

# New Vaccine Technologies Carry and Deliver

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BD MEDICAL PHARMACEUTICAL SYSTEMS

Vaccine developers are using novel drug delivery methods that offer advantages over traditional techniques such as improved immunogenicity, better stability, specific control over antigen release, and a wider pool of targeted diseases.

**V**accines have helped slash infectious diseases mortality rates throughout the Western world. Vaccine programs also have made progress in reducing deaths in developing nations, but immunization coverage there is still limited. Death rates are slowly rising, traditional vaccine development approaches have reached a standstill, and new challenges are emerging. Developers need new tools to tackle old threats such as malaria and HIV, new threats from biological terrorism, and new targets such as cancer.

“If you think about the history of vaccine delivery, it has pretty much been static for the past 60 years. I really think new delivery methodologies are going to pave the way for better and more effective vaccines

with better protection for both developed and developing nations,” predicts Kenneth (Bill) M. Yates, president of drug delivery specialist DelSite (Irving, TX, [www.delsite.com](http://www.delsite.com)).

Coming up with new and improved vaccines may be a matter of aligning our ever-growing understanding of biotechnology, micro-/nanotechnology, and the immune system. “To improve vaccines, we need to combine technologies such as antigens, adjuvants, and delivery,” stresses Thomas R. Tice, PhD, executive vice-president and chief scientific officer of Brookwood Pharmaceuticals, Inc. (Birmingham, AL, [www.brookwoodpharma.com](http://www.brookwoodpharma.com)), an innovator of new drug delivery technologies.

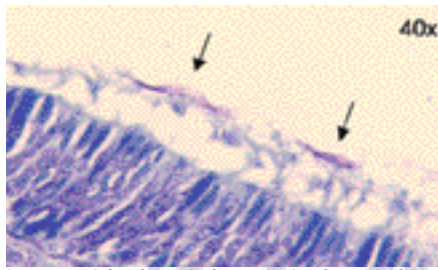
In the pipeline at several pharmaceutical and biotech companies are vaccines that use carriers—either genetically engineered microorganisms or micro-/nanoparticles—to deliver the immunostimulating payload. These drug delivery methods, many of which are in early-phased clinical trials, claim advantages over traditional vaccines such as improved immunogenicity, better stability, specific control over antigen release, and a wider pool of targeted diseases.

## Timing is everything

**Improving immunogenicity.** Knowing exactly how and when the immune system is triggered is key information for vaccine developers. It’s no wonder then, that researchers are exploiting what they know about the body’s internal rhythms to increase the potency and efficacy of vaccines.

For example, scientists are using nano- and microparticles as delivery vehicles for genetic instructions to produce a specific antigenic protein. If the release of DNA is in sync with the body’s immune system, these gene-based vaccines can induce a long-lasting more effective immunity. Says Jorge Heller, a former principal scientist at A.P. Pharma (Redwood City, CA, [www.appharma.com](http://www.appharma.com)) who developed such genetic vaccines, “DNA vaccine delivery systems work better than conventional vaccines because you can get both a humoral response [*i.e.*, the presence of antibodies in the blood stream] and a [helper] T-cell-mediated response. Traditional vaccines only give you a humoral response.”

DNA-carrying microparticles are injected into the body and are taken up by



Intranasal *in situ* gelation powder formulation in rats

host cells. The DNA enters the nucleus and programs the cells to become, in effect, bioreactors that churn out foreign antigenic protein. Because the host cells produce the antigen, the process won't cause disease side effects. Says Alan Engbring, spokesperson for DNA delivery specialist, Vical Inc. (San Diego, CA, [www.vical.com](http://www.vical.com)), "We are effectively using the cells in the body as factories to manufacture the proteins. We're providing the DNA that programs those cells to produce a protein."

The microparticles are sized less than 10  $\mu\text{m}$  so that they will be taken up by either antigen-presenting cells (*e.g.*, dendrites, macrophages) or other types of cells (*e.g.*, astrocytes). In the case of macrophages, the microencapsulated antigenic protein is engulfed by B-cells or helper T-cells to give an immune response. "The microparticles are like a Trojan horse," explains Brookwood Pharmaceuticals' Tice, codeveloper of the "VAXCAP" vaccine delivery technology, which is in early-phase clinical trials.

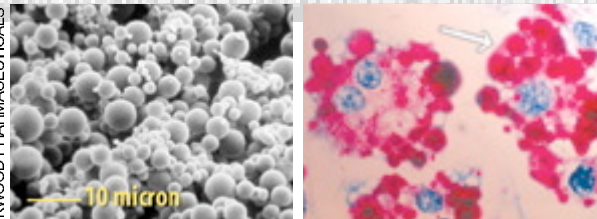
Under certain pH conditions, DNA can be destroyed. To avoid this effect, A.P. Pharma researchers have experimented with molecularly engineered poly(orthoesters) microspheres that preserve the genetic material until its release. Because DNA is deactivated in less than 5 pH, the microspheres are designed to maintain a neutral interior during a specifically timed erosion period. To achieve better immunogenicity, for example, the microspheres degrade and release the DNA in response to the phagosome's pH, according to Heller.

The delivery of vaccines through mucosal sites, such as the nasal cavity, is another technique that, if timed correctly, could provide systemic and mucosal protection at the site of infection. Because of lymphocyte trafficking, "you can see some cross-protection against infections at other mucosal sites such as the lungs or

the gastrointestinal tract," notes Yates of DelSite.

In addition, vaccinologists believe that such delivery routes could offer cross-protection against multiple strains of a given pathogen because of mucosal antibody secretion. An example of this effect is MedImmune's "FluMist" nasal-delivered influenza vaccination which is said to offer better cross-protection against various strains influenza than traditional injectable vaccines.

But, it has been hard to get the vaccine to stay in the nose long enough to be effective. "With a vaccine, the longer you have exposure, the better chance you have of developing an immune response," says Dennis Discher, professor of chemical and



(left) VAXCAP microparticles. The antigen is embedded in a matrix of resorbable poly(lactide-coglycolide) polymer. (right) VAXCAP microparticles taken up by macrophages. The arrow indicates the location of some of these microparticles. After uptake, the microparticles will release antigen within the macrophages followed by resorption of the polymer matrix.

biomolecular engineering at the University of Pennsylvania and researcher at The Nanotechnology Institute (Philadelphia, PA, [www.nanotechinstitute.org](http://www.nanotechinstitute.org)), a research group using self-assembled polymers to deliver small drug molecules.

In the past, some researchers have used a modified live virus to infect the mucosa. "In our case, we're trying to prolong the residence time so that we can use inactivated or other types of antigens, whether they be a protein, a conjugate, or a particulate," Yates explains.

To achieve this effect, DelSite's "GelVac" powder formulation instantly gels upon contact with nasal fluids, entrapping the vaccine antigens. Then, a natural matrix forms that slowly releases the antigen. At present, this technology, which is in Phase I trials without an antigen, is being incorporated into vaccines that use attenuated viruses, live vectors, or DNA.

**In one fell swoop.** Controlling antigen release with gene-based vaccines can also improve booster regimens.

By mixing microparticles with various degradation rates, vaccines could include "automatic boosters" that eliminate repeat visits to a healthcare provider. To achieve this type of delivery, Brookwood Pharmaceuticals uses two types of microparticles with specific hydrophobicity and absorption properties to control the length of time before each microparticle releases its antigen. "We can make one injection that takes care of the prime and a booster, for instance," says Tice of Brookwood Pharmaceuticals.

### Eliminating cold chains

Because most vaccines are injected and use live or weakened pathogens to initiate an immune response, they must be refrigerated until the time of use. Maintaining an unbroken chain of cold storage from manufacturing to administration poses a particular challenge for developing nations where refrigeration is not always readily available. And, in the event of a biological attack, the need for a cold chain could impede stockpiling and slow mass vaccinations.

Certain drug delivery methods could provide solutions for this problem. Because DNA vaccines do not use pathogens, for example, they don't need cool storage during shipment. "We have found that they are very stable without refrigeration," notes Heller.

DelSite's intranasal powder technology also is designed to overcome the refrigeration dilemma. "Because it's a powder formulation, it won't require cold storage and it should also confer longer stability," explains Yates. Vaccines developed with this technology will be a single-unit dose, so patients will be able to self-administer the treatment in cases in which broad, quick coverage is needed (*e.g.*, in a pandemic or bioterrorist attack).

Formulation scientists also are getting in on the cold-chain-elimination act. *Pharmaceutical Technology* recently reported ("In the Field," April 2005, p. 28) on a technology pioneered by Cambridge Biostability (CBL, Cambridge, UK [www.biostability.com](http://www.biostability.com)) that addresses the refrigeration prob-

lems by combining antigens in dry, sugar-glass microspheres.

The microspheres are suspended in stable, injectable formulations and have an anticipated shelf life of 12–18 months at room temperature. Accelerated antiaging trials have proven 6-month stability of vaccines kept at 55 °C. Though real-time lifetime studies have yet to be performed, “the shelf life essentially will be indefinite,”

says the company’s chief scientist and principal investigator, Bruce Roser, PhD. The company and its partners currently are working to stabilize formulations to combat various biothreats, cancers, and other diseases.

### New disease targets

Put simply, the easy vaccines have already been made. “The higher value, new vac-

cines are where the newer technology seems to be more acceptable,” notes Tice. “We tend to see more activity in cancer than in tetanus vaccines.”

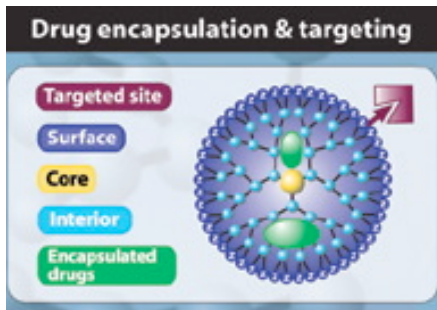
To expand the pool of diseases for which vaccines are available, drug developers are searching for flexible systems that can deliver a variety of antigenic material. One delivery technique that could bring new disease targets to the table is the microorganism vector. Scientists select a bacterial (*e.g.*, salmonella) or a viral organism (*e.g.*, equine encephalitis) and reengineer its genome to carry genetic instructions for the protein the immune system must recognize. “We’re using the cell’s own biological machinery as if it were creating a new virus to resemble a viral particle,” notes Peter F. Young, president and CEO of AlphaVax, Inc. (Research Triangle Park, NC, [www.alphavax.com](http://www.alphavax.com)). The body recognizes the carrier as an invader—though it can no longer cause disease—and builds immunity to the antigens.

Selecting the proper vector for delivery is crucial to the success of this technology. “The vector is going to determine its utility as an immunological agent. There are many variables that can be fatal flaws in a vector,” Young points out. Some companies have withdrawn from this market because their vectors could only carry limited types of antigens. According to AlphaVax, its “ArV” vector technology uses the Venezuelan equine encephalitis (VEE) virus because it has several unique biological properties. For example, once injected into the body, the virus has an intrinsic immunotopism that makes dendritic cells take up the particles and present them to the immune system, provoking an immune response. The vaccine replica particles are engineered so that the virus is not replicated in the body. AlphaVax has several vaccine candidates in Phase I and II clinical trials, including vaccines that counteract biothreats, HIV, cancer, and parainfluenza virus.

Both microorganism and microparticle carriers could be suitable for another new application: combination vaccines against biothreats, in which one vaccination protects against anthrax, smallpox, and Ebola, for example. This type of combination therapy might be possible with microencapsulation, according to Brookwood Pharmaceuticals, if drug developers mi-



DENDRITIC NANOTECHNOLOGIES, INC.



Dendrites could be used to encapsulate antigenic material or active therapies in their void space.

ciated with drugs such as paclitaxel,” explains Stephen Benoit, co-founder and CEO of NanoMed Pharmaceuticals Inc. (Lexington, KY and Kalamazoo, MI, [www.nanomedpharm.com](http://www.nanomedpharm.com)).

NanoMed’s technology produces nanoparticles that mask the delivery-limiting characteristics of the drugs; enable targeted delivery of the drugs to specific tissues and cells (e.g., tumors); and provide a sustained release of the drugs in tissue which could reduce dosage frequency, peripheral toxicity, and adverse effects.

Other new target diseases include infectious diseases such as anthrax, severe acute respiratory syndrome (SARS), and even HIV because it’s too dangerous to vaccinate against them using a dead or an attenuated virus. NanoMed Pharmaceutical’s nanoparticle-based dendritic cell-targeted HIV-1 therapeutic vaccine under development has generated neutralizing antibodies to key epitopes on the Tat protein.

Benoit explains that this “suggests that we may be able to significantly delay or perhaps avoid the progression of HIV or AIDS.” The therapeutic vaccine is expected to enter clinical trials within 12–18 months.

### Will processing and production make or break new vaccines?

As with all pharmaceuticals under development, the full-scale manufacturing processes for new vaccines must be planned during early-phase clinical trials.

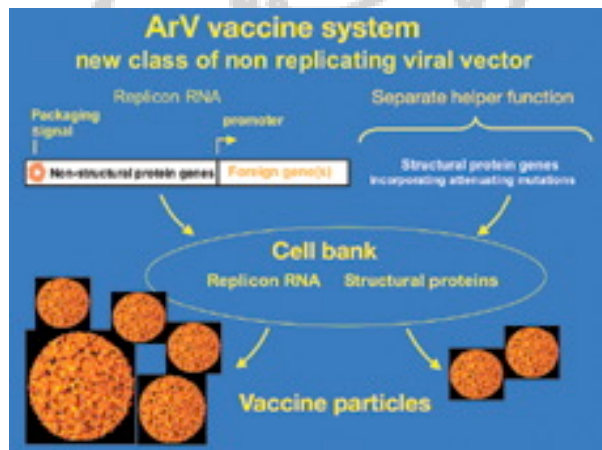
The paradox of producing a sterile pharmaceutical made with a bacterial carrier and DNA that cannot be destroyed is a challenge. “At the end of the day, you want to have a sterile solution. But you cannot sterilize the solution in a normal way because it would destroy the carrier organism,” says Maik W. Jomitz, group vice-president for product management at Sartorius North America (Edgewood, NY, [www.sartorius.com](http://www.sartorius.com)). “What has to happen is you must be very, very clean right from the upstream side [to the filling point] so that you avoid any kind of contaminant inside the fluid, if it’s an injectable.”

Because the technology is based on the delivery of DNA, Brookwood’s VAXCAP

microencapsulation production process is based on this principle. “We prepare the microcapsules aseptically. This is important for DNA vaccines because sterilizing (e.g., with ionizing irradiation) DNA will inactivate it,” notes Tice. The company can scale its process to 10 kilos or more.

Nanomedicine developer NanoMed Pharmaceuticals, is using a proprietary manufacturing technique that also prevents damage to the carriers during large-scale production. The company’s “Nanotemplate Engineering” manufacturing technology begins by melting a pharmaceutical matrix and adding the appropriate polymeric surfactants. Once cooled, the solid, spherical, uniformly dispersed nanoparticles are suspended in a liquid that can be directly filtered through a standard 0.2- $\mu$ m filter, and lyophilized or spray-dried for formulating as a tablet or stored as a powder. “Our nanoparticle formulations can be put in a vial for injection, formulated for nasal delivery, or tableted. The technology is really quite flexible,” says Benoit. According

ALPHAVAX, INC.



Viral vector vaccine system.

microencapsulate the antigens simultaneously or mix microparticles containing each vaccine antigen. In the case of bacterial vectors, Avant Immunotherapeutics (Needham, MA, [www.avantimmune.com](http://www.avantimmune.com)) is in preclinical development with cholera and typhoid fever bacteria as delivery vehicles for a plague–anthrax vaccine. The tableted carrier binds to and burrows into the gastrointestinal wall before it self-destructs. According to the company, the vector elicits a broad immune response without causing disease symptoms or side effects.

Several companies have their eyes on gene-based therapies for cancer vaccines that prevent the recurrence of tumors in patients who have undergone traditional oncological therapies. But in addition to prevention, the same carrier delivery method might reduce side-effects in traditional tumor-fighting treatments. For example, NanoMed Pharmaceuticals, Inc. is working to reformulate approved anticancer drugs using nanoparticles as delivery vehicles. “*In vitro* data suggest that the nanoparticles may enable us to overcome multidrug resistance problems that are typically asso-

### Immunology glossary

**Antibodies** are specialized proteins produced by B-cells after stimulation by an antigen.

**Antigens** are molecular proteins or carbohydrate substances that the body recognizes as foreign and combats with the production of antibodies.

**Antigen-presenting cells (APCs)** are cells such as macrophages, dendritic cells, or other body cells that “present” antigen in a form that T-cells recognize to trigger an immune response.

**B-cells** are lymphocytes that produce antibodies in response to infection.

**Dendritic cells** are white blood cells that function as APCs to capture antigens and present them to the T-cells where an immune response is initiated.

**Helper T-cells** increase the production of antibodies by B-cells and regulate the activities of all effector cells.

**Humoral** relates to immunity that involves antibodies secreted by B-cells and circulating in bodily fluids.

**Lymphocytes** are a type of white blood cell.

**Macrophages** are white blood cells that consume foreign material, cell debris, and invading microorganisms.

**T-cells** are white blood cells that are responsible for cell-mediated immune responses.

Source: *Webster’s New World Medical Dictionary*, (Hungry Minds, Inc., New York, NY, 2000).

to the company, the process is rapid, cost-effective, and scalable.

Other nanopharmaceutical makers have struggled with scaling up their particle development processes because some characteristics cannot be reproduced reliably. Companies have experienced a frustratingly vast distribution in the sizes and structures of their nanoparticles. But dendrimers, Donald Tomalia, PhD, founder of Dendritic NanoTechnologies, Inc., (DNT, Mount Pleasant, MI, www.dnanotech.com) points out, can be reproduced with accurate size, shape, and surface-chemistry specifications. "They're just like a chemical compound," he notes. "You can run a mass spectrometer on them and they're just as reproducible as the purest pharmaceutical that a Big Pharma company would sell."

The hollow, tree-like nanoparticles can be loaded with high levels of toxic anticancer drugs, for example, and release drug at the site of the disease. With this passive targeting technique, healthy cells located near a tumor wouldn't be de-

stroyed and severe side effects would be reduced. Certain companies, such as drug delivery specialist Starpharma (Melbourne, Australia, www.starpharma.com), have accurately produced multikilogram quantities of dendrimers for use as drug carriers.

Commercializing dendrimers and other nanoparticles may require new analytical methods, however. "The analysis of nanoparticles is very different from traditional quality assurance methods used by pharmaceutical companies," notes Tomalia. "You may have to use more mass spectroscopy, gel electrophoresis, and size-exclusion chromatography, for example." DNT and the Nanotechnology Characterization Laboratory currently are working to develop better analytical technologies to characterize nanostructures.

Separation specialists also have recommended membrane chromatography for vaccine processing. Membrane manufacturers take microporous membrane structures and modify inner surfaces of the pores with the same types of ion exchange

chemistries that would be on chromatography beads. Instead of having Q-ion exchange resin in the column, the relevant chemistry is on the internal pore surface of the membrane.

"With this method, you can have a significantly open porosity," notes Jerold M. Martin, Pall Life Sciences's (East Hills, NY, www.pall.com) senior vice-president and global technology director. "You can speed up the process, and you actually have more capacity as well. Then, it's very easy for the molecules to bind and elute." According to Martin, several vaccine manufacturers have already introduced membrane chromatography as a purification step in the downstream processes for DNA and viral vaccines currently in clinical development.

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