetic antibacterial clays.

are relatively easy to create. The mixture is left to react for approximately a week, followed by removal of excess salts. Creating pyrite is a more complicated process, however. Research interest in pyrite mostly splits into nanotechnology and geology. For nanotechnology applications, synthesized pyrite is very small nanospheres, which react too fast for Morrison's use cases, but larger crystals used in geological studies react too slowly. As a result, the team developed their own technique to obtain the necessary size of pyrite spheres to mimic what they observed in nature. Through a trial-and-error process, the team discovered that reacting a mixture of polysulfide, polyvinylpyrrolidone, and ferrous chloride with ferrous iron (Fe^{2+}) resulted in uniform spheres approximately two micrometers in size.

When the synthesized smectite and pyrite are combined within the clay, the pyrite acts as a semiconductor, allowing electrons to flow through and produce iron and reactive oxygen species (ROS), which are highly reactive chemicals containing oxygen, such as hydrogen peroxide. This creates a cycle in which pH—which affects a wound's ability to heal—is held steady by the smectite clay. Levels of iron and ROS are also pivotal factors in clay's healing properties, as both iron and ROS are destructive to biomolecules. "By carefully tuning these parameters in the laboratory, we can tailor the clays to react in any way we want," says Morrison.

In natural samples, 95 percent smectite to 5 percent pyrite is the optimal ratio to achieve clay with antibacterial properties. Clay is exposed to the elements in natural environment, however. Natural antibacterial clays are hydrothermally altered, meaning interactions with water, such as frequent rain, oxidize the pyrite to release Fe^{2+}, which is then absorbed back into the clay. To imitate this process, Morrison's team reacted a small amount of the synthetic smectite with Fe^{2+}, leading to a continuous cycle of pyrite reducing ferric iron (Fe^{3+}) to Fe^{2+}.

The synthetic clay mixtures Morrison's team created successfully eliminated the full panel of the six ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter*) pathogens that result in the most global deaths due to antibiotic-resistant infections. With help from the Laboratory's Animal Care Facility, the team conducted topical application tests on hairless mice. The team pipetted Methicillin-resistant *Staphylococcus aureus* (MRSA)—a type of bacteria that causes antibiotic-resistant infections—onto two-centimeter-long wounds on the backs of groups of test mice and untreated control mice. For the test mice, they applied their synthetic antibacterial clay topically to the wound on days two and four after the infection and covered the site with a wound pad. The team also obtained data for control mice with wounds not infected with MRSA.

The Benefits of Iron

Typically, a robust biofilm forms over infected wounds. It is known that topical antibiotics have a hard time penetrating through the biofilm to fight the infection, but the antibacterial clay application showed much different results: The wounds treated with clay decreased in size by 71 percent over a period of eight days, and the bacterial cell concentrations were reduced by 98.2 percent. Meanwhile, the untreated wounds remained infected throughout the same period, and ultimately exhibited only a 36 percent decrease in wound area, reaching a plateau by day four. The uninfected control wounds showed no signs of injury by day eight, as expected. "When looking at a chronic nonhealing [infected] wound, normally, iron is the enemy because its presence can exacerbate the harmfulness of a pathogen," says Morrison. Although iron availability, like other metals, limits bacterial growth, too much of it is toxic to healthy cells. The researchers were surprised to find that overloading the wound with the high level of iron absorbed into the clay is effective as an antibacterial treatment.

Antibacterial treatments typically scavenge iron away from the bacteria to prevent such toxicity to healthy tissue. However, in the case of synthetic clays, the constant supply of soluble Fe^{2+} is rapidly taken up by pathogenic bacteria, which does not have a strong way to regulate this interaction, before the iron has a chance to irreparably damage the healthy neighboring cells. Though surrounding host cells experience acute toxicity, they recover once the bacterial infection is eliminated and the chronic wound cycle is broken. The continuous creation of Fe^{2+} through the interaction cycle of smectite clay and pyrite results in an abundance of Fe^{2+} with which the bacteria can react, restricting the infection's progression.

An important benefit of this synthetic approach is its tunability. "We want to reach that Goldilocks zone where we have just enough of the reactive species to kill the pathogen but not enough to damage skin cells," says Morrison. The antibacterial clays sustain longer reaction times that can more effectively heal a wound by providing constant antibacterial elements over the course of the healing process. To achieve the same effect, using metals alone would require higher, more toxic concentrations of metals.

A Humanitarian Security Concern

Morrison says Lawrence Livermore has a unique interest in work on synthetic clays for its security applications. Three decades have passed since the discovery of any new antibiotics, and the rise of resistance to existing antibiotics is becoming a national—even global—security issue. "Antibiotic resistance has been a problem since the discovery of antibiotics, but the issue seems to become a little worse every year," he explains. "We don't want to be caught in a scenario where there are no working antibacterials because we didn't start investing in a solution early enough."

Nicole Collette (left) manages the Animal Care Facility, where she and her team conducted the model tests for Keith Morrison (right) and his research collaborators.

Livermore provided Morrison and his team with several key resources to enable the team's groundbreaking study. In addition to access to the Animal Care Facility, where facility manager Nicole Collette and her team ran the mouse model tests, the Laboratory's Biosciences and Biotechnology Division provided Morrison's group with their own Risk Group 2 biosafety laboratory. The facility has biosafety controls to avoid contamination by agents associated with human diseases. To synthesize and characterize the minerals, the team used x-ray diffraction and scanning electron microscopy facilities in the Nuclear and Chemical Sciences Division. Optimizing access to and use of the resources at Livermore, the team currently has a patent pending for the clay synthesis method, formulation, and application process. Ahead of achieving clinical applications, Morrison and his team are exploring work with the cosmetic industry to present synthetic antibacterial clay as a skin product not marketed to treat an underlying disease, such as for acne treatment or as a facial mask. The licensing of the team's patent for such commercial purposes could help fund future clinical trials.

The researchers hope their efforts will eventually have important impacts on reducing bacterial infections and mortality. Beyond topical application, Morrison also envisions other ways in which synthetic antibacterial clays can combat antibiotic-resistant infections, including a potential agricultural implementation. For example, the clays' antibacterial properties enable treatment of the solid municipal waste and sewage used to fertilize agricultural fields, which often contribute to antibiotic-resistant infections in humans in surrounding cities and towns. The clays can also occasionally be mixed into animal feed on farms to help prevent intestinal infections. "If adding too many antibiotics to cattle and pigs can be avoided, that's a win for everyone," says Morrison.

—Anashe Bandari

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