A reconstructed 3D image showing the accumulation of 30-nm nanoparticles (green) in a pancreatic tumour.

NANOTECHNOLOGY

Carrying drugs

Traditional chemotherapies can be toxic but nano-sized carriers can keep them out of healthy tissue and take old drugs to new places.

BY KATHERINE BOURZAC

When Joseph DeSimone makes nanomedicines, he compares himself to a baker. He mixes drugs with different chemical 'batters', puts them in tiny moulds, cures them and then turns them out. He can mould almost any shape: discs, cubes, long sticks, roughened doughnuts, or particles shaped like pollen, viruses or red blood cells. But unlike a baker's cakes, brags DeSimone, a chemical engineer at the University of North Carolina in Chapel Hill, every particle in a batch will be identical, regardless of the recipe.

The materials scientists and chemists who work in nanotechnology are creative designers, but they're also control freaks. The ability to make particles to exact specifications, and to control their form at the nanoscale with great precision, enables researchers to control

their function. DeSimone's various shapes can squeeze through blood vessels or worm their way to the core of a tumour. And shape is just one of many properties he and others can engineer at the nanoscale. Nanoparticles with carefully controlled chemistry, size, surface charge and other properties can carry drugs to new places and give them new functions. Nano-engineered drug carriers can slip selectively into cancerous tissue, or protect the drugs they carry from being destroyed before they reach their destination.

Nanomedicine has the potential to address one of the biggest problems in cancer therapy: how to get enough of the right drug to the right place, without causing side effects or inducing resistance. As researchers learn more about the tumour microenvironment, and about how to engineer and manufacture nanoparticles that carry drugs, they are gaining more control over

the course of cancer therapy — an advance that is expected to lead to more effective treatments with fewer side effects. It is also resulting in therapies that were previously thought impossible, such as drugs that change their properties depending on where they are in the body, or that target proteins once deemed undruggable. Some labs are even testing ideas inspired by robotics and computing, such as nanoparticles that communicate with each other to increase accumulation in a tumour.

SIZE MATTERS

One of the greatest uses of nanomedicine in cancer treatments so far has been keeping toxic drugs out of healthy tissues, says Rakesh Jain, a cancer biologist at Massachusetts General Hospital in Boston who is also affiliated with several drug companies. Many traditional chemotherapies are too toxic to be given in large doses or combined with other toxic drugs. They may have precise chemical targets but they are poor at targeting specific tissues — they make their way blindly through healthy tissues and cancerous ones alike, causing harmful side effects.

Doxorubicin is used to treat a range of cancers, but it can also cause life-threatening heart damage. One of the earliest successes of nanomedicine was Doxil, a doxorubicin-carrying nanomedicine, approved in 1995, that keeps the drug out of the heart.

By the mid-1980s, researchers knew that 100-nanometre particles are too big to exit healthy blood vessels but can easily escape the leaky, hastily built vasculature that feeds tumours. Doxil was engineered to take advantage of this. To keep doxorubicin out of the heart, researchers loaded it into a lipid bubble about 100 nanometres in diameter. Lipids don't allow for much engineering control, but they readily self-assemble into bubbles. When shaken together in a water-based solution, the lipid molecules coalesce around doxorubicin particles to create drug-delivering nanoparticles. Then, to help the nanoparticles evade the immune system, researchers coat them with polyethylene glycol. Once these Doxil particles accumulate in the tumour, the drug leaks out of its carrier and attacks nearby cells.

Patients receiving Doxil have one-third the congestive heart failure incidence of those given conventional doxorubicin, resulting in "a quantum jump in quality of life", Jain says. But keeping drugs out of the wrong places is much easier than getting them into the right ones. Drugs the size of Doxil are passively excluded from healthy tissue but cannot actively make their way deep into a tumour, instead clustering at its perimeter. As a result, nanomedicines offer little or no survival benefits compared with conventional formulations, Jain says. "Now we have to improve delivery in tumours."

The nanomedicines currently being developed are more sophisticated than Doxil, but many maintain the basic design of a spherical

carrier with a coating. To improve delivery, companies such as BIND Biosciences of Cambridge, Massachusetts, are also tuning other properties such as charge, chemistry and shape. Chief executive Scott Minick describes BIND's approach as "medicinal nanoengineering". Minick was previously chief executive of Sequus Pharmaceuticals, the company that developed Doxil. But unlike Doxil, which uses simple lipids as its drug carrier, BIND's nanomedicines use polymers, which are easier to engineer. This approach means the company can build drug bubbles and direct where they go, control how quickly they release a drug, and target cancer cells according to their surface markers.

The leading candidate¹, BIND-014, is a 100-nanometre polymer sphere loaded with docetaxel, a drug that kills dividing cells. Like Doxil, BIND-014 relies on its size to leave the tumour vasculature. Unlike Doxil, however, the polymer centre has been engineered to control drug release, among other things. The outer layer is made up of two additional components: polyethylene glycol to help it evade the immune system, and binding molecules that seek out markers found only on the surface of tumour cells (see 'Nano drug carrier').

The early results of BIND-014's phase I clinical trials look promising, Minick says. "This patient population is late-stage, terminally ill, and we don't expect to see signs of efficacy," he says. Even so, there are hints that the drug is working: although the trial is still recruiting, the company has reported that, following a course of BIND-014, tumours shrank in two of 17 patients.

To design its therapies, BIND Biosciences tweaks the size, charge and other properties of each part of its drug carriers, giving it control over circulation time and drug release rate, for example. This approach allows it to make effective nanomedicines without knowing all the biological details of why a particle works as well as it does. Researchers elsewhere, however, are more deliberately taking advantage of advances in understanding the biophysics of the tumour microenvironment.

SMALL AND SQUISHY

Designing nanomedicines to seep out of the bloodstream into tumour blood vessels is only the first step in cancer drug delivery². Although a size of 100 nanometres works well for some things, it's still quite large for a drug. "Once a big nanoparticle leaks out, it's pretty much stuck," says Jeffrey Hubbell, a chemical engineer at the Ecole Polytechnique Fédérale de Lausanne in Switzerland.

Once a large particle leaves the leaky blood vessels, it has difficulty moving deep into a tumour. Making the particle smaller would improve its mobility, and is also an advantage when fighting certain tumours — particularly pancreatic and some breast cancers — that are threaded with a tough tangle of collagen,

COMMUNICATING CHEMOTHERAPIES

Reconnaissance nanoparticles lodge in a tumour and signal drug carriers to swarm to the site and accumulate at higher concentrations than are otherwise possible.

which presents a physical barrier to drugs.

But reliably making polymer nanoparticles much smaller than 100 nanometres is tricky. Kazunori Kataoka, a materials scientist at the University of Tokyo, Japan, developed the first polymer drug carrier in the mid-1980s. His company, NanoCarrier, based in Kashiwa, has now developed a 30-nanometre polymer to transport the chemotherapy drug cisplatin; it is currently undergoing phase II clinical trials in patients with pancreatic cancer.

Cisplatin usually has severe kidney toxicity, requiring patients to drink painfully large amounts of water during treatment. Kataoka says that's not the case in the NanoCarrier trials because the carrier's size allows it to move into and accumulate in the pancreatic tumour, instead of accumulating in the kidney. "We've already successfully extended survival," he says, which is heartening given how difficult pancreatic cancer is to treat. In a small phase I trial, the drug more than doubled survival time from five months to more than twelve.

Back in North Carolina, DeSimone's work moulding strangely shaped particles has a similar motivation: controlling a drug's size and shape to help it enter tumours. "We want to figure out how cancer cells get in places they're not supposed to be, and mimic that," he says, so that he and others can make drugs to follow them there.

DeSimone drew inspiration for his particle-moulding method from semiconductor manufacturing plants, which make tiny transistors by the trillion. He can precisely vary just a single property, such as stiffness, and then test how the particles move through the body. Using this manufacturing method, which has been licensed by Liquidia Technologies of Research Triangle Park, North Carolina, he can find out, for example whether a squishier

drug is better at squeezing into the centre of a tumour.

SILENCE WILL FALL

One of the most promising applications for nanoengineered drug carriers is gene silencing, in which small bits of RNA are deployed to shut down crucial cancer genes through a process known as RNA interference. Researchers know how to make RNA sequences that theoretically can turn off any given gene. But without a good delivery vehicle to test the effects of these silenced genes, finding promising therapeutic targets in animals — let alone making an RNA therapy that will work in people — is slow going, says William Hahn, an oncologist at Harvard Medical School in Boston, Massachusetts. Nanocarriers may be just the technology to push this technique forward.

Designing a nanocarrier suited to transport gene-silencing RNA is tricky, however. It must make it all the way inside a cancer cell, which requires an escort smart enough to evade destruction. Typically, if it's not cleared out by the liver, an RNA-carrying particle will bind to the cancer cell membrane, which then folds inward and pinches itself off into an acidic, destructive bubble called an endosome inside the cell.

Researchers are working on several tricks to get round this. Chemical engineer Mark Davis at the California Institute of Technology in Pasadena has developed a polymer carrier that absorbs positive charges as they are pumped into the endosome. This creates an osmotic pressure that eventually bursts the cancer cell's bubble, freeing the silencing RNA before it is destroyed.

Traditional therapies work by binding to proteins and disabling them. Unfortunately, most cancer genes produce proteins that are

NANOMEDICINE IN CLINICAL TRIALS

Several nanoscale drug carriers are currently in clinical trials.

Company	Drug	Formulation	Status	Description
Calando Pharmaceuticals	CALAA-01	A polymer nanocarrier containing gene-silencing RNA	Phase I	A polymer nanocarrier holds RNA that silences a gene in solid tumours needed for DNA synthesis and replication
BIND Biosciences	BIND-014	A polymer nanocarrier targeted to cancer cells carries docetaxel	Phase I	Targets solid or metastatic prostate cancer cells by binding to prostate-specific membrane antigen
Nippon Kayaku	NK105	A polymer nanocarrier containing paclitaxel	Phase III	Looking for progression-free survival in patients with metastatic or recurrent breast cancer
NanoCarrier	Nanoplatin (NC-6004)	A polymer nanocarrier containing cisplatin	Phase I/II	Evaluating Nanoplatin in combination with gemcitabine in patients with advanced or metastatic pancreatic cancer, with the aim of reducing kidney toxicity compared with cisplatin alone
Cerulean Pharma	CRLX101	A pH-sensitive polymer nanocarrier releases camptothecin in the acidic environment of cancer cells	Phase II	Separate studies testing CRLX101 in advanced non-small cell lung cancer and in ovarian cancer

considered undruggable by traditional means. Some of these proteins hide inside the cancer cell, out of the reach of antibody drugs that can only get to the surface. Other proteins have a physical shape that provides no foothold for a drug of any kind. Nanoengineering, Davis says, could break through these defences. With the right carrier, there is no need to go after the undruggable proteins — gene-silencing RNA can instead go directly to the genes that make them.

It can also target several cancer genes at once. “The goal has got to be to hit multiple targets simultaneously” so the tumour cannot develop resistance, says Davis. If a tumour mutates in the course of treatment, oncologists will be able to order therapies that target those new mutations.

THE LOGICAL NEXT STEP

Nanotechnology researchers such as Davis, and companies like BIND, are focused on getting more effective therapies into the clinic as quickly as possible. But most nanomedical research has been done *in vitro* and in animals; little is known about how these drugs work in people, Davis says, although a series of clinical trials is under way (see ‘Nanomedicine in clinical trials’).

Other researchers are using the tools of physical science, from robotics to computer science, to realize more fanciful ideas about future drugs. One prototype, made by George Church’s group at Harvard University in Cambridge, Massachusetts, is a drug-stuffed ‘lock box’ that opens only after performing a simple logic operation akin to those done by computer circuits. The box is made of DNA, a material Church and his colleagues chose for its design possibilities. Using a technique called DNA origami, the DNA self-assembles into a barrel shape, with locks and hinges that cause it to spring open when particular surface markers on cancer cells — the ‘keys’ — unlock them.

Church calls his DNA drug carrier a nanobot because it performs a computational logic function: when two input signals are present (two molecular markers on the targeted cancer

cell), the box generates an output (opening to release its drug payload). In one recent experiment³, the team designed a cylinder of DNA that contains a cancer drug, like gems in a jewellery box. The nanobot had two locks designed to be opened by two proteins on the surface of aggressive leukaemia cells. The researchers showed that the leukaemia cells could unlock the nanobot, but other cells in the bloodstream could not.

The logic-function idea was motivated by a clinical need. Most targeted therapies seek out just one cell surface marker. Logic-gated systems like Church’s, however, might allow for

communicate with each other to ‘swarm’ to tumours.

“Ninety percent of cancer deaths are caused by metastases,” says Bhatia. Finding those secondary tumours is difficult, especially when they’re new. “We want to inject a therapy that will figure out where the metastasis is” and then communicate that information to other drugs, she says, so more of the drug reaches its target. Early demonstrations⁴ showed that drug-carrying nanoparticles accumulate in a tumour in much larger numbers than they would without such communication — in one experiment, there was a 40-fold increase (see ‘Communicating chemotherapies’).

Bhatia is looking to expand this idea by incorporating design tricks from robotics. Like ants, whose individual actions are simple but who *en masse* can build a complex anthill, groups of individual robots can be programmed to swarm and perform tasks collectively. Instituting simple rules such as “maximize your distance from all neighbours” has allowed roboticists to make groups of robots that fly like bees in a swarm. If Bhatia can apply this to drugs, she might achieve even greater drug accumulation.

The work is unorthodox, but that doesn’t bother Bhatia. “We want to evolve nanomedicine away from formulations where everything is exactly the same,” she says. Indeed, the whole field is still evolving. As researchers improve their ability to control, manufacture and innovate at the nanoscale, they are opening up new paths for cancer therapy. Some may prove fruitless, but others could yield new ways to make cancer therapy less painful and more effective. ■

NANO DRUG CARRIER

BIND-014 carries its payload in a tangle of drug-releasing polymer. The PEG coating helps it circulate and binding ligands help it find the tumour.

specifically targeted drugs that go after cancer cells, such as leukaemia, but avoid healthy cells that might have a surface marker in common with the cancer cells. But the research is still in the early stages, as it is difficult to produce enough DNA boxes to test the method in animal models.

Sangeeta Bhatia, a biomedical engineer at the Massachusetts Institute of Technology, is also taking inspiration from information technology and other fields. She is emulating natural systems and robotics to make smart cocktails of cancer therapeutics that

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