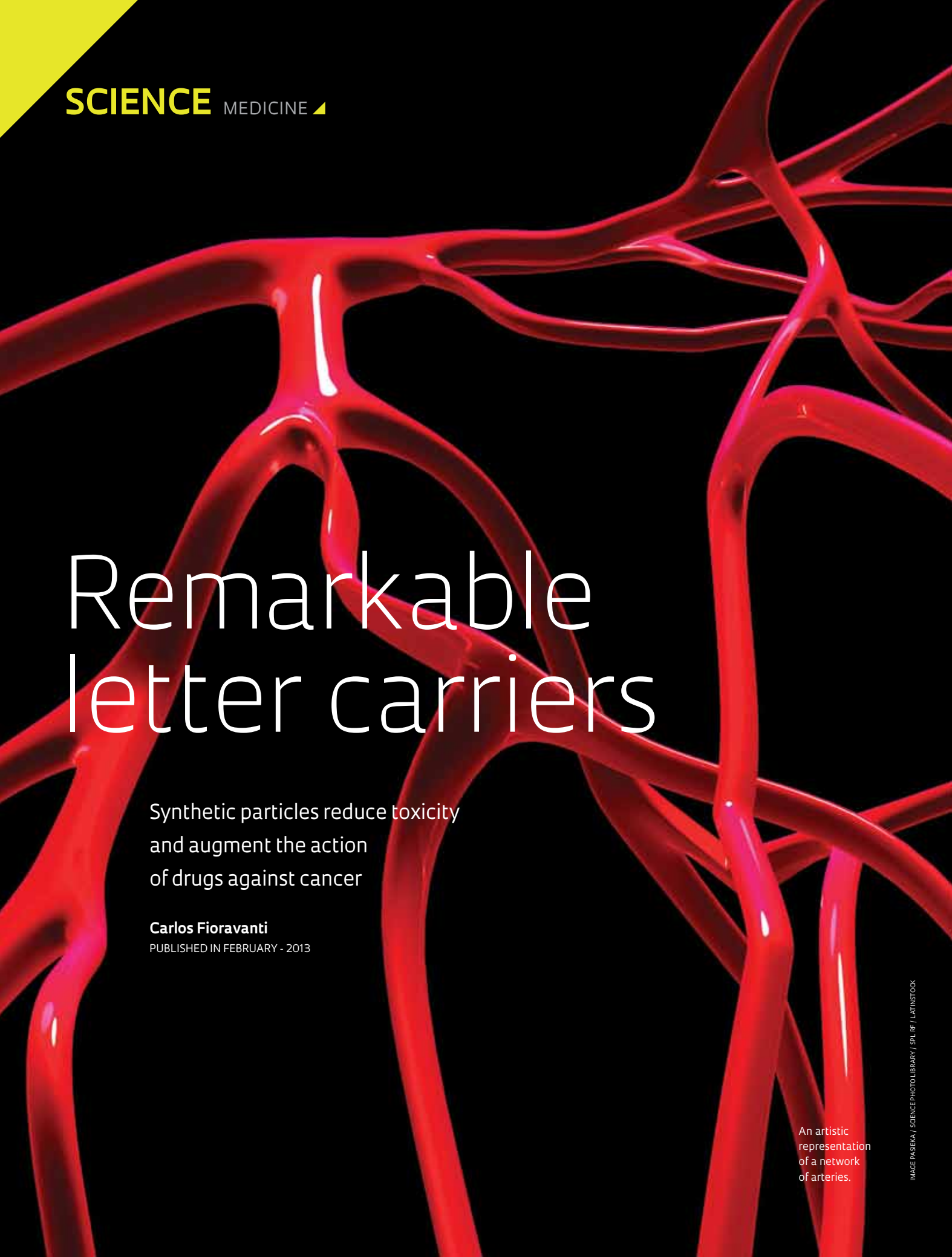

# Remarkable letter carriers

Synthetic particles reduce toxicity and augment the action of drugs against cancer

**Carlos Fioravanti**

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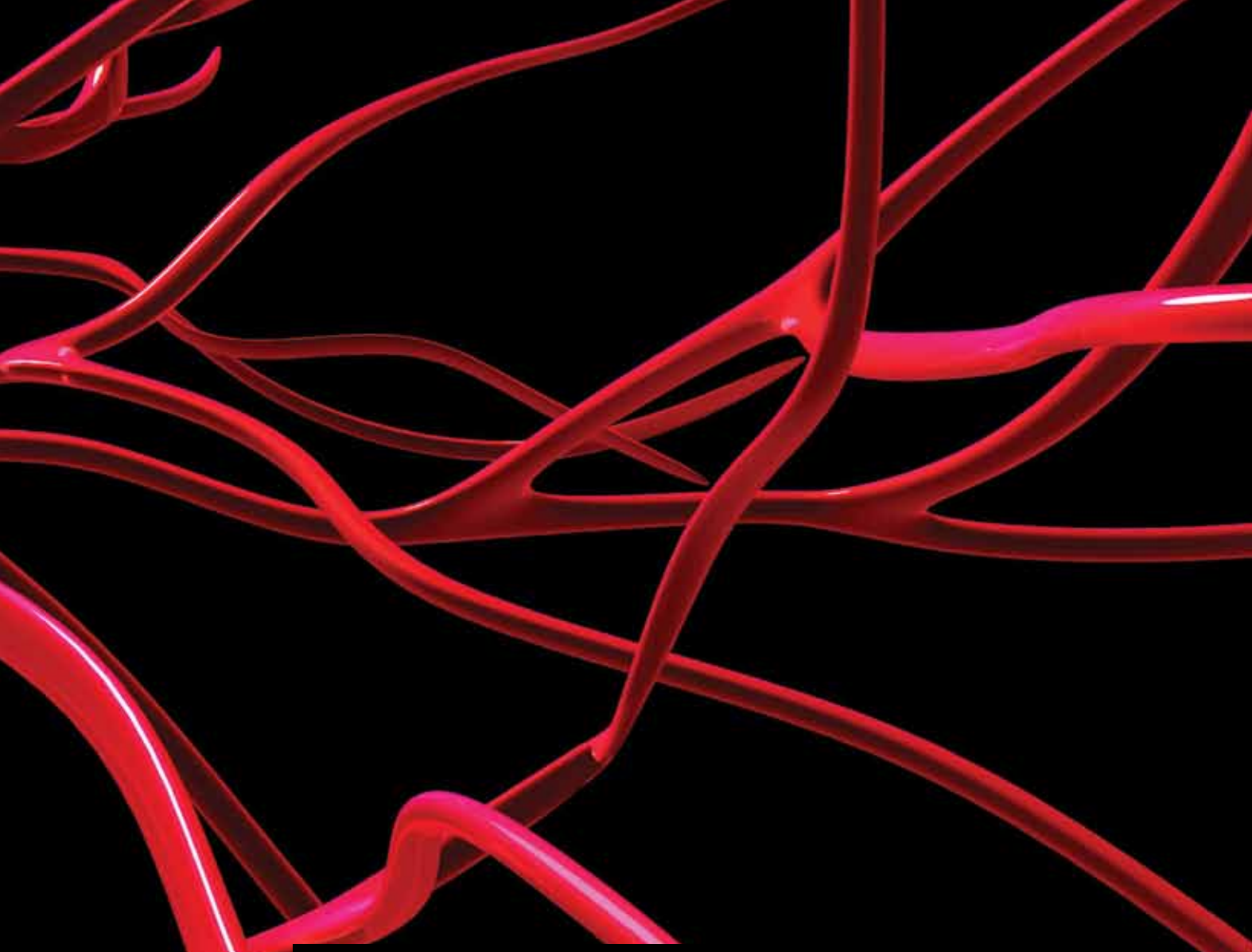
An artistic representation of a network of arteries.

IMAGE PASIEKA / SCIENCE PHOTO LIBRARY / SPL RF / LATINSTOCK



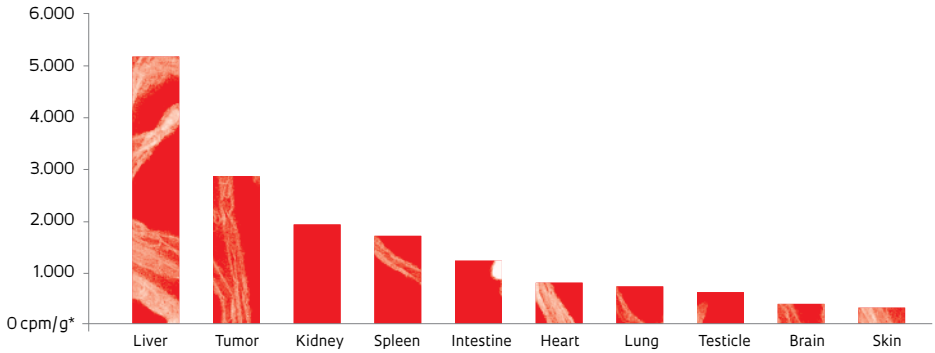
Twenty-one years ago, when Dr. Raul Maranhão presented his cancer treatment strategy—based on artificial, compact, cholesterol particles—in national and international scientific journals, he did not imagine that he would encounter so many surprises, disappointments, setbacks and detours in moving his idea forward. Systematically, he experimented using animal models and then on limited groups of people with cancer, concluding that lipid nanoemulsions (known as LDEs) could act as a drug platform. LDEs would be able to carry drugs to predefined targets and reduce toxicity, which is a serious problem and very common in anti-cancer therapies. Often, the unwanted effects of these therapies are severe enough to limit their use, forcing a reduction in dosage or cessation of treatment. "We mastered some chemotherapeutics," said Dr. Maranhão, with equanimity earlier this year from his basement office at the University of São Paulo (USP) Heart Institute (Incor).

He then brought up on his computer screen a table summarizing the undesirable side effects that arose in 46 people treated with carmustine, a highly toxic anti-tumor drug, combined with spheres of artificial cholesterol. With this treatment, the most common side effects—such as nausea, vomiting, alopecia (hair loss), anemia, severe loss of immune cells and hepatic or renal changes evaluated at grades 1, 2 and 3—were minimal, even at a dosage of 350 milligrams, nearly three times more than the dosage normally administered. "The results are very consistent, with no loss of pharmacological action," he says.

Making these strategies work is not easy. There is a global battle to reduce drug toxicity, which could result in more treatment options for physicians and less discomfort for people undergoing treatment. Teams from the State University of Campinas (Unicamp) and the Butantan Institute are also making progress with other types of particles that are proving effective in improving the action of drugs and vaccines. In each case, new combinations must pass all the safety and toxicity tests in animal models and in humans, and if

## PRINCIPAL DESTINATIONS

Concentration of cholesterol particles (LDE) with taxol, labeled with radioactive elements in the tissues and organs of mice




* Cpm / g: particle radioactivity per gram of tissue

**SOURCE** LIPID LABORATORY / INCOR-USP

they perform satisfactorily in all of these experiments, they will be approved for widespread use.

Dr. Maranhão had to overcome many scientific, technical and bureaucratic obstacles before successfully concentrating the drug-containing spheres in tumors *(see graph above)*. Cholesterol-rich LDEs, with a structure similar to low density lipoprotein (LDL) and a diameter of 20 to 60 nanometers (one nanometer is one millionth of a millimeter), are captured by cells through LDL receptors, which are abundant in tumor cells. "We deceived the tumor cells by providing a raw material they need in order to multiply, combined with a drug that will destroy them," he says.

Two other anti-tumor drugs, paclitaxel (Taxol) and etoposide, provided them with more work to do. With Hélio Stefani, a colleague from the USP School of Pharmaceutical Sciences, Dr. Maranhão soaked the LDE spheres with fatty acids to augment the adherence of the anti-tumor drugs. "In tests in mice and people with cancer," he said, "the toxicity decreased dramatically." However, this does not always work. In fact, the toxicity of methotrexate did not decrease when combined with LDE spheres. "I cannot explain why, but it may be possible to reduce the dosage, since the combination with the LDEs augments the absorption of the drug by tumor cells."

### TESTING IN HOSPITALS

Intense scientific work and the results of initial clinical trials conducted since 1990 on approximately 200 people have served as the motivation behind two broader clinical trials that are in progress at public hospitals in the city of São Paulo. In one study, 23 people, after undergoing other treatments for prostate, breast, ovarian and lung cancer, some with bone metastases, are now receiving Taxol and LDE at the Arnaldo Vieira de Carvalho Cancer Institute. In two men, the level of prostate specific antigen (PSA) decreased from 100 to 10 nanograms per deciliter of blood, indicating the regression of prostate cancer after seven months of treatment, according to Dr. Sylvia Graziani, a physician at the Institute's oncology clinic. "In ovarian and breast cancers, we observed a stabilization of the disease and a significant improvement in clinical status due to the absence of adverse effects common to chemotherapy," she says. "I saw a number of patients having lunch while undergoing chemotherapy," says Dr. Maranhão. The medications used to prevent vomiting, a common side effect of treatment, have been eliminated.

"LDEs have a high affinity for inflamed tissues and areas of intense cell division," says Dr. Maranhão. This characteristic led to other potential uses, such as treating atherosclerosis, a chronic inflammatory disease characterized by the buildup of fatty plaque in the arteries and veins. In one experiment, the arteries of rabbits with cholesterol plaques, somewhat similar to tumors, absorbed three times more LDEs than other tissues or organs. "The LDEs with paclitaxel cleaned the rabbit arteries," he said, as he showed a succession of photos and graphics. The information he collected was used as the basis for testing the safety of combining taxol with LDE in a group of 10 people selected for treatment at the Dante Pazzaneze Institute of Cardiology in São Paulo. The results, detailed in an article in the final stages of preparation, indicate that this strategy can reduce the inflammation that accompanies the formation of fatty plaques in the coronary arteries of people who have already suffered a heart attack. "We have almost zero toxicity," he

**IMAGES 1** LQES / UNICAMP **2 & 3** INCOR / USP

## Carbon nanotubes activate immune cells and augment the response to antigens

concludes. This evidence is also impressive because it supports the use of an anti-tumor agent such as taxol to treat heart disease.

### NEW VACCINES IN SIGHT

Particles that carry drugs can do more than expected, and they can change other characteristics that often hinder drug efficacy. The Unicamp team of Chemist Oswaldo Alves has apparently overcome a limitation of camptothecin, an anti-tumor agent that is difficult to apply because of its insolubility, according to tests performed on tumor cells at the Federal University of São Paulo (Unifesp).

"We made a slurry with silica nanoparticles, and the camptothecin entered the cells as if it were soluble," says Amauri Jardim de Paula, one of the Alves researchers. "Today, we can synthesize nanostructures with a high degree of control over the chemical and physical properties on the surface and inside so that the surface can attract and the interior repel water molecules."

In the cover story of the October 2012 issue of the *Journal of the Brazilian Chemical Society*, Alves makes an important statement concerning the safety of using mesoporous silica nanoparticles: these nanostructures do not destroy red blood cells under real-life conditions when immersed in plasma, regardless of the electrical charge on the surface of the particles; previous studies performed on a cell solution have suggested that this could be the case.

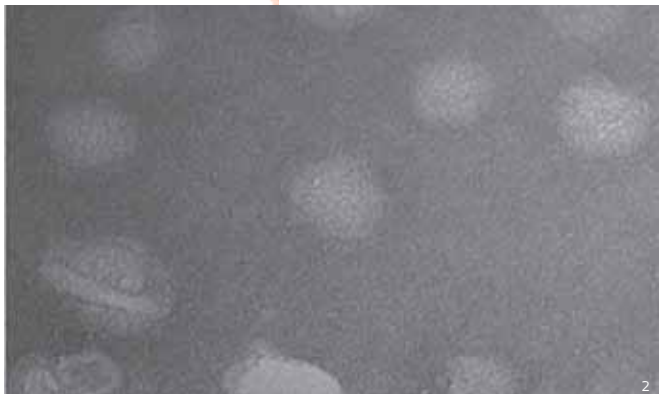
A type of nanostructured porous silica called SBA-15 is proving effective in transporting vaccines orally, according to studies conducted since 2002 in collaboration with the University of São Paulo, the Butantan Institute and the Laboratório Cristália. Tests with the hepatitis B vaccine in mice indicate that viral antigens carried by silica spheres were able to pass through the extremely acidic environment of the stomach, which most proteins cannot withstand, and be absorbed in the intestines.

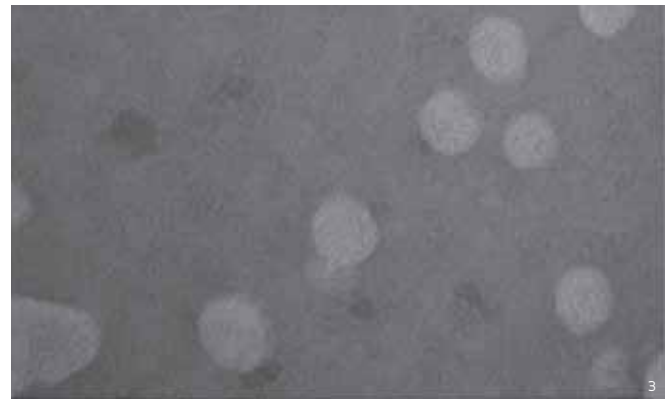
"We are now in the process of planning tests on human volunteers," says Osvaldo Sant'Anna, a Butantan researcher who is excited about the possibility of expanding oral vaccinations that are currently limited to the Sabin polio vaccine. He believes that if the tests go forward with positive results, silica particles could carry more than one vaccine at a time, in a manner more benign to the body than current techniques of application (injections), and they could yield considerable savings by eliminating the use of needles and syringes. These silica particle-based delivery vehicles could also increase the number of people vaccinated and enable the application of doses smaller than those presently administered.

Diego Stéfani Teodoro Martinez, of the Alves team, prepared carbon nanotubes of 10 to 40 nanometers in diameter and up to 10 micrometers in length soaked in antigens. This combination increased the body's response compared to the response induced by antigens alone in preliminary tests in mice at the Butantan Institute, in partnership with Sant'Anna. "Nanotubes apparently have an immunostimulatory effect," says Martinez, "possibly because they activate macrophages, a type of immune cell, and the release of cytokines," which are the communication molecules of the immune system.

Other combinations of carriers and drugs may also yield impressive results. Unicamp, USP and

Special vehicles: purified carbon nanotubes (*in red*) and lipid nanoemulsion alone (*left*) and with methotrexate (*right*).



Unesp groups coordinated by Wagner Fávaro and Nelson Duran, both of Unicamp, were able to reduce the dosage of the P-MAPA immunomodulator agent by a factor of approximately 10 without a loss of efficacy against bladder cancer by using a commercial polymer known as poloxamer or pluronic in preliminary tests in animal models.

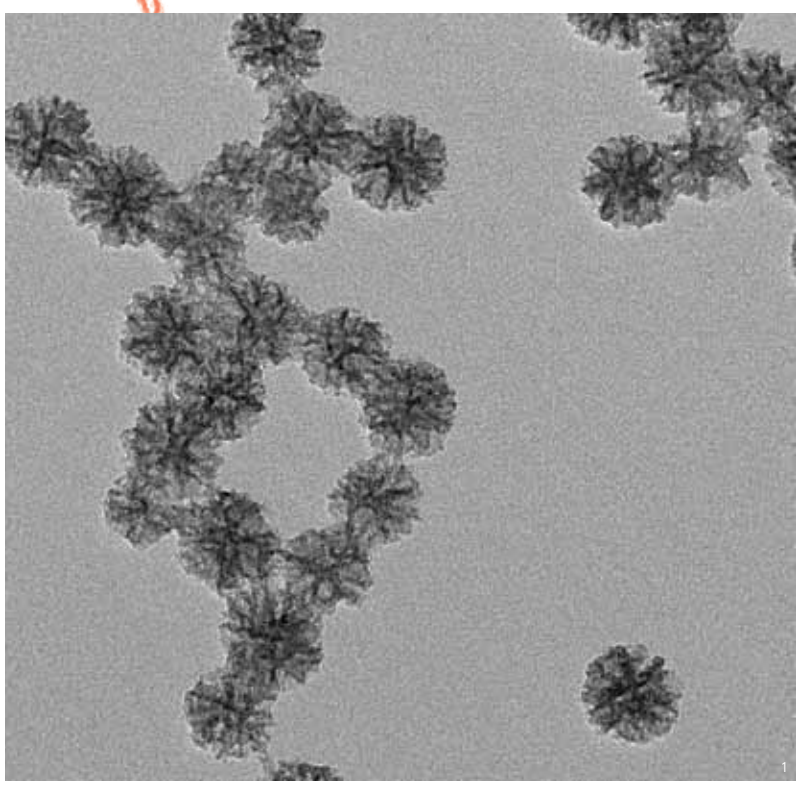
"The pluronic must have facilitated the entry of the P-MAPA into the layer of cells lining the inside of the urinary bladder," says Fávaro. "It's an excellent result." If confirmed in future studies, this effect may allow a considerable savings in drug costs and improve drug action, in addition to reducing the risk of any toxic effects. The P-MAPA and LDE spheres apparently do not lead to adverse reactions, nor do they present health risks, according to the tests performed thus far.

In studies conducted at Unicamp and Colorado State University in the US—with funding from the research network Farmabrasilis, FAPESP, the National Council for Scientific and Technological Development (CNPq), the US National Institutes of Health (NIH) and Unesp of Botucatu—P-MAPA has been shown to halt the progression of bladder cancer and slow the growth of bacterial colonies that cause tuberculosis in animal models. In studies on rats with bladder cancer, P-MAPA exhibited superior efficacy compared to the BCG vaccine, the best available treatment against this disease, as detailed in an article published in the June 2012 issue of the journal *Infectious Agents and Cancer*.

Results that now appear simple to understand were, at first, difficult to interpret. In 2006, Oswaldo Alves was faced with the following dilemma: should I purchase or synthesize the carbon nanotubes? He decided to buy them, but he noted that the nanotube sample was not a pure substance, as it should be, and this caused Alves to doubt the results of the experiments—positive or negative—uncertain whether the outcomes he observed were caused by the action of the nanotubes or the impurities.

**"Almost no one is questioning the origin and purity of the nanostructure samples used in experiments," says Alves**

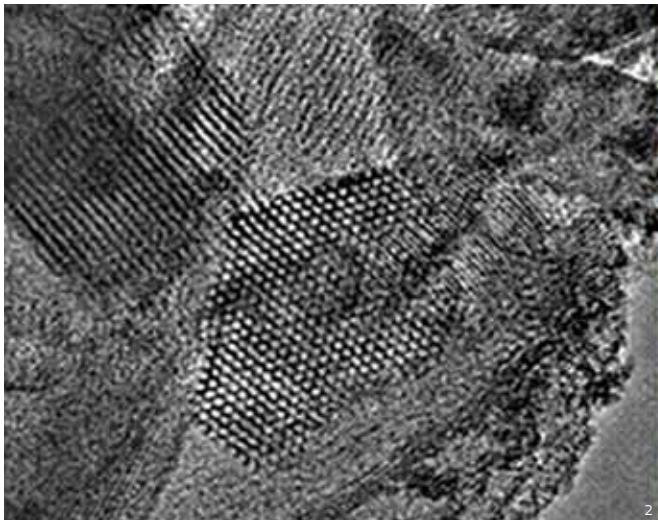
"Purity has been a neglected subject," he says. "Because of the rush to publish results, almost no one is questioning the origin of the nanostructure samples used in experiments; there is an assumption [that] they are pure." Alves and his group developed their own purification techniques, and they now remove 98% of the metallic and carbonaceous residue from the imported nanomaterials. The resulting purified substances from Unicamp's Solid State Chemistry Laboratory are so different from the original that they have been named LQES-1 and LQES-2. Another ongoing challenge, he said, is "to identify other chemists, physicists, biologists and physicians who want to work with nanostructures and believe that it is possible to bring quality to Brazilian science."

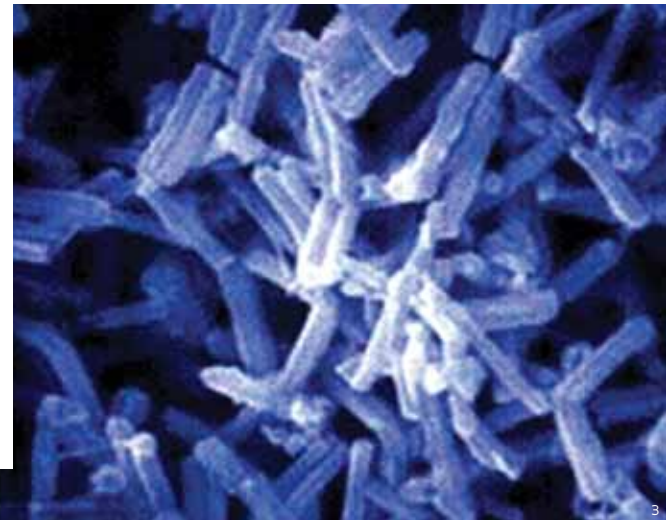


"We cannot move forward alone," says Dr. Maranhão, who has also faced many dilemmas. One of them was where to publish the results: "if I reveal too much, having sought the most widely read journals, I could be pushed aside by other groups, which could advance more quickly". Deciding not to publish was not feasible because publishing scientific articles affords researchers visibility and credibility. The solution he found was to publish in medical journals of medium impact "without much fanfare."

When Dr. Maranhão started, there were no patent laws for new drugs. At that time, the concept of medical nanotechnology did not exist, and the national companies were not interested in developing national drugs in Brazil. He tried a few foreign companies, hoping to find partners

**IMAGES 1** LQES / UNICAMP **2 & 3** INCTTOX / THE BUTANTAN INSTITUTE





Two types of mesoporous silica nanoparticles: one type from Unicamp, with pores that repel water and a surface that attracts water (*opposite page*); and another type from Butantan-USP, with hexagonal pores (*above, left*) and in the shape of cylindrical tubes (*right*).

that would help in the production and development of nanoparticles, but company mergers and acquisitions disrupted the negotiations. Dr. Maranhão saw that he would have to set up and install his own production laboratory next to his room at InCor, and he paid a fortune for imported chemotherapy even as India and China began to sell at much lower prices. "It's a chess game," he says. "Often, the strategy precedes the science."

## THE NEXT BATTLE

Dr. Maranhão explored areas unknown to him and formed teams, another risk, because the pace of the work and the methodological rigor of the group members may differ. "Luckily I've never had problems with my collaborators," he says, "and I owe a lot to my medical colleagues and professors, such as Sylvia Graziani, Noedir Stolf, Vânia Hungria, Eloisa Bonfá, Roberto Hegg, Jesus Carvalho, Durvanei Maria and many others who have collaborated on this project for many years and whose work is of the highest quality."

Yes, he says he did have problems with the necessarily anonymous reviewers of his funding requests, who criticized his lack of focus—because the research involved exploring other LDE medical applications—or asked for details that he did not have or that did not concern him. "We are our own executioners," says another USP professor who has followed Dr. Maranhão's work for many years.

To anyone who has discovered an apparently fantastic molecule and thinks the next stages of research and development will be simple, Dr. Maranhão's message is: "Do not be overly ambitious. If you really want to accept this challenge, you must be in it for the long haul." The work is not over yet; the next battle in sight will be to register the LDE drug combinations with government regulators.

"In the United States," he says, "since we have already made the medical and scientific arguments, and given the urgency of finding new drugs against cancer, our registration request could easily be fast-tracked." Fast-track is a fast and simplified method for obtaining approval on new drugs—a process that has been adopted by the US federal government. In Brazil, there is no fast-track, and the registration process usually requires a lot of paperwork and takes many years before the final stamp-of-approval is obtained. A Brazilian company is reported to have sent 70 kilos of documents to the agency responsible for this area in Brasilia and waited seven years to register a Brazilian drug similar to Viagra. ■

## Projects

**1.** *Lipid nanoparticles: applications in the study of the physiopathology, diagnosis and therapeutics of degenerative diseases* - No. 06/58917-3; **Grant Mechanisms** Thematic Project; **Coordinators** Raul Cavalcante Maranhão (USP); **Investments** R$1,406,940.52 (FAPESP).

**2.** *New therapeutic strategies for non-muscle invasive urinary bladder cancer* - No. 12/20706-2; **Grant Mechanisms** Regular Line of Research Project Award; **Coordinators** Wagner José Fávaro (Unicamp); **Investments** R$133,260.00 (FAPESP).

**3.** *Production of mesoporous silica nanostructures to transport hydrophobic antitumor agents* - No. 09/10056-8. **Grant Mechanisms** Doctorate; **Coordinators** Amauri Jardim de Paula (Unicamp); **Investments** R$110,201.13 (FAPESP)

## Scientific articles


FÁVARO, W.J. et al. Effects of P-MAPA immunomodulator on toll-like receptors and p53: potential therapeutic strategies for infectious diseases and cancer. **Infectious Agents and Cancer**. v. 7, No. 14. 2012 (online).

KRETZER, I.F. et al. Drug-targeting in combined cancer chemotherapy: tumor growth inhibition in mice by association of paclitaxel and etoposide with a cholesterol-rich nanoemulsion. **Cellular Oncology**. v. 6, pp. 451-60. 2012.

PAULA, A.J. et al. Suppression of the hemolytic effect of mesoporous silica nanoparticles after protein corona interaction: independence of the surface microchemical environment. **Journal of the Brazilian Chemical Society**. v. 23, No. 10, pp. 1807-14. 2012.