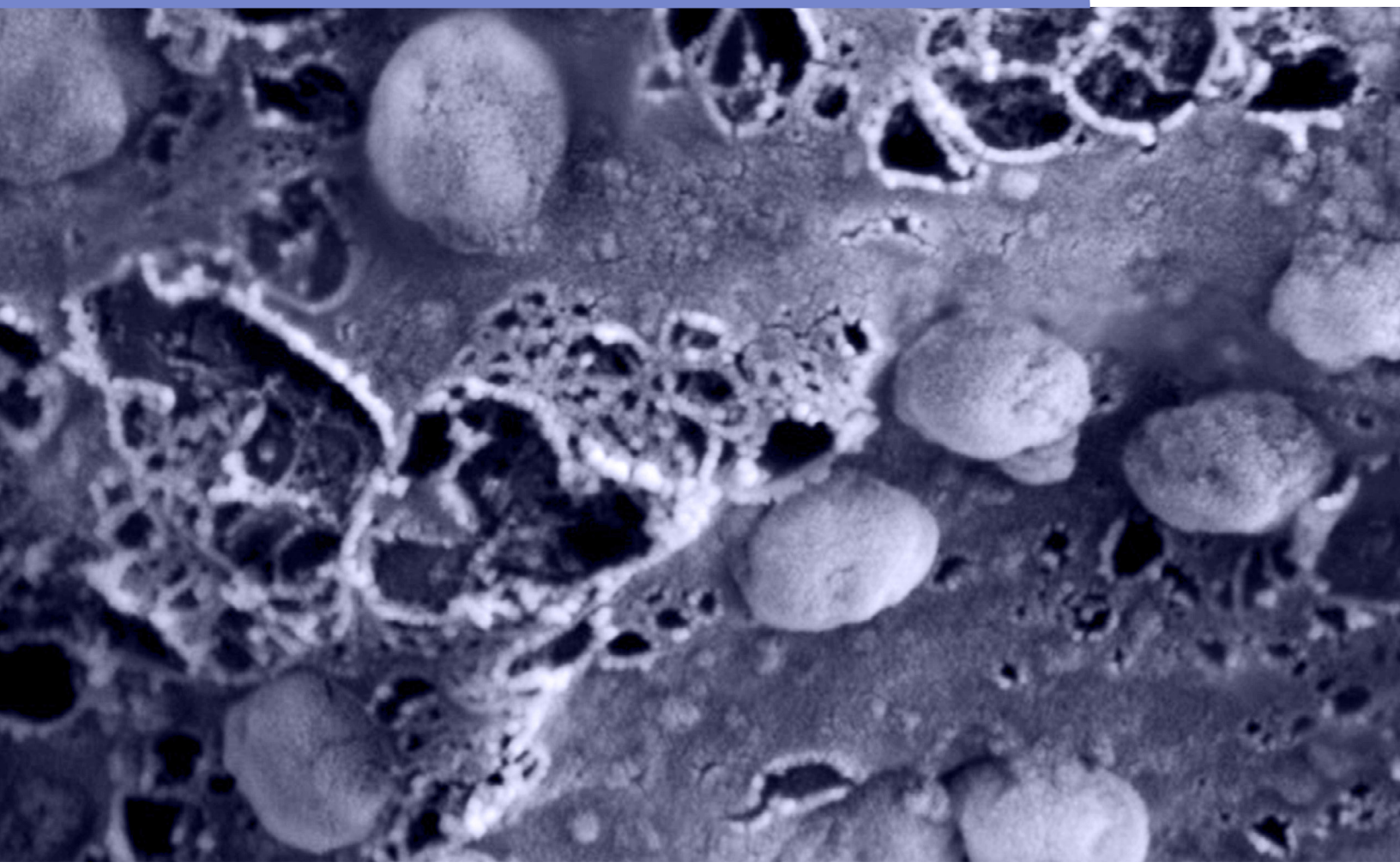
Delivering the Goods, Bringing the Heat

Nanoparticles Show Their Mettle as Platforms for Theranostic Imaging, Targeted Drug Delivery, and Photothermal Therapy



FORTIS LIFE SCIENCES

A SPONSORED PUBLICATION FROM

GEN Genetic Engineering & Biotechnology News

FORTIS
LIFE SCIENCES

Custom fabrication and functionalization of nanoparticles for therapeutics

Leverage our unique experience in nanoparticle development and GMP manufacturing to ensure rapid development and commercialization of your nano-enabled products.

We offer solutions from proof of concept to scaled manufacturing of therapeutic systems for:

- Targeted delivery
- Controlled release
- Photothermal therapeutics
- Theranostics
- Gene therapy

Learn more at: fortislife.com/drug-delivery-nanomaterials

nanoComposix

Delivering the Goods, Bringing the Heat

Nanoparticles Show Their Mettle as Platforms for Theranostic Imaging, Targeted Drug Delivery, and Photothermal Therapy

CONTENTS

Introduction

When we battle disease, we need options other than brute force. We need precision weapons. And for many therapeutic applications, the precision weapons of choice are functionalized nanoparticles.

In modern medicine, as in modern warfare, the idea is to apply the necessary force—no more, no less—and to do so only when and where necessary. Now, the necessary force may be a flow of therapeutic molecules or a surge of thermal energy. In either case, it can be meted out by nanoparticles. Moreover, the nanoparticles may be equipped with the functional equivalents of camouflage, shielding, and target acquisition systems. The results? More loiter time and less collateral damage.

Nanoparticles may also perform reconnaissance and elevate command and control. Both capabilities involve communications of some sort. For example, fluorescence, magnetic, and light-scattering technology may enable reporting and imaging. In addition, targeted electromagnetic energy may be used to trigger therapeutic release or power thermal ablation.

All of these possibilities are discussed in this eBook. For example, it presents a Fortis Life Sciences white paper that cites advances in the use of silica and metal-based nanoparticles to improve therapeutic delivery and enable novel photothermal treatments. Summarizing the findings of 16 peer-reviewed articles, the white paper feels almost as comprehensive as a nanoparticle catalog. (For an actual catalog—one that includes solid nanoparticles, microporous nanoparticles, nanoshells, layered nanoparticles, and more—visit https://nanocomposix.com/collections/all.)

Besides describing the depth and scope of the existing nanoparticle arsenal, this eBook presents a range of next-generation nanoparticle developments. Specifically, five articles from GEN suggest how nanoparticle design is becoming so sophisticated it may soon qualify as "personalizable."

For example, one article describes "smart transformable nanoparticles." These are nanoparticles that can alter their size and shape (and hence, their functionality) upon stimulation from their surrounding environment. Another article describes a fluorescence quenching assay that can assess the integrity of the cell membrane coatings that can give nanoparticles biomimetic properties. (Such properties include the ability to pass as human immune cells.) Yet another paper describes how the self-assembly of plasmonic gold nanoparticles can be induced in targeted cells, specifically, cancer cells.

Overall, this eBook locates nanoparticles in the current order of battle. The battle in question is, of course, the battle between medical science and disease. Although disease is a formidable and shifty foe, medical science is poised to make good use of nanoparticles, especially now that nanoparticles are becoming more configurable, targetable, and even personalizable.

FORTIS LIFE SCIENCES

GEN Genetic Engineering & Biotechnology News

Source: Sanjeri/Getty Images

Smart Transformable Nanoparticles Show Promise as Tumor Theranostics

Scientists from China and the United States have examined how biology triggers morphological changes in certain types of nanoparticles. These types of particles are called smart transformable nanoparticles because they can alter their size and shape upon stimulation from their surrounding environment.

The particles are particularly promising for tumor theranostics because their physical properties will adapt to the physiology. These adaptations improve particle circulation, biodistribution, tumor penetration, tumor retention, and subcellular distribution for targeted therapy.

The team published its study ("Smart transformable nanoparticles for enhanced tumor theranostics") in Applied Physics Reviews.

"The physical morphologies of nanoparticles, especially size and shape, always significantly influence their biological behaviors. In the past, nanoparticles with constant physical morphologies have been widely investigated and applied in tumor theranostics," the investigators wrote.

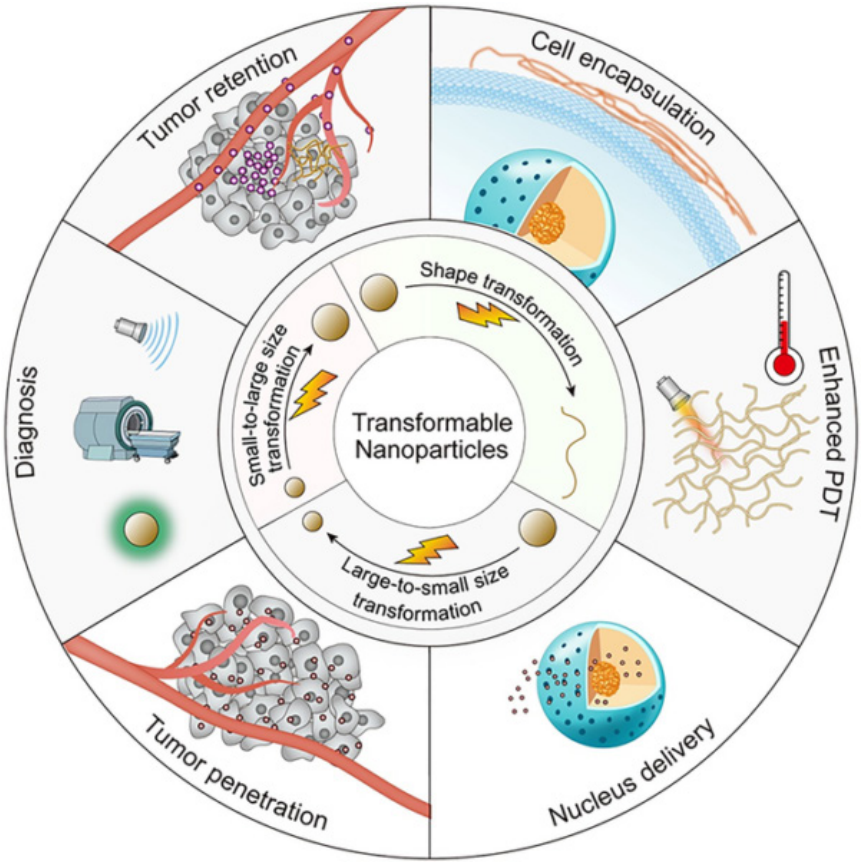
"With the increased in-depth knowledge of tumors and physiological microenvironments, nanoparticles are required to self-adjust their physical morphologies during their circulation

in varying physiological microenvironments and when reaching tumor site that possess distinct microenvironments. Therefore, smart transformable nanomaterials, which can alter their morphologies under different physiological conditions, show great potential in advanced tumor theranostics.

"This review summarizes the influence of nanoparticles' physical morphologies on their biological behaviors under different physiological conditions, highlights the designs of transformable nanoparticles serving as a guideline for their construction, intensively discusses the recent biomedical applications of these smart transformable nanoparticles for tumor theranostics, and also proposes future challenges and perspectives in the development of smart transformable nanoparticles for tumor theranostics."

"Smart transformable nanoparticles can alter their morphologies under different physiological conditions as the therapeutic demands," said co-author Jianxun Ding, PhD, from the Chinese Academy of Sciences. "In our study, we reveal the structural designs for these smart systems as well as the in-depth mechanisms of the transformations."

The researchers present the designs of transformable nanoparticles as a guideline for their



Smart transformable nanoparticles could undergo size or shape transition as the requirement of different conditions, showing great potential in future tumor theranostics.

[Jianxun Ding/Chinese Academy of Sciences]

construction and discuss the biomedical applications in the realm of theranostics. Ding and his colleagues developed novel classifications for nanoparticle transformation design and the mechanisms contributing to the change.

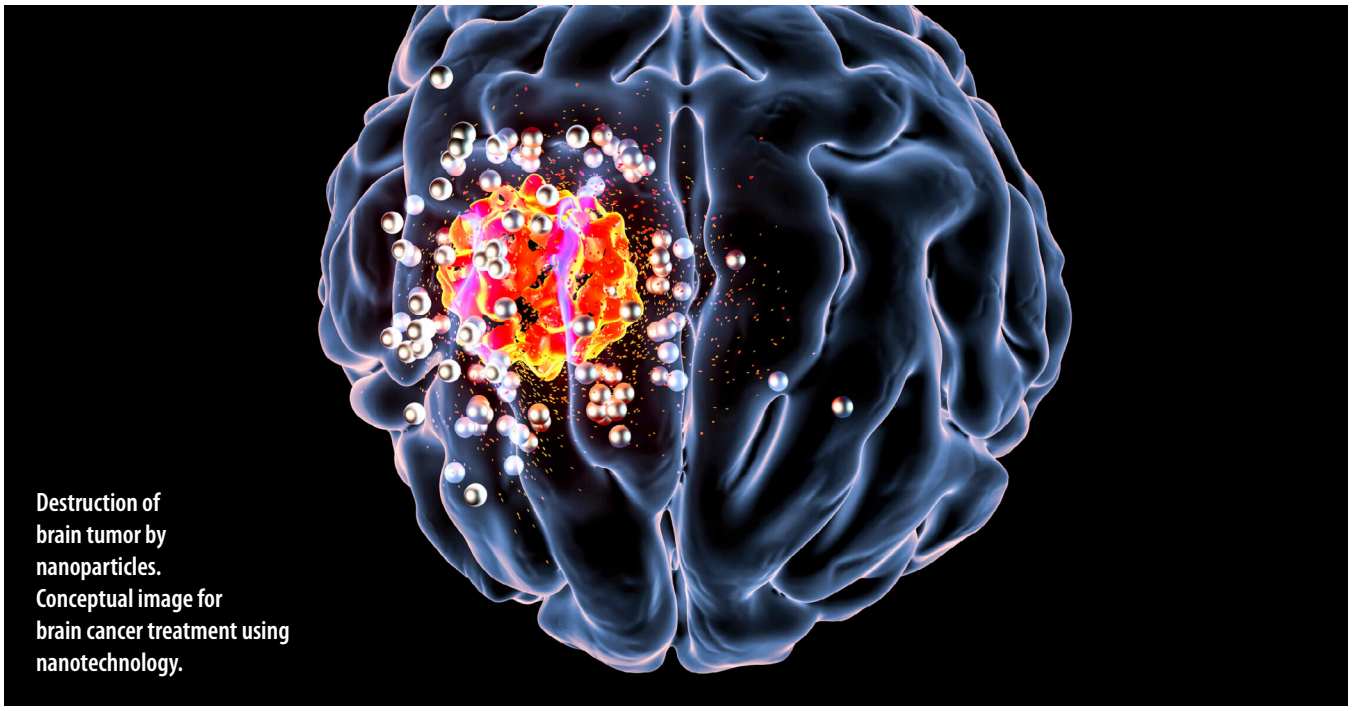
For instance, the researchers divide the design transformation into two broad categories: size and shape. For size-transformable nanoparticles, the alterations are further divided into small-to-large and large-to-small transformations. The study discloses detailed and rational designs of transformable nanoparticles based on their structures.

As for the mechanisms contributing to nanoparticle transformation, "we believed the structure and stimuli both made a great contribution," noted Ding. "For example, different pH values decided the accurate site for the transformation, which correlate to varying physiological, extracellular, and endo/lysosomal conditions."

Nanoparticles with constant physical morphologies have been widely investigated and applied in tumor theranostics in the past, while more recent studies of nanoparticle transformation phenomena have focused primarily on the response to stimuli. Until now, however, there has not been an in-depth discussion on the designs and applications of morphology-transformable nanoparticles.

"Our review covers the structure design, mechanism for transformation, and biomedical application of smart transformable nanoparticles, and includes perspectives on their limitations as well," explained Ding. "We believe this review will shed light on this important field."

"Smart transformable nanoparticles can alter their morphologies under different physiological conditions as the therapeutic demands."



Destruction of brain tumor by nanoparticles. Conceptual image for brain cancer treatment using nanotechnology.

Source: Sanjeri/Dr Microbe/Getty Images

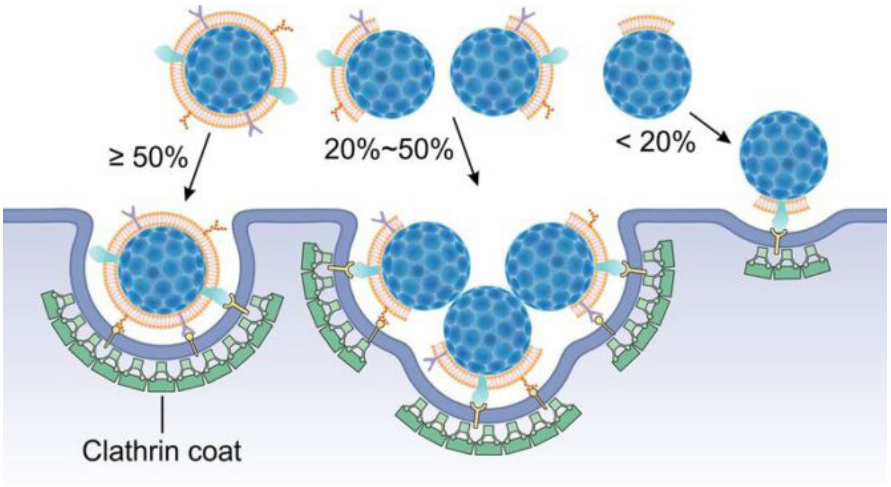
Biomimetic Nanoparticles for Targeted Cancer Therapy

Cell membrane coated biomimetic nanoparticles (NPs) have been widely studied in nanomedicine because of their unique properties such long blood circulation, specific molecular recognition, and efficient cancer targeting, indicating a great potential in targeted cancer therapy. However, the integrity of the cell membrane coating on NPs, a key metric related to the quality of these biomimetic-systems and to the resulting biomedical function, has remained largely unexplored.

Now researchers at the University of Eastern Finland report the development of a fluorescence quenching assay to probe the integrity of the cell membrane coating. Their study ("Cell membrane coating integrity affects the internalization mechanism of biomimetic nanoparticles"), published in Nature Communications, shows that the great majority of the cell membrane coated NPs were only partially coated when traditional coating techniques were applied.

The scientists, who carried out their work in the department of applied physics under the direction of Vesa-Pekka Lehto, PhD, says that this information is essential as the coating degree impacts the biological fate of NPs.

"Here, we report a fluorescence quenching assay to probe the integrity of cell membrane coating. In contradiction to the common assumption of perfect coating, we uncover that up to 90% of the biomimetic NPs are only partially coated. Using in vitro homologous targeting studies, we demonstrate that partially coated NPs could



[caption pulled from body: please check]

Researchers reported that "a fluorescence quenching assay to probe the integrity of cell membrane coating. In contradiction to the common assumption of perfect coating, we uncover that up to 90% of the biomimetic NPs are only partially coated. Using in vitro homologous targeting studies, we demonstrate that partially coated NPs could still be internalized by the target cells."

still be internalized by the target cells," write the investigators.

"By combining molecular simulations with experimental analysis, we further identify an endocytic entry mechanism for these NPs. We unravel that NPs with a high coating degree (≥50%) enter the cells individually, whereas the NPs with a low coating degree (<50%) need to aggregate together before internalization.

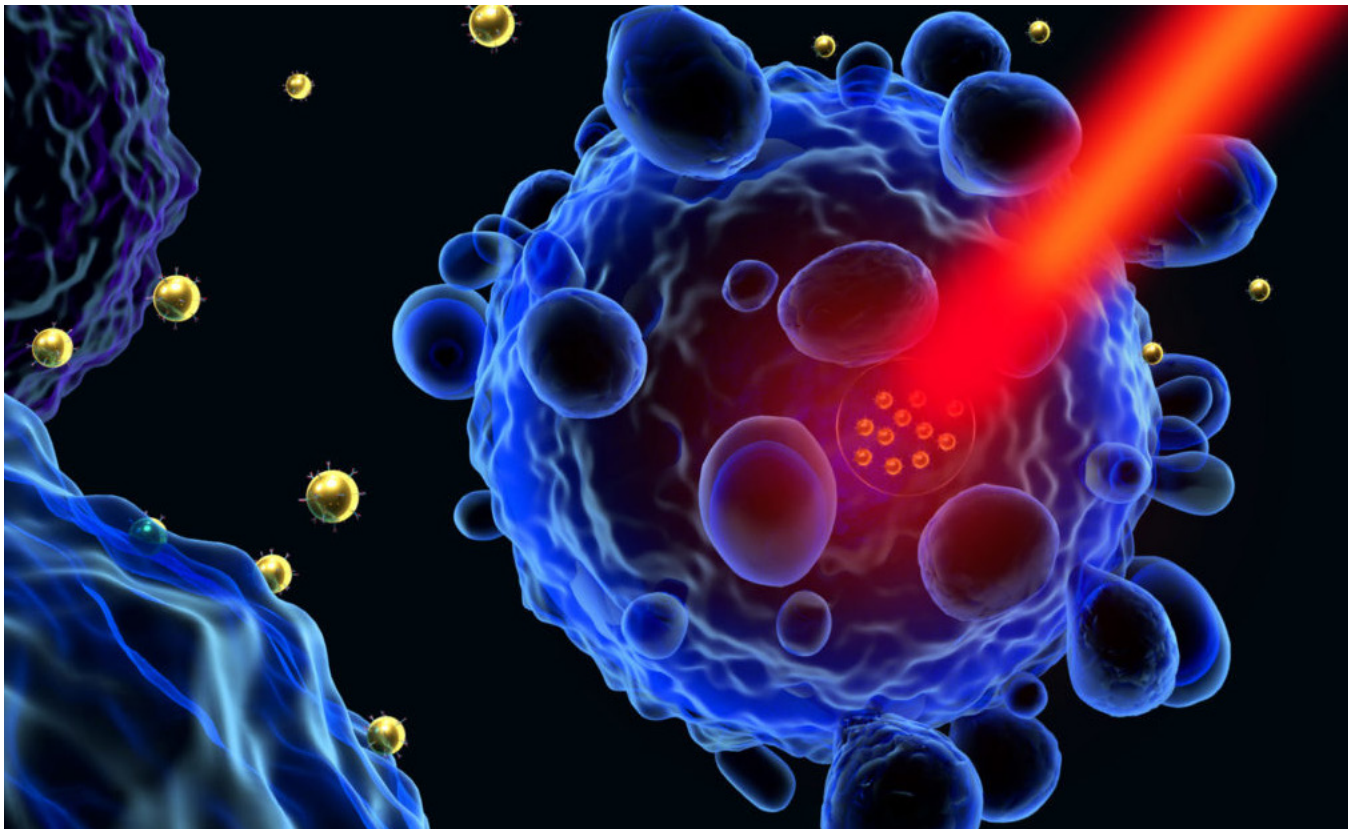
"This quantitative method and the fundamental understanding of how cell membrane coated NPs enter the cells will enhance the rational designing of biomimetic nanosystems and pave the way for more effective cancer nanomedicine."

"The present methods for characterizing the cell membrane coating are only qualitative and fail to statistically evaluate the degree and variability of the coating," notes Lizhi Liu, a researcher and first author of the publication. "When we applied the developed quantification method to evaluate the success of the commonly used protocols to produce fully coated NPs, we found that the fraction never exceeded 20%."

"Our discovery is a big surprise to whole scientific community in nanomedicine because it has been generally accepted that the cell membrane coating is perfect. Despite of the partial coating, biomimetic NPs could still be internalized by the target cells via different pathways," adds Wujun Xu, PhD, senior research and team leader and one of the corresponding authors of the paper.

To explain this, the authors proposed a new endocytic entry mechanism for these partially coated NPs by computational simulations. Specifically, the NPs with a high coating degree (≥ 50%) entered the cells individually, whereas the NPs with a low coating degree (< 50%) needed to aggregate together before internalization.

"The present study highlights some of the limitations of the current cell membrane coating protocols and motivates the efforts to improve the protocols. The developed quantification method is a practical tool to assess the success of these efforts and establish a standard for comparing the different coating designs," says Vesa-Pekka Lehto.



Source: Meletios Verras/Getty Images

Gold Nanoparticles Produced in Cancer Cells with Novel Technique

University of Maryland Baltimore County (UMBC) scientists and collaborators published a study, "Intratumoral generation of photothermal gold nanoparticles through a vectorized biomineralization of ionic gold," in Nature Communications that reportedly demonstrates for the first time a technique for biosynthesizing plasmonic gold nanoparticles within cancer cells, without the need for conventional bench-top lab methods.

The approach has the potential to notably expand biomedical applications, according to the researchers, especially in x-ray imaging and cancer therapy.

Conventional laboratory-based synthesis of gold nanoparticles requires ionic precursors and reducing agents subjected to varying reaction conditions such as temperature, pH, and time. This leads to variation in nanoparticle size, morphology, and functionalities that are directly correlated to their internalization in cells, their residence time in vivo, and clearance.

To avoid these uncertainties, the research paper demonstrates that biosynthesis of gold nanoparticles can be achieved efficiently and directly inside cancer cells without requiring conventional laboratory methods, notes Dipanjan Pan, PhD, professor of chemical, biochemical, and

environmental engineering at UMBC.

The researchers examined how various cancer cells responded to the introduction of chloroauric acid to their cellular microenvironment by forming gold nanoparticles. These nanoparticles generated within the cell can potentially be used for various biomedical applications, including in x-ray imaging and in therapy by destroying abnormal tissue or cells.

In the paper, Pan and his team describe their new method of producing these plasmonic gold nanoparticles within cells in minutes, within a cell's nucleus, using polyethylene glycol as a delivery vector for ionic gold.

"Various cancer cells have been demonstrated to have the capacity to form plasmonic gold nanoparticles when chloroauric acid is introduced to their cellular microenvironment. But their biomedical applications are limited, particularly considering the millimolar concentrations and longer incubation period of ionic gold," write the investigators.

"Here, we describe a simplistic method of intracellular biomineralization to produce plasmonic gold nanoparticles at micromolar concentrations within 30 min of application utilizing polyethylene glycol as delivery vector for ionic gold. We have characterized this process for intracellular gold nanoparticle formation, which progressively accumulates proteins as the ionic gold clusters migrate to the nucleus. This nano-vectorized application of ionic gold emphasizes its potential biomedical opportunities while reducing the quantity of ionic gold and required incubation time."

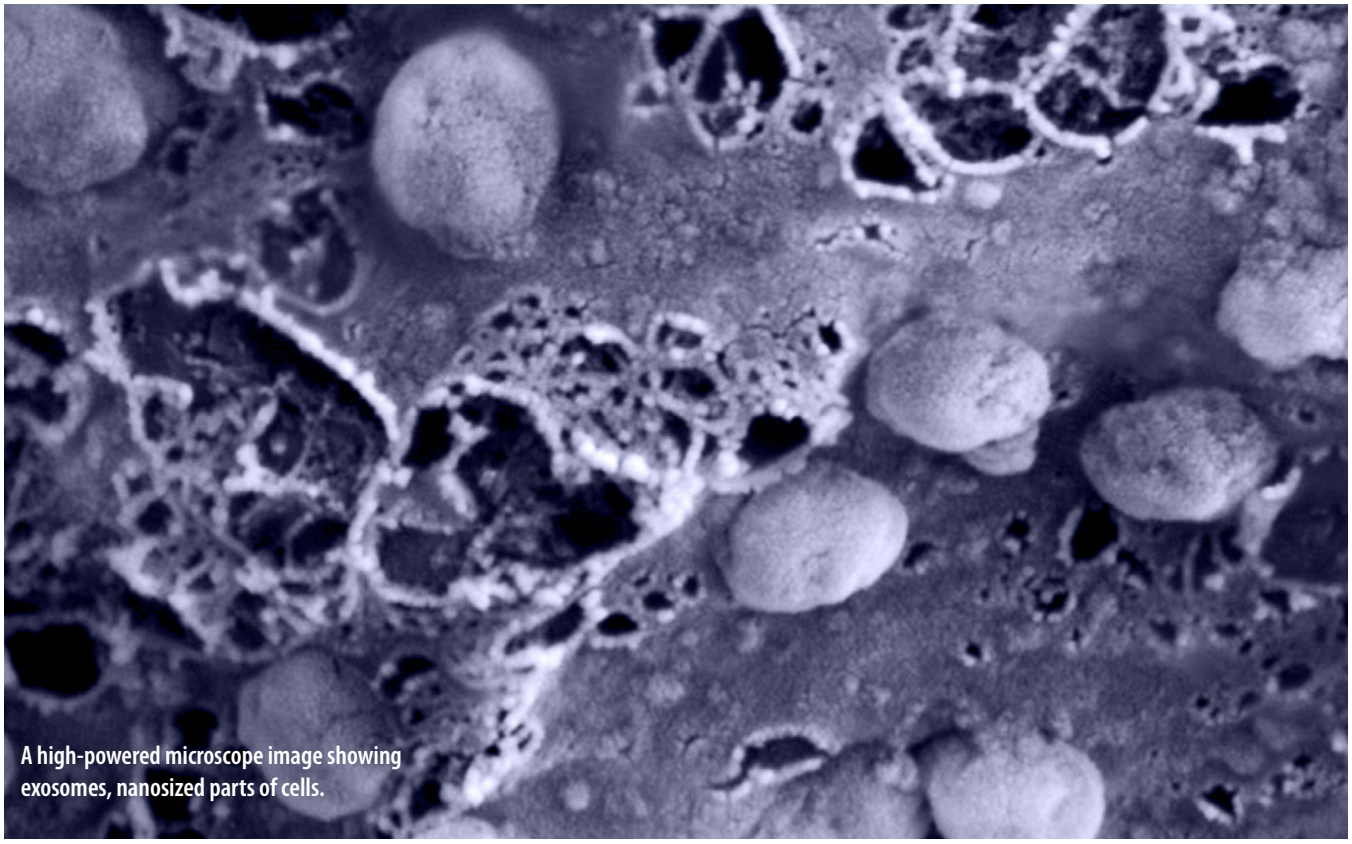
"To demonstrate its biomedical potential, we further induce in-situ biosynthesis of gold nanoparticles within MCF7 tumor mouse xenografts which is followed by its photothermal remediation."

"We have developed a unique system where gold nanoparticles are reduced by cellular biomolecules and those are able to retain their functionality, including the capacity to guide the remaining cluster to the nucleus," explains Pan.

The team also worked to further demonstrate the biomedical potential of this approach by inducing in-situ biosynthesis of gold nanoparticles within a mouse tumor, followed by photothermal remediation of the tumor. According to Pan, the mouse study exemplifies how "the intracellular formation and nuclear migration of these gold nanoparticles presents a highly promising approach for drug delivery application."

He calls gold the quintessential noble element that has been used in biomedical applications since its first colloidal synthesis more than three centuries ago.

"To appreciate its potential for clinical application, however, the most challenging research ahead of us will be to find new methods of producing these particles with uncompromised reproducibility with functionalities that can promote efficient cellular binding, clearance, and biocompatibility and to assess their long-term term effects on human health," he continues. "This new study is a small but important step toward that overarching goal."



A high-powered microscope image showing exosomes, nanosized parts of cells.

Source: Surya Shrivastava/ City of Hope

Novel Delivery System Suppresses HIV in Mice

HIV, the virus that causes AIDS, is one of the world's most serious public health challenges. Despite advances in our scientific understanding of HIV and its prevention and treatment, there are many who still do not have access to prevention, care, and treatment, and there is still no cure. A new mouse study by researchers at City of Hope and Menzies Health Institute Queensland at Griffith University may provide hope. The researchers report they have developed an anti-HIV protein that suppressed HIV levels in the bone marrow, spleen, and brain of mice and prevented replication.

Their findings are published in Nature Communications in a paper titled, "Exosome-mediated stable epigenetic repression of HIV-1."

"Human Immunodeficiency Virus (HIV-1) produces a persistent latent infection," the researchers wrote. "Control of HIV-1 using combination antiretroviral therapy (cART) comes at the cost of life-shortening side effects and development of drug-resistant HIV-1. An ideal and safer therapy should be deliverable in vivo and target the stable epigenetic repression of the virus, inducing a stable 'block and lock' of virus expression. Towards this goal, we developed an

"This innovative technology could become a viable way to deliver therapies not only for HIV but also for other diseases, including ones that affect the brain, such as Alzheimer's and Parkinson's..."

HIV-1 promoter-targeting Zinc Finger Protein (ZFP-362) fused to active domains of DNA methyltransferase 3 A to induce long-term stable epigenetic repression of HIV-1."

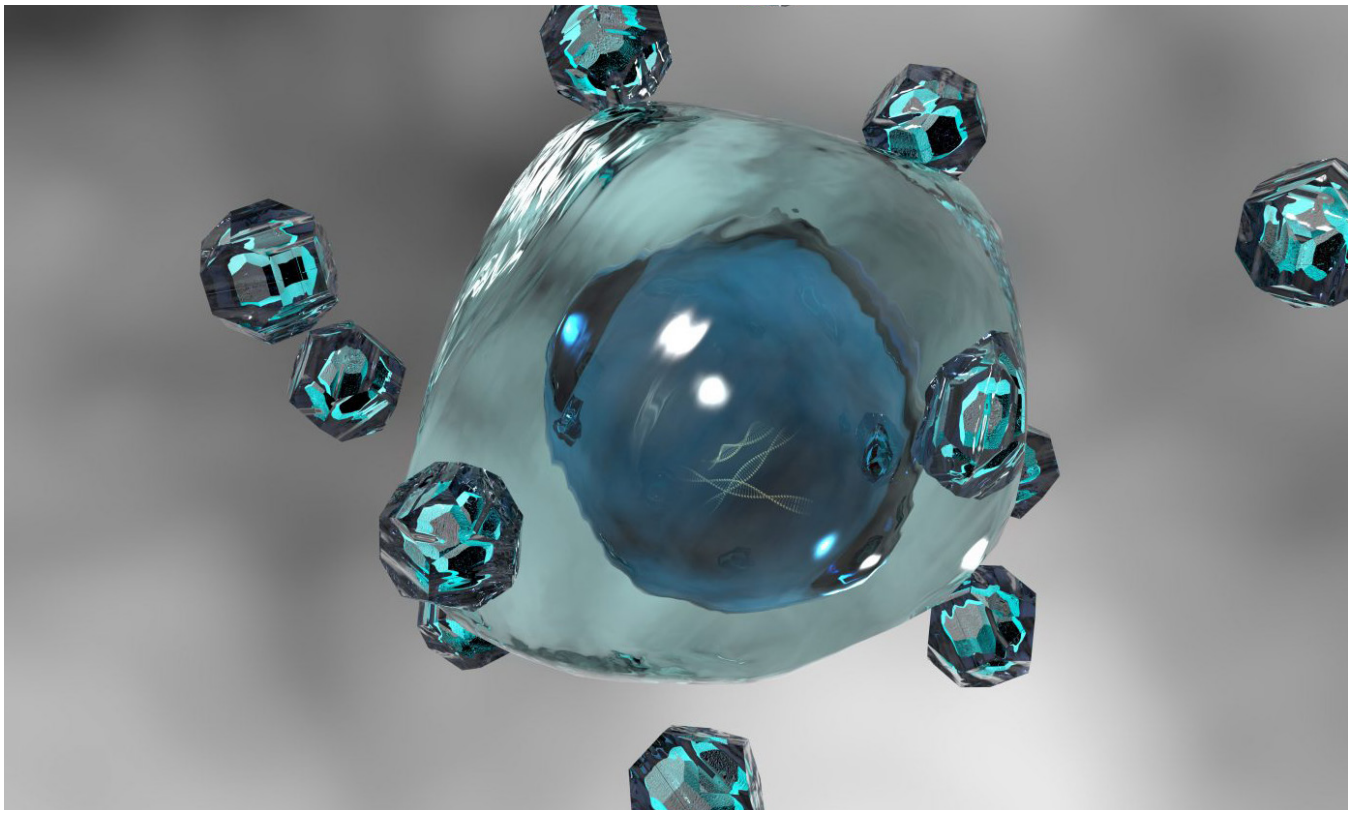
Their delivery system may pave the way for future therapies that suppress infectious diseases.

"This innovative technology could become a viable way to deliver therapies not only for HIV but also for other diseases, including ones that affect the brain, such as Alzheimer's and Parkinson's," explained Kevin Morris, PhD, senior author of the study and professor from City of Hope's Center for Gene Therapy and Griffith University's School of Pharmacy and Medical Sciences.

"The ZPAMt HIV protein repressor we developed is packaged into exosome nanoparticles and can enter cells where it epigenetically silences HIV," Morris said. "We show that these nanoparticles can systemically 'block and lock' HIV expression. This is the first time that block and lock has been successfully delivered to treat HIV in vivo in the brain."

This finding is both imperative and innovative for diseases such as HIV due to its ability to hide in the brain, making it difficult to treat because of the blood-brain barrier.

"The anti-HIV-1 therapeutic exosomes presented in this study, with additional preclinical safety studies, have the potential to be adopted for clinical trials along with cART for people living with HIV-1, which may reduce the stringency of drug regimen and enhance their quality of life," concluded the researchers. "Furthermore, we have shown a viable route to achieve specific hyper-methylation of an integrated provirus, which can be extended beyond HIV-1/AIDS therapy."



Source: Meletios Olique/Getty Images

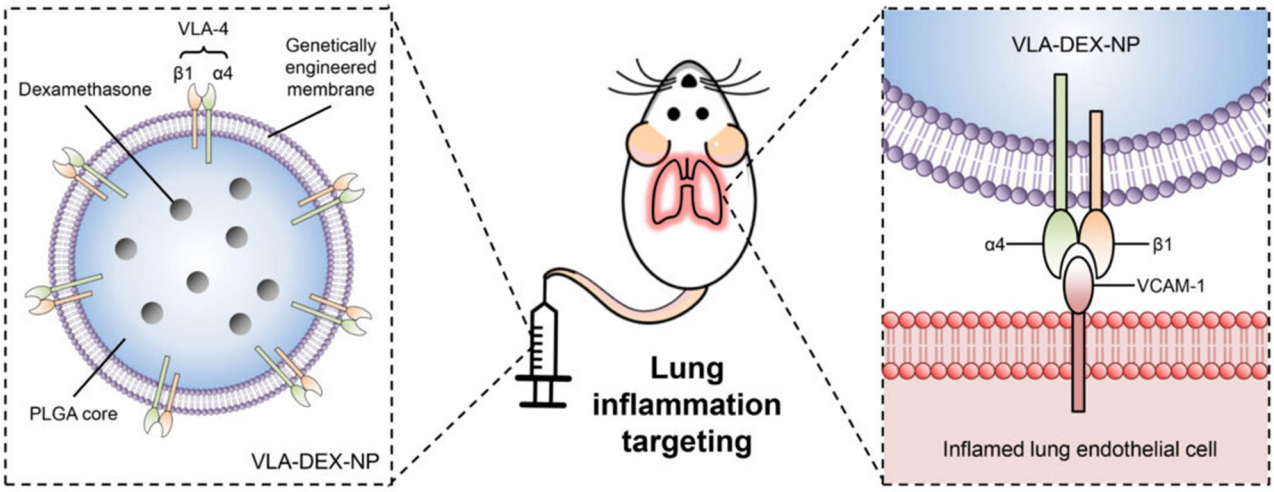
Versatile Nanosponge Drug Delivery Platform Proves Effective in Inflamed Lungs

Nanoparticles disguised as human immune cells can enhance the healing powers of a variety of drugs by traveling specifically to the affected cells before they release their cargo of concentrated drugs.

Scientists at the department of nanoengineering and the Moores Cancer Center at the University of California, San Diego (UCSD), have coated drug-filled nanoparticles with the membrane of immune cells and showed that these nanosponges target and deliver drugs to inflamed sites in the lungs where they are needed.

To prove the efficacy of their strategy, the researchers filled the coated nanoparticles with the potent anti-inflammatory drug dexamethasone (DEX) and intravenously injected them in mice with inflamed lungs to show that this treated the inflammation completely at drug concentrations that are not effective through standard delivery methods.

The researchers reported their findings in the Science Advances article, "Genetically engineered cell membrane-coated nanoparticles for targeted delivery of dexamethasone to inflamed lungs."



Genetically engineered cell membrane-coated nanoparticles deliver drugs to inflamed lungs.
[Source: Zhang Lab/UCSD]

The study was funded by the National Institutes of Health (NIH) and the Defense Threat Reduction Agency Joint Science and Technology Office for Chemical and Biological Defense (DTRA CB).

Earlier work in the lab of UCSD nanoengineering professor Liangfang Zhang, PhD, used nanoparticles coated with cell membranes derived from the body's cells to absorb toxins produced by MRSA, treat sepsis, and target cancer cells.

"In this paper, we used a genetic engineering approach to edit the surface proteins on the cells before we collected the membranes. This significantly advanced our technology by allowing us to precisely overexpress certain functional proteins on the membranes or knockout some undesirable proteins," said Zhang, senior author on the paper.

The strategy exploits the naturally occurring target-ligand binding affinity between a protein (VACM1) upregulated on inflamed endothelial cells to attract immune cells, and its binding partner on immune leucocytes (VLA-4), to produce the biomimetic nanoparticle capable of targeting inflammation.

"We engineered cell membranes to express the full version of VLA4 all the time," said Joon Ho Park, PhD, a graduate student in Zhang's lab and first author on the paper. "These membranes constantly overexpress VLA4 in order to seek out VCAM1 and the site of inflammation. These engineered cell membranes allow the nanoparticle to find the inflamed sites, and then release the drug that's inside the nanoparticle to treat the specific area of inflammation."

Nanoparticle delivery allows fast, concentrated, and specific delivery of a drug allowing lower doses to have effects comparable to standard methods of delivery. The authors showed that DEX accumulates at the inflamed lung tissue at higher levels, faster than standard drug delivery approaches.

"We're delivering the exact same drug used in the clinic, but the difference is we're concentrating the drugs to the point of interest," said Park. "By

"This is a versatile platform, not just for lung inflammation but any type of inflammation that upregulates VCAM1..."

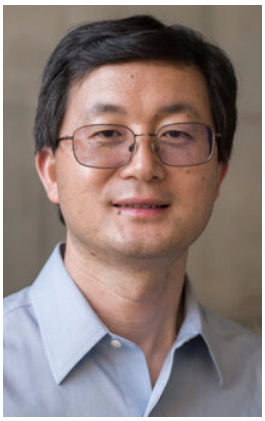
having these nanoparticles target the inflammation site, it means a larger portion of the medicine will wind up where it's needed, and not be cleared out by the body before it can accumulate and be effective."

This platform strategy can be applied to treat a variety of diseases caused by local inflammation.

"This is a versatile platform, not just for lung inflammation but any type of inflammation that upregulates VCAM1," said Park. "This technology can be generalized; this engineered cell membrane-coated nanoparticle doesn't have to overexpress VLA4, it could be swapped out to another protein that can target other areas of the body or accomplish other goals."

Park and the team engineered VLA4 overexpressing cell membranes by packaging VLA4 genes into a viral vector and introducing these into lab-grown mouse host cells.

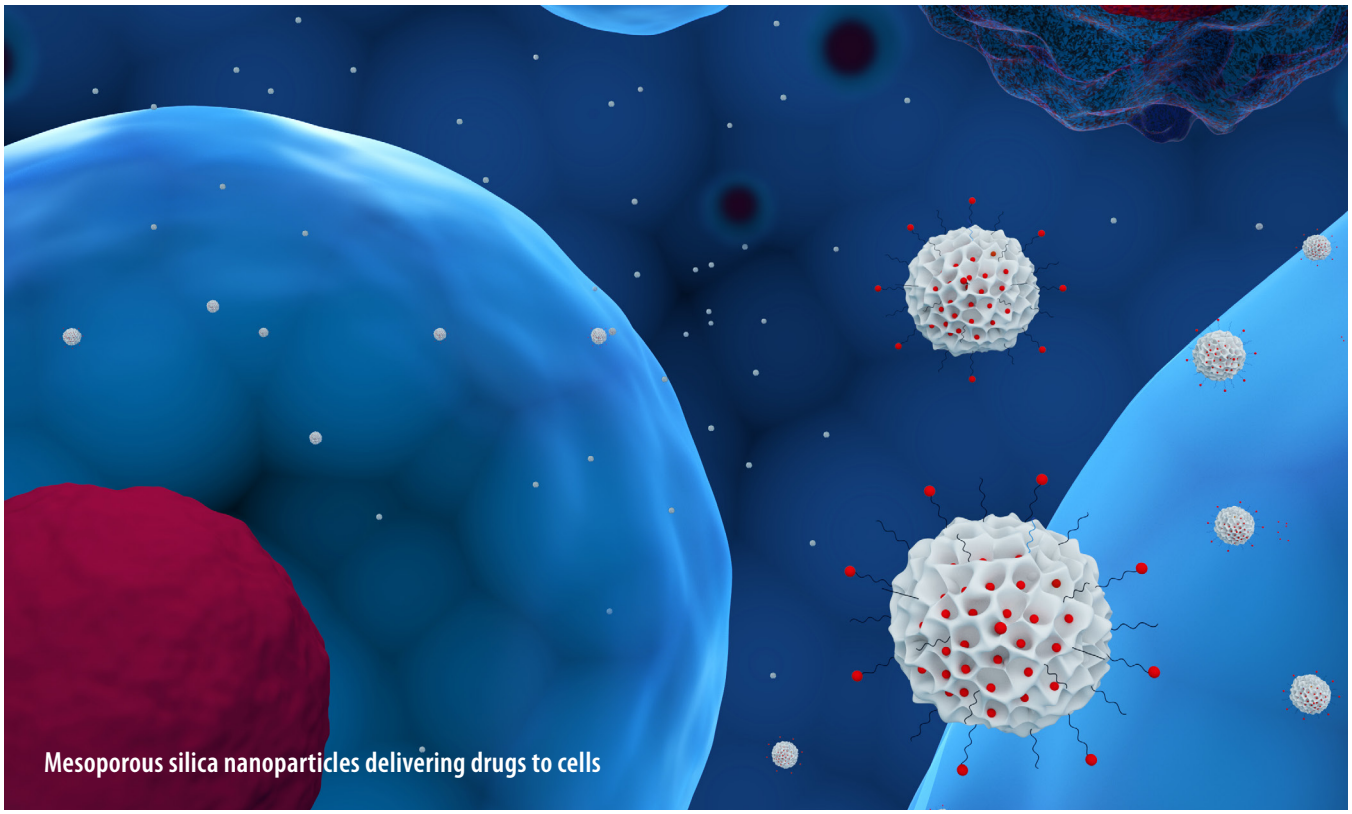
In future studies, the team will examine the process using human cell membranes engineered to express the human version of VLA4.



Liangfang Zhang, PhD [left], professor of nanoengineering, University of California, San Diego, is senior author on the study; Joon Ho Park [right], a graduate student in Zhang's lab, is first author on the paper

"By leveraging the established gene editing techniques, this study advances the cell membrane-coated nanoparticles to a new level and opens up new opportunities for targeted drug delivery and other medical applications," said Zhang.

It may still be a while before the technology can be tested in human clinical trials, but the scientists believe these early results are encouraging.



Mesoporous silica nanoparticles delivering drugs to cells

Applications of Nanoparticles in Targeted Drug Delivery and Photothermal Therapy

A compendium of recently published peer-reviewed articles

The use of nanoparticles for targeted drug delivery, controlled release of therapeutic agents and photothermal therapy (PTT) is growing rapidly. These particles offer multi-functional capabilities that include the ability to be tracked within the body, deliver a specific compound at a controlled rate, target a particular location, and remotely kill cells once in place. Biomolecules such as antibodies, oligonucleotides, or peptides can also be bound to the nanoparticle to direct it to a precise location while long circulation times can be achieved by coating particles with poly-ethylene glycol (PEG). In addition to drug delivery, these versatile particles can be engineered to include fluorescence, magnetic, and light scattering properties. Once at their intended destination, nanoparticles can act as a reporter, release a compound, or be remotely heated to damage nearby biological structures.

Nanoparticles can carry thousands of drug molecules embedded within or attached to their surface. For the sustained release of a therapeutic,

the core of the particle can be filled with either a solid or high concentration liquid formulation of the drug; the shell layer controls the rate at which the drug diffuses out of the core. Silica shells that are porous with a well-defined thickness can provide precise control over the diffusion delivery rate. The silica shell layer can also be chemically modified to have an affinity for the drug itself. In this case, the large surface area of the porous shell can hold and then release the therapeutic compound. Alternatively, particles can be triggered to release their payload because of changes in the local environment or by external stimuli. The trigger can be pH, heat, light, or the presence of salts or other signaling molecules. Once the trigger is initiated, the drug is released from the particle providing further localization of the therapeutic treatment.

This compendium highlights recently published articles that describe the use of silica and metal-based nanoparticles to improve therapeutic delivery and enable novel photothermal treatments.

Drug Delivery

Zhao, et al., reported the development of mesoporous silica nanoparticles (MSNs) loaded with isoimperatorin, a traditional Chinese medicine, and encapsulated in lymphoma cancer cell membrane to construct a targeted drug delivery system. Ca^{2+}-dependent proteins expressed on cancer cell membranes can mediate the adhesion and targeting of tumor cells. As shown using cell culture and animal models, the novel delivery platform had characteristics of immune escape, anti-phagocytosis, high drug loading rate, low pH value sensitivity, good biocompatibility, and active targeting of the tumor site, blocking the lymphoma cell cycle and promoting mitochondrial-mediated apoptosis.

In a subsequent study, Zhao, et al., constructed a similar anti-lymphoma drug delivery system using MSNs loaded with harmine, a type of beta-carboline alkaloid extracted from the seeds of Tribulus terrestris. While harmine has demonstrated anti-tumor activity, it does not exhibit targeting behavior and is characterized by poor pharmacokinetics, which limits clinical application. In contrast, the MSN-harmine complex passively targeted the tumor tissue, showed pH-responsive drug release, was non-toxic and demonstrated efficient anti-lymphoma properties.

MSNs have also been used to enable the combination of cancer immunotherapy and photodynamic therapy (PDT), a noninvasive therapeutic modality used against tumors accessible to a light source, to generate a systemic antitumor immune response against disseminated cancer. This application used MSNs for theranostic positron emission tomography (PET)-guided PDT and neoantigen-based cancer vaccination. Multiple neoantigen peptides, a CpG oligodeoxynucleotide adjuvant, and photosensitizer chlorin e6 were loaded into the MSN nanoplatform; PET imaging revealed effective accumulation of the MSNs in tumors following intravenous administration. Subsequent PDT with laser irradiation recruited dendritic cells to PDT-treated tumor sites and elicited neoantigen-specific, tumor-infiltrating cytotoxic T-cell lymphocytes. Strong antitumor efficacy of PDT-immunotherapy against

locally treated tumors and distant, untreated tumors was demonstrated in several mouse models. The authors suggest that MSNs offer a promising platform for combining imaging and PDT-enhanced personalized immunotherapy for the treatment of advanced cancer.

Silica nanoparticles are also being explored as physiochemical permeation enhancers that facilitate the oral delivery of protein therapeutics. Oral dosage forms of insulin and other protein drugs would markedly improve patient experience, compliance, and disease outcomes, but the physiology of the gastrointestinal tract prevents the use of this route of administration. Lamson et al., have described the use of anionic nanoparticles to bind intestinal surface receptors that mediate the opening of tight junctions, increasing intestinal permeability and enabling the oral delivery of proteins. The authors report that the permeation-enhancing effect is a function of nanoparticle size and charge, with smaller (≤ 200 nm) and more negative particles such as silica conferring enhanced permeability.

Nanoparticles are also playing an important role in the development of theranostics which combine therapeutics and diagnostics for image-guided therapy. Mukerjee et al. recently described the synthesis and screening of antibody-conjugated silica-coated iron oxide nanoparticles for prostate-specific membrane antigen (PSMA)-specific cell targeting. PMSA is a recognized target for the delivery of cancer therapeutics and imaging agents due to its high expression on the surface of prostate cancer cells. The authors generated an optimized PSMA-targeted magnetic iron oxide nanoparticle with silica applied to the nanoparticle surface. Silica was selected for nanoparticle shelling because it is inert, biocompatible, easily modified, thermally stable, and provides a rigid and stable coating that maintains nanoparticle solubility and stability after routine manipulations. These studies summarize a successful strategy for generating and evaluating a series of antibody-conjugated iron oxide nanoparticles that target PSMA and advance the potential of targeted theranostic agents for future treatment of prostate and other cancers.

While silica-coated nanoparticles are biocompatible and widely used in theranostics, imaging, and drug delivery, they can trigger a reaction of the innate immune system. Park et al., explored the use of noncovalent surface functionalization of silica nanoparticles with purified proteins to inhibit nanoparticle-induced complement activation and macrophage uptake, two innate immune reactions related to nanomedicines. Silica nanoparticles were tested alone and after coating with bovine serum albumin, human serum albumin, fibrinogen, complement factor H, or immunoglobulin G proteins. All coatings except IgG protected against complement activation to varying extents; these coatings also blunted macrophage uptake. In addition to mitigating innate immune reactions, the authors note that these methods are scalable and might constitute a strategy for improving the immunological safety profile of silica and silica-coated nanoparticles as well as other types of inorganic nanoparticles.

In addition to silica-based nanoparticles, metal nanomaterials are being used extensively for drug delivery applications. Because nanoparticle-based drug delivery systems offer such a wide

variety of functionalities, however, pre-clinical assessments can be challenging to compare to conventional formulations. Their size, charge, and surface functionalization can impact targeting and pharmacokinetic behavior. To address this challenge, Zazo, et al., used physiologically based pharmacokinetic (PBPK) modeling to provide a mechanistic approach for studying drug biodistribution in individual organs and tissues and predict human pharmacokinetics from preclinical studies. The PBPK model was used to simulate stavudine biodistribution after the administration of a 40 nm gold nanoparticle-based drug delivery system in rats. The model confirmed that the stavudine-gold nanoparticle met important characteristics for a drug delivery system, including payload, sustained release, and increased in vitro and in vivo drug concentrations in cells and tissues.

Gold nanoparticles are being used for the precision delivery of powerful antibiotics. Fuller, et al., reported the use of a technique called electrospinning to immobilize different antibiotics on a fibrous mesh scaffold along with either cationic or anionic gold nanoparticles to target the delivery of the antibiotic. Delivering the antibiotics directly to the infection site rather than via an oral dosage can be beneficial as oral dosages are distributed nonspecifically throughout the body, requiring a high dose to ensure the proper concentration at the infection site. If an antibiotic can be delivered directly to the site of infection, the dosage can be lowered, reducing side effects and complications. The nanomesh structures had different antibiotic release profiles, with citrate-capped gold nanoparticles combined with colistin having the highest sustained release over 14 days. As a proof of concept, the authors believe these results describe an opportunity for fabricating meshes in which gold nanoparticles as a drug release mechanism for antibiotics.

Ongoing work on the use of gold nanoparticles to deliver colistin, considered a last line of defense in treating infections, is focused on enabling the same therapeutic effect but at a lower dosage to minimize dose-dependent side effects. By delivering colistin coated on an anionic gold nanoparticle, the minimum inhibitory concentration of E. coli has been reduced sixfold compared to the antibiotic alone in studies conducted in nutrient broth. Given these results, the anionic colistin-coated gold nanoparticles show great promise for the delivery of this powerful antibiotic at a lower dosage with improved efficacy.

Goyal, et al., reported the use of layer-by-layer assembled silica core/gold shell nanoshells (LbL-NS) as vehicles for efficient intracellular delivery of microRNAs (miRNAs). miRNAs are short non-coding RNAs whose ability to regulate the expression of multiple genes offers the potential to treat disease; unfortunately, miRNAs cannot passively enter cells due to their hydrophilicity and negative charge. LbL-NS were produced by coating negatively charged nanoshells with alternating layers of positive poly-L-lysine (PLL) and negative miRNA. The outer layer consisted of PLL to facilitate entry of the tumor suppressor miR-34a into triple-negative breast cancer (TNBC) cells and protect the miRNA. The authors reported that the LbL-NS efficiently delivered miR-34a to TNBC cells to suppress cancer cell growth, warranting their further investigation as tools for miRNA replacement therapy.

Fuller, et al., reported the use of a technique called electrospinning to immobilize different antibiotics on a fibrous mesh scaffold along with either cationic or anionic gold nanoparticles to target the delivery of the antibiotic.

Advancements in engineering a compact near-infrared plasmonic nanostructure with image-enhancing agents for combined imaging and therapy have been reported by Henderson et al. The authors developed a compact (sub-100 nm) multi-layer core-shell nanoparticle suitable for near-infrared (NIR) photothermal therapy that can provide simultaneous contrast enhancement for T1 magnetic resonance imaging (MRI) and fluorescence optical imaging (FOI). The structure encapsulates both types of contrast agents in the internal silica layer between the gold core and shell.

Photothermal Therapy

One of the most promising therapeutic applications of nanoparticles is the ability to locally generate heat. Plasmonic nanoparticles can be engineered to efficiently absorb light and convert the absorbed energy to heat, which is then released into the surrounding environment. By changing the size and shape of the plasmonic nanoparticle, the peak absorbance wavelength can be moved into the NIR region of the spectrum where skin and other biological tissues are relatively transparent. Magnetic nanoparticles can also be used for heating where instead of light, an oscillating electromagnetic field is used to generate heat-inducing eddy currents in the nanoparticles causing them to heat. While the equipment utilized to generate the magnetic field is complex, this treatment can be applied to areas in the body that are difficult to penetrate with light.

Among the many and varied applications of this nanoparticle-based approach is photothermal therapy (PTT) for cancer. PTT, which involves the application of plasmonic nanoparticles as light-triggered thermal transducers, has emerged as a promising cancer treatment strategy. A significant amount of research is underway to optimize PTT.

Jorgensen, et al., developed a single particle and PET-based platform to correlate the heat

generation of plasmonic nanoparticles with their potential as cancer-killing agents. Heat generation and absorption cross-section of single irradiated nanoparticles were quantified in vitro using a temperature-sensitive lipid-based assay and compared to their theoretically predicted photo-absorption. In vivo, heat generation of irradiated nanoparticles was evaluated in human tumor xenografts in mice using 2-deoxy-2-[F-18] fluoro-D-glucose (18F-FDG) PET imaging. To validate the platform, the authors quantified the photothermal efficiency of NIR resonant silica-gold nanoshells (AuNSs) and benchmarked the results against the heating of colloidal spherical, solid gold nanoparticles (AuNPs). Heat generation of the resonant AuNSs was superior compared to the non-resonant AuNPs both in vitro and in vivo. These results indicate that PET imaging can be used to reliably monitor early treatment response to PTT and offer a way to benchmark novel plasmonic nanoparticles for use in this novel treatment modality.

An ongoing challenge with plasmonic PTT is the need to minimize the damage to the surrounding tissue. Adjacent tissue can be damaged by absorption of laser light, thermal conductivity, nanoparticles diffusing from the tumor, or a combination of these factors.

He, et al, sought to better understand light–tissue interactions and thermal responses in porcine brain tissue samples including the brain stem, cerebrum, and cerebellum, under laser treatment. The authors found that different tissues have differential optical and thermal properties and confirmed the enhancement of heating by adding plasmonic gold and silver nanoparticles. When the temperature under laser irradiation was measured, a significant difference between the heating of the brain stem and the other types of tissue was observed. The effect of plasmonic PTT on the tumor and the side effects on the healthy brain tissue are related in a non-trivial manner, depending on the tissue properties. As such, the authors conclude that a personalized analysis of the local brain tumor environment is needed to balance the effect and side effects prior to plasmonic PPT.

Heat distribution during PTT can be heterogeneous throughout the tumor volume, leading

to the tumor only being partially treated. Simon, et al., have investigated whether giving the treatment repeatedly, referred to as fractionated PTT, increases efficacy in mice bearing subcutaneous tumors. The study used silica-gold nanoshells in two fractionated protocols – either two or four laser treatments of a murine subcutaneous colorectal tumor model. Efficacy was evaluated by monitoring tumor growth and PET imaging of 18F-labeled glucose analog 18F-FDG. While the authors found no significant differences in tumor growth and survival in single-dose PTT or fractionated PTT, some animals showed inhibited tumor growth or complete tumor disappearance with fractionated PTT; these animals also showed a significant decrease in tumor uptake of 18F-FDG after therapy. Given these results, it is clear that many factors can affect the outcome of PTT and continued optimization is needed.

The potential of gold nanoshells and nanorods for tumor ablation in combination with NIR light is driving the development of superior nanomaterials and improved methods for optimizing irradiation regimens, which could improve PTT. Von Maltzahn et al, described the development of novel plasmonic nanomaterials to improve the specificity of cancer ablation by homing to tumors and acting as antennas for accepting externally applied energy. The team synthesized PEG–protected gold nanorods that exhibit superior spectral bandwidth, photothermal heat generation per gram of gold, and circulation half-life compared with gold nanoshells, the prototypical tunable plasmonic particles. A single intravenous injection of PEG-nanorods enabled the destruction of all irradiated human xenograft tumors in mice. The authors believe that these results highlight the potential of integrating computational therapy design with nanotherapeutic development for precision tumor ablation.

Because the success of PTT treatment depends on many biological factors, routine use will necessitate robust patient monitoring and evaluation. The PET tracer 18F-FDG has been successfully used in cancer imaging; a decrease in 18F-FDG uptake in tumors following treatment could be interpreted as a loss of tumor viability. 18F-FDG evaluation can, however, be impaired by tumors that have a low baseline uptake or reside in or in the vicinity of tissues that have a naturally high glucose metabolism. To address this, Simon et al., evaluated diffusion-weighted imaging (DWI), an MR technique that characterizes the diffusion of water molecules within tissues, for early response monitoring of PTT in tumor-bearing mice using silica-gold nanoshells (NS). NS-treated mice experienced inhibited tumor growth and significantly prolonged survival compared to control mice. Changes in 18F-FDG uptake and the apparent diffusion coefficient, the way DWI is quantified, correlated significantly with survival, demonstrating that both methods can be used for the early evaluation of PTT.

Conclusion

As demonstrated in the publications reviewed in this compendium, nanomaterials like gold nanospheres, gold nanoshells, gold nanorods, and iron oxide nanoparticles as well as silica and mesoporous silica nanoparticles can offer exceptional performance and unique capabilities

in the applications of drug delivery and photothermal therapeutics

At Fortis Life Sciences, we utilize our proprietary nanoparticle manufacturing techniques to provide solutions for the rapid development and commercialization of medical devices and therapeutic products. There are many nanomaterial companies and many GMP manufacturers but very few specialize in both. We leverage our diverse expertise to help partners bring high-impact nano-enabled products to market. Review our large library of off-the-shelf nanoparticles for research use or contact us today to speak with an expert regarding GMP requirements or custom developments.

References

1. Global Nanoparticle Drug Delivery Market, Dosage, Price and Clinical Pipeline Outlook 2028 Report. Published by Kuick Research, 2022.
2. Zhao Q, Sun X, Wu B, et al. Construction of homologous cancer cell membrane camouflage in a nano-drug delivery system for the treatment of lymphoma. J Nanobiotechnol 19, 8 (2021). https://doi.org/10.1186/s12951-020-00738-8
3. Zhao, Q, Wu B, Shang Y, et al. Development of a nano-drug delivery system based on mesoporous silica and its anti-lymphoma activity. Applied Nanoscience. 2020. https://doi.org/10.1007/s13204-020-01465-0.
4. Xu C, Nam J, Hong H, et al. Positron emission tomography-guided photodynamic therapy with biodegradable mesoporous silica nanoparticles for personalized cancer immunotherapy. ACS Nano. 2019. DOI: 10.1021/acsnano.9b06691.
5. Lamson NG, Berger A, Fein KC, et al. Anionic nanoparticles enable the oral delivery of proteins by enhancing intestinal permeability. Nature Biomedical Engineering. https://doi.org/10.1038/s41551-019-0465-5.
6. Mukherjee A, Darlington T, Baldwin R, et al. Development and screening of a series of antibody-conjugated and silica-coated iron oxide nanoparticles for targeting the prostate-specific membrane antigen. ChemMedChem. 2014. 9, 1356. DOI: 10.1002/cmdc.201300549.
7. Park JH, Jackman JA, Ferhan AR, et al. Cloaking silica nanoparticles with functional protein coatings for reduced complement activation and cellular uptake. ACS Nano. 2020. 14:11950. https://dx.doi.org/10.1021/acsnano.0c05097.
8. Zazo H, Colino C, Gutierrez-Millan C, et al. Physiologically based pharmacokinetic (PBPK) model of gold nanoparticle-based drug delivery system for stavudine biodistribution. Pharmaceutics. 2022. https://doi.org/10.3390/pharmaceutics14020406.
9. Fuller MA, Carey A, Whiley H, et al. Nanoparticles in an antibiotic-loaded nanomesh for drug delivery. RSC Advances. 2019. 9, 30064. DOI: 10.1039/c9ra06398f.
10. Fuller M, Whiley H, Koper I. Antibiotic delivery using gold nanoparticles. SN Applied Sciences. 2020. 2:1022. https://doi.org/10.1007/s42452-020-2835-8.
11. Goyal R, Kapadia CH, Melamed JR, et al. Layer-by-layer assembled gold nanoshells for the intracellular delivery of miR-34a. Cellular and Molecular Bioengineering. 2018. https://doi.org/10.1007/s12195-018-0535-x.
12. Henderon L, Neumann, O, Kaffes C, et al. Routes to potentially safer T1 magnetic resonance imaging contrast in a compact plasmonic nanoparticle with enhanced fluorescence. ACS Nano. 2018. DOI: 10.1021/acsnano.8b03368.
13. Jorgensen JT, Norregard K ,Tian P, et al. Single particle and PET-based platform for identifying optimal plasmonic nano-heaters for photothermal cancer therapy. Science Reports. 2016. 6:30076. DOI: 10.1038/srep30076.
14. He Y, Laugesen K, Kamp D, et al. Effects and side effects of plasmonic photothermal therapy in brain tissue. Cancer Nanotechnology. 2019. 10:8. https://doi.org/10.1186/s12645-019-0053-0.
15. Simon M, Norregaard K, Jorgensen JT, et al. Fractionated photothermal therapy in a murine tumor model: comparison with single dose. International Journal of Nanomedicine. 2019. 14 5369–5379. http://doi.org/10.2147/IJN.S205409.
16. von Maltzahn G, Park J-H, Agrawal A, et al. Computationally guided photothermal tumor therapy using long-circulating gold nanorod antennas. Cancer Research. 2009. 69: (9). DOI: 10.1158/0008-5472.CAN-08-4242.
17. Simon M, Jorgensen JT, Norregaard K, et al. 18F-FDG positron emission tomography and diffusion weighted magnetic resonance imaging for response evaluation of nanoparticle-mediated photothermal therapy. Scientific Reports. 2020. 10:7595. https://doi.org/10.1038/s41598-020-64617-w.