

Stopping tooth decay before it starts—without killing bacteria

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Oral bacteria are ready to spring into action the moment a dental hygienist finishes scraping plaque off a patient's teeth. Eating sugar or other carbohydrates causes the bacteria to quickly rebuild this tough and sticky biofilm and to produce acids that corrode tooth enamel, leading to cavities. Scientists now report a treatment that could someday stop plaque and cavities from forming in the first place, using a new type of cerium nanoparticle formulation that would be applied to teeth at the dentist's office.

The researchers will present their progress toward this goal today at the American Chemical Society (ACS) Fall 2020 Virtual Meeting & Expo.

The mouth contains more than 700 [species of bacteria](#), says Russell Pesavento, D.D.S., Ph.D., the project's principal investigator. They include

beneficial [bacteria](#) that help digest food or keep other microbes in check. They also include harmful streptococcal species, including *Streptococcus mutans*. Soon after a cleaning, these bacteria stick to teeth and begin multiplying. With sugar as an energy source and building block, the microbes gradually form a tough film that can't easily be removed by brushing. As the bacteria continue metabolizing sugar, they make acid byproducts that dissolve [tooth enamel](#), paving the way for cavities.

Dentists and consumers can fight back with products including stannous fluoride to inhibit plaque, and [silver nitrate](#) or silver diamine fluoride to stop existing tooth decay. Researchers have also studied nanoparticles made of [zinc oxide](#), copper oxide or silver to treat dental infections. Although bactericidal agents such as these have their place in dentistry, repeated applications could lead to both stained teeth and bacterial resistance, according to Pesavento, who is at the University of Illinois at Chicago. "Also, these agents are not selective, so they kill many types of bacteria in your mouth, even good ones," he explains.

So, Pesavento wanted to find an alternative that wouldn't indiscriminately kill bacteria in the mouth and that would help prevent tooth decay, rather than treat cavities after the fact. He and his research group turned to [cerium oxide nanoparticles](#). Other teams had examined the effects of various types of cerium oxide nanoparticles on microbes, though only a few had looked at their effects on clinically relevant bacteria under initial biofilm formation conditions. Those that did so prepared their nanoparticles via oxidation-reduction reactions or pH-driven precipitation reactions, or bought nanoparticles from commercial sources. Those prior formulations either had no effect or even promoted biofilm growth in [lab tests](#), he says.

But Pesavento persevered because the properties and behavior of nanoparticles depend, at least

partially, on how they're prepared. His team produced their nanoparticles by dissolving ceric ammonium nitrate or sulfate salts in water. Other researchers had previously made the particles this way but hadn't tested their effects on biofilms. When the researchers seeded polystyrene plates with *S. mutans* in growth media and fed the bacteria sugar in the presence of the cerium oxide nanoparticle solution, they found that the formulation reduced biofilm growth by 40% compared to plates without the nanoparticles, though they weren't able to dislodge existing biofilms. Under similar conditions, silver nitrate—a known anti-cavity agent used by dentists—showed no effect on [biofilm](#) growth.

"The advantage of our treatment is that it looks to be less harmful to [oral bacteria](#), in many cases not killing them," Pesavento says. Instead, the nanoparticles merely prevented microbes from sticking to polystyrene surfaces and forming adherent biofilms. In addition, the nanoparticles' toxicity and metabolic effects in human oral cells in petri dishes were less than those of silver nitrate.

Pesavento, who was awarded a patent in July, would like to combine the nanoparticles with enamel-strengthening fluoride in a formulation that dentists could paint on a patient's teeth. But, he notes, much work must be done before that concept can be realized. For now, the team is experimenting with coatings to stabilize the [nanoparticles](#) at a neutral or slightly basic pH—closer to the pH of saliva and healthier for teeth than the present acidic solution. His team has also begun working with bacteria linked to the development of gingivitis and has found one particular coated nanoparticle that outcompeted stannous fluoride in limiting the formation of adherent biofilms under similar conditions. Pesavento and his team will continue to test the treatment in the presence of other bacterial strains typically present in the mouth, as well as test its effects on human cells of the lower digestive tract to gain a better sense of overall safety for patients.

More information: Nanoceria reduce in vitro *Streptococcus mutans* biofilm adherence:
Applications in oral medicine:

Abstract

Streptococcus mutans has long been a target of interest for antimicrobial therapy in the field of oral medicine. *S. mutans* produces robust tooth-borne biofilms that serve a key etiological factor in the progression of dental caries and odontogenic infections. Silver-based bactericidal agents have proved effective at reducing oral *S. mutans* proliferation, yet their repeated administration has raised concerns of emerging bacterial resistance and deleterious effects on oral microbiota. Tooth-applied biofilm inhibitors with non-lethal mechanisms of action have offered a novel approach for both limiting biofilm formation and reducing effects on the entirety of the oral microbiome. The use of nanoparticles in this capacity has received considerable interest in recent years. Although several studies have focused on the antimicrobial effects of cerium oxide nanoparticles (nanoceria, CeO₂-NP) few have focused on their effects on clinically relevant bacteria under the initial biofilm formation conditions. In this work, nanoceria derived solely from Ce(IV) salt (i.e., ceric ammonium nitrate, CAN; ceric ammonium sulfate CAS) hydrolysis were found to reduce adherent in vitro *S. mutans* growth in the presence of sucrose by approximately 40% while commercial dispersions of "bare" nanoceria (3 nm, 10-20 nm, 30 nm), Ce(NO₃)₃ (CN) or ammonium salts (AN, AS) alone were either inactive or observed to slightly increase biofilm formation under similar in vitro conditions. Planktonic growth and dispersal assays support a non-bactericidal mode of biofilm inhibition active in the initial phases of biofilm production. Human cell proliferation assays suggest only minor effects of hydrolyzed Ce(IV) salts on cellular metabolism at concentrations up to 1 mM Ce, with less observed toxicity compared to equimolar concentrations of AgNO₃ - a long used intraoral antimicrobial agent. The results presented herein have potential applications in the field of oral medicine.

Provided by American Chemical Society

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