BioSpectrum the business of Bio & Health Sciences

Scientist uses Raman laser for early detection of bacteria

12 May 2017 | Interviews

The research paper co-authored by Dr Balaprasad Ankamwar of Savitribai Phule Pune University is published in the journal of UK's Royal Society of Chemistry

The professional life of a scientist revolves around studies and experiments. Each experiment, whether successful or a failure, does not change the scientist's perseverance. It simply proves or disproves the hypothesis that lead to the study, giving collected data to work further and taste success. And the script is same for Dr Balaprasad Ankamwar, Associate Professor at Bio-Inspired Materials Research Laboratory, Department of Chemistry, Savitribai Phule Pune University (Formerly, University of Pune). With interest in crystal growth, calcium carbonate, synthesis of Graphene, conducting metal composites, Dr Ankamwar owes his interest to nanotechnology, biosynthesis of nanomaterials, semi conducting nanomaterials, chemical and biomedical applications of nanomaterials to his 'Nano Guru' Murali Sastry, CEO of IIT-B-Monash Research Academy.

Along with Ujjal Kumar Sur and Pulak Das, Dr Ankamwar has found a fast and innovative way to identify presence of bacteria, even in small quantity using surface-enhanced Raman scattering (SERS) phenomenon. The research paper, titled 'SERS study of bacteria using biosynthesized silver nanoparticles as the SERS substrate', was published in Analytical Methods (www.rsc.org/methods), a journal published by The Royal Society of Chemistry, United Kingdom, and is among the top 25 accessed research works of 2016.

Speaking to BioSpectrum, Dr Ankamwar shares the experiment and observations.

How far have you have come with your current study?

It is still a very early stage. We have only dealt with two non-infectious strains — *Staphylococcus aureus* and *Escherichia coli* . The problem we are facing is with infectious bacterial or viral strains that cannot be used in an open environment. We need special safety environment like laminar flow hoods or sealed polymer containers to carry out such tests. We are not sure how effectively our technique will work under these conditions and need to check that in the coming time.

Is the technique very expensive?

Recently, I made an enquiry regarding the instrument involved in our physics department. It is really very costly. I don't know the exact price, may be more than Rs 2 crore. When I inquired from a Swedish group and a seller in Delhi for the instrument for my project, I was given an estimation of Rs 50 lakh for a table top model. The high cost is mainly because of the laser in the instrument. However, the seller agreed to give a concession of Rs10-15 lakh if I bought more lasers.

How many lasers are required for the technique?

One laser is required for our work. We can use other types of lasers also but are yet to check on that. We are presently using the three lasers of SPPU physics department for the experiment. I need to test it for other lasers also and add the information to our earlier collected knowledge.

How many bacterial strains have you used for your study so far?

We have only used two strains so far — *E. coli* and *Staphylococcus aureus.* We have not done studies on other strains yet, but we plan to work on mycobacterial species. But since it is an infectious agent, we need to work out the safety measures for that. Our research started with study of bacteria particularly *E. coli*, which is associated with urinary tract infection (UTI), a common disease among people of all age groups in developing countries. We were thinking on that line when my friend Dr Vijay Rani Sood, Professor and HOD, Applied Sciences and Humanities Department, Uttar Pradesh Technical University (UPTU) suggested that we can try with Raman spectroscopy and we got amazing results with that. Even though a table top model is available in our physics department, the only constraint is the use of proper and safe environment while dealing with infectious bacterial or viral strains. The method can be used to detect many diseases as Raman shift gives number of signals corresponding to bacteria. We need a good databank of various strains with subsequent Raman data for each so that when we are running samples, we can easily relate and compare the data with the information we already have in our databank.

How much time is required to implement the study further?

It may take one or two years before we can have some substantial results. Right now, there are many limitations. Rules and regulations have to be followed, many permissions to be taken and MoUs have to be signed. The biotechnology and microbiology department of SPPU can supply us with many samples of different strains and I can go ahead with the testing, with non- infectious samples easily and health centres can provide samples for our tests, as clinical samples.

Are you planning to use your study in developing diagnostic kits in the future?

Colorimetric assays have been more commonly used for diagnostic purposes as compared to spectroscopic ones like the Raman technique. These instruments are very expensive. Only when we are able to make an economical tool for our tests can it be used as diagnostic kits for general public use. We are using different substrates like glass, silica and other materials for exploring options for our assays. We need time to work in this direction. We need authorisation from physicians for handling pathogenic strains. If kits are to be developed, then hospitals and research centres can help us, as it can be of great use in detection especially in an epidemic situation. A lot of cost is required in the sample testing process and infrastructure is costly.

Have you thought of any collaboration?

We applied for the UK's Longitude Prize. It is a £10 million prize fund rewarded to a diagnostic test that helps solve the problem of global antibiotic resistance. It is run by Nesta and supported by Innovate UK as funding partner. They have two main conditions for eligibility, namely to prepare a model of our study for detection purpose and collaborating with a UK-based firm to take our technique further. If selected, the organisers will cover all expenses as they take us around the world with our experiment. As there is a lot of competition for this award, we are working on it. We have also been asked regarding the current status of commercialisation for our product.

Have you approached the private sector for support?

Not yet. I thought to approach the government sector first. There is slow pace with the government and I have already lost 2-3 months. I am looking forward to support from private sector. My only concern is that the results I get should be authentic, while dealing with the various biosafety arrangements.

Since how long have you been working on this idea?

I started working on nanoparticles at National Chemical Laboratory (NCL), Pune, with Dr Sastry in 2003 but took up the SERS project three years back. A databank will be the guideline for samples taken from patients. Suppose we take a pre-cancerous sample and we get some signals, we can compare with the existing data obtained from other techniques. Then we can think further. Recently, doctors could not diagnose the ailment my friend's wife was suffering from despite taking various tests done. The SERS test can help detecting the bacteria fast and pre-diagnostic test can be of use. Diseases at different stages can be studied and the results can be stored in a databank and the information can be then used for diagnosing conditions of other patients.

Do you think your study will move fast once it reaches the foreign shore?

Yes. I am working on the prototype and model to take part in the Longitude Prize. They are supporting my idea. Recently I met a scientist at Bhabha Atomic Research Centre (BARC) and he told me that the cost of the table top model of the instrument can come down to Rs 10-15 lakh but I don't know whether the quality of tests would be affected. The table top model has its own advantages, as it can be carried around easily. A concern I have is that once we start getting data from various strains from SERS spectra, then we need to be sure of the correct interpretation, which is only possible if we have a good database. Only then we can compare our results and come out with the right diagnosis and information regarding a particular sample and condition. Although database resources for standard Raman spectra of biological samples like different bacteria are gradually becoming available, it is not always the situation where the peaks illustrated in a Raman spectrum will also be observed in the SERS spectrum of the same sample. Therefore, one can differentiate known or unknown pathogens rapidly within a few seconds such as bacteria using the SERS spectra.

What are the limitations of your experiment?

The SERS based detection method of bacteria cannot differentiate one strain from another within bacterial species. The SERS spectra lack the molecular level specificity compared to genome sequencing or mass spectrometric based proteomics analysis. Further improvement in the instrumentation of the micro-Raman system as well as data analysis may enhance the differentiation power. We hope that we can detect a single bacterium using our SERS substrate in the near future. At present, we are involved in the SERS-based detection technique of Mycobacterium groups with species such as *Mycobacterium tuberculosis*, which is known to be resistant to most of the common drugs. We are also studying the effect of antibiotic exposure on drug-sensitive bacteria studied by SERS using our biosynthesized Ag nanoparticles. We expect that one can detect a single bacterium using our biosynthesized SERS substrate in the near future.

Did you get financial support?

We were funded by the University Grants Commission (UGC), New Delhi and University Grants Commission-Department of Atomic Energy-Consortium for Scientific Research (UGC-DAE CSR), Kolkata centre, Collaborative Research Schemes. Indian National Science Academy (INSA), New Delhi and Board of College and University Development (BCUD), University of Pune also supported it. Apollo Hospital, Kolkata provided us clinical bacterial isolates from patients with Urinary Tract Infections.

The process

The researchers used biosynthesised silver nanoparticles obtained from the leaf extract of *Neolamarckia cadamba* as a surface-enhanced Raman scattering (SERS) active substrate to detect bacteria, even in small quantity, within a very short time of 1–5 sec. The developed SERS substrate provided a highly reproducible, stable and uniform Raman signal with a large enhancement factor and almost zero fluctuation. They worked on two bacterial strains, Staphylococcus aureus and Escherichia coli. The detection method is useful for analysis of slow-growing bacteria, which typically may take weeks during laboratory tests. Neolamarckia cadamba was used as an eco-friendly and cost-effective biosynthesised silver nanoparticles as the source of reducing and stabilising agents and the technique helped in rapid microbial diagnostics. The mixture is exposed to Raman laser in a process called SERS spectroscopy and the signals get enhanced manifold in the presence of nanoparticles. The laser falls on the bacteria present at the interface of silver nanoparticles and different bacteria give different signals. The bacteria is identified by looking at the Raman shift it produces. The result will help in detection of infection at its inception.

SERS spectroscopy as a spectroscopic analytical tool provides large enhancement of weak Raman signal and facilitates suitable identification of chemical and biological systems. It is a powerful tool to identify pathogens like bacteria with different cell wall compositions. The SERS spectrum generated by illuminating the whole bacterium as it interacts with the silver nanoparticles revealed the molecular composition within ten nanometers of the outermost bacterial envelope. The study also shows that biosynthesised silver nanoparticles used as the SERS substrate is very stable under atmospheric conditions even after three months.