Nanotechnology Characterization Laboratory

NCL News

Accelerating the transition of concepts to clinical applications

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Nanoemulsions for the Detection and Treatment of Brain Cancer

Dr. Mansoor Amiji, Professor of Pharmaceutical Sciences at Northeastern University and a recipient of a platform grant from the National Cancer Institute's Alliance for Nanotechnology, is studying nanoemulsions for the detection and treatment of brain cancer. In this interview, Dr. Amiji discusses nanoemulsions, and how the Nanotechnology Characterization Laboratory (NCL) is helping him advance this novel experimental approach toward clinical trials.

Simple to prepare from non-toxic components of oil, water, and surfactants, Dr. Amiji's nanoemulsions have the potential to carry hydrophobic imaging and therapeutic compounds across the blood-brain barrier. The hope is that this novel formulation will help detect and treat even the smallest tumors embedded in remote regions of the brain.

NCL News: What is a nanoemulsion and how is it made?

Dr. Amiji: An emulsion is a mixture of two otherwise immiscible substances, in which one substance is dispersed into another in the form of very small droplets. A nanoemulsion is a mixture of oil, water, and other ingredients, including surfactant, that increase the stability of the mixture. The dispersed nano-sized droplets of oil are made simply by sonication – exposure to ultra-high frequency sound waves or high-pressure homogenization. The nanoemulsions are able to suspend or dissolve agents that might otherwise be difficult to dissolve in a water-based solution. In this case, we have added both imaging agents, such as gadolinium ions, and anti-cancer drugs, such as paclitaxil and ceramide.

NCL News: What are some of the applications that you envision for the nanoemulsions?

Dr. Amiji: One of the major challenges in cancer is how to effectively treat brain tumors. They have the highest mortality rate compared to other types of cancer, and a lot of children are affected by this devastating disease. In fact, it is the leading cause of death from cancers among children up to the age of 10 years. So it's clearly a disease that has a tremendous need for novel therapies.

The advantages of nanoemulsions include the fact that we can select different oils and components to impart specific properties. In particular, we hope to engineer these emulsions to cross different types of biological barriers upon administration, and then release the drug or imaging agent at target sites that we're interested in. In the case of brain tumors, this is very important because a lot of drugs, including chemotherapeutic agents, are not able to cross what's called the blood-brain barrier.

Nanoemulsions make it possible to exploit not only the properties of the oil and the surfactant, but also the size of the oil droplet, which allows them to gain access to different sites in the body.

In This Issue:

NCL News: Why did you choose a nanoemulsion approach as opposed to other nanoparticles?

Dr. Amiji: With the emulsion, we can select unique types of oil, including oils that are rich in unsaturated fatty acids. The hypothesis is that the body does not produce these essential unsaturated fatty acids, and therefore must establish several mechanisms for transporting and absorbing them from nutritional sources.

Making use of biological transport mechanisms such as pumps and receptors, we can deliver molecules of interest, including drugs and imaging agents, to various areas of the body. For instance, for different types of oil, which have different types of fatty acids, we see very different levels of the drug in the brain.

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NCL News: Why did you choose to work with the NCL?

Dr. Amiji: I have several projects funded by the NCI's Alliance for Nanotechnology. We also have a training grant from them for doctoral students who will be developing nanotechnology for medical applications. The third arm of support set up by the Alliance is the Nanotechnology Characterization Laboratory.

Because nanotechnology is still a developing field, we need a facility like NCL that can take concepts from academia or from small companies and characterize these materials in a way that they would eventually be acceptable for submission as an investigational new drug (IND) application to the FDA. In academia, we are somewhat insulated from the processes and skills needed to get FDA approval of new drugs.

I was very much attracted to the idea of getting nanoemulsion technology into the clinic quickly. We don't want it to be an academic project. With the help of the NCL, we could understand exactly what were going to be the obstacles to getting these nanotechnology products to the clinic to benefit patients, and how to overcome those obstacles.

NCL News: What were some of the key findings made by NCL?

Dr. Amiji: The key finding so far, and the work is ongoing, has been that these emulsions are safe. They haven't shown any overt toxicity in animals. They have also confirmed that the nanoemulsions are stable – they do not separate over a defined period of time.

Because it's a multi-component system with two anti-cancer drugs and an MRI contrast agent, each one has to be tested independently and in combination to see how well they are delivered to the brain, and whether there is any synergistic effect with the combination therapy. Preliminary in vivo MRI studies indicate good contrast enhancement, better than the commercially-available Magnevist preparation. For the therapeutic function, we are just beginning to do more of the efficacy studies. To conduct these studies, the NCL intends to test the formulation on a transgenic mouse model that spontaneously develops brain tumors. These kinds of animal resources would be almost impossible to get our hands on as academic researchers.

NCL News: How have the NCL's findings contributed to improvement of the nanoemulsion product?

Dr. Amiji: We were making small batches in the lab, usually about 10 milliliters, with a method for reducing particle size down to thr nanometer scale. But at NCL identified that the particle size distribution might be inconsistent when we scale up using this particular method. When we get to the point where we have to make larger quantities of these emulsions, we would have to use other approaches, such as microfluidic systems, to generate a more consistent nanoparticulate size distribution.

We are working with NCL and the National Institute of Standards and Technology (NIST) to get a handle on how to make larger quantities of nanoemulsion under current good manufacturing practice (cGMP) standards established by the FDA. In an academic lab, access to this type of knowledge is critical to advance an idea to clinical trials.

The NCL also found that the process by which we had been formulating the product was leading to a lot of free gadolinium ions, which can be very toxic. They suggested that we could make the gadolinium-chelation complex first and then add that to the emulsion rather than adding free gadolinium to an emulsion containing the chelating agent. We modified the recipe accordingly, and we've completely eliminated the problem of free gadolinium.

NCL News: Can the nanoemulsion technology transfer to other types of CNS diseases?

Dr. Amiji: Sure. We are looking at various other diseases, such as Parkinson's disease, Alzheimer's disease, attention deficit disorder, and other types of applications where there is clearly a greater understanding of what's happening in the brain, but promising therapies have not materialized because the drug candidates do not cross the blood-brain barrier.

NCL News: Do you envision that, with different compositions of nanoemulsion, you could deliver drugs to different parts of the body?

Dr. Amiji: Absolutely, that's another area that we are exploring. Composition of the oil and how much of these unsaturated fatty acids we have in the oil will dictate where else it will go. The other important thing we can do is to put different kinds of molecules on the surface of these particles, similar to what has been done with liposomes. This type of surface engineering does make it possible not only to get drugs to enter into the brain, but to have the drugs absorbed in the gastrointestinal tract, so the patient can take a pill rather than have an injection.

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NCL News: What's next for the nanoemulsion project?

Dr. Amiji: The next step will be to continue working with NCL to show that the product is safe, efficacious, and potentially useful in the treatment of brain cancer, and then find a pharmaceutical or biotech company that will be interested in taking the product through clinical trials and FDA approval.

These are fairly expensive studies to conduct. So it's important that we have NCL as a resource, especially for those of us in academia. The NCL characterization studies allow potential investors to have more confidence in funding these projects. They can be a bridge for academic groups trying to transform their ideas into actual commercial products.

If I were to take this concept to a company, their first question would be, "Well, how many patients a year are affected by brain tumor?" The answer is, it's only about 12,000 a year. It's really not a big market. For a company, they might not seem a worthy investment at such an early stage. But if NCL can help take it a step further with the pre-clinical safety and efficacy studies, that reduces the risk and increases the chances of finding someone to license the technology, especially if the technology platform can be validated for broader applications. ■

NCL Connections

NCL and NTP

Nanotechnology is rapidly becoming accepted and incorporated in industrial and biomedical practice. Both the general public and policymakers, however, are raising concerns that nanoparticulate materials may pose unknown risks from biomedical uses and environmental exposure. NCI's Nanotechnology Characterization Laboratory (NCL) and the National Toxicology Program (NTP), have come together to share knowledge and data, and to coordinate research on the potential risks of nanotechnology in medicine and the environment.

The NTP is a department-wide program in the U.S. Department of Health and Human Services comprising several agencies, including the National Institute of Environmental Health and Safety (NIEHS), the Food and Drug Administration (FDA), and the National Institute of Occupational Safety and Health. The NTP was established 30 years ago to coordinate toxicology research and testing by the federal government, and to establish guidelines for industry on how to conduct toxicology studies as they relate to environmental and occupational exposure.

The program took on nanomaterials about three years ago, when it became apparent that there would be many potential opportunities for public exposure to these materials from environmental, occupational, and medical sources. At the time, the various federal agencies, including NIEHS, NIOSH, and the FDA, did not have much experience in dealing with nanomaterials, so they established the NTP's Nanotechnology Safety Initiative, led by Dr. Nigel Walker, to coordinate their efforts in gaining knowledge and setting guidelines for the toxicological study of nanotechnology products. Today, the NTP is studying some of the most commonly used nanotechnology products, such as metal oxides, quantum dots, and dendrimers.

With NTP focusing on the health effects of long-term, low-dose environmental exposure and the NCL focused on the shorter-term, higher-dose exposure to biomedical nanomaterials, it became clear that the two groups could learn from each other. "There was a real need to coordinate our activities because essentially, we are doing complementary activities. If we can leverage what we are doing for the NCL and vice versa, then we can get more bang for the buck," said Dr. Walker. Now in the early stages of the collaboration, the two groups look at both sides of nanotechnology. "One of the areas where we are looking at coordinating our efforts is dendrimers, where the NCL has already conducted early studies on their clinical toxicity, but there is no assessment of longer-term risk or hazard." The two groups are also looking at how different labs are conducting nanomaterial characterization around the country. The goal is to make sure everyone is working from the same guidelines, tailored to environmental or clinical toxicology, to ensure reproducibility and easier comparison of results.

Most of the toxicology work conducted under NTP is done by outside laboratories through contracts and grants. The studies are carried out in many of the same facilities used by the pharmaceutical industry under federal Good Laboratory Practice (GLP) guidelines. The NTP designs studies before handing them off to contractors, and evaluates the data upon completion of the work. If the NTP needs to look at inhalation toxicity, for example, they contract out the work to organizations with expertise in inhalation studies, and similarly for reproductive toxicity and immunotoxicity. It will be critical to ensure each contractor adheres to the same set of guidelines and standards for characterizing nanomaterials – an

area that may be new to many of them. That's where the experience of the NCL can be of great help.

"It was very clear to us that when looking at in vitro and in vivo data, you need to know how to handle, evaluate, and characterize nanomaterials, and the NCL is very good at that," said Dr. Walker. "They have really developed that area in terms of the assay cascades and protocols. The NTP will be leveraging the NCL's information for longer-term studies without having to reinvent it."

For the NCL, there is the opportunity to extend the knowledge base to cover the entire life cycle of the products it studies. "We can find toxicity under laboratory conditions where material is being made under milligram quantities," said Dr. Anil Patri, Senior Scientist at the NCL. "It's quite another thing to extrapolate to that material being synthesized in kilogram and ton quantities." The potential for exposure in a manufacturing environment brings up a host of new issues related to toxicology. Dr. Patri continued, "With dendrimers, for example, we are looking at drug delivery and imaging applications, but when they enter the clinic they are also excreted. Then you are looking at the potential exposure from rivers, water supply, etc. At one end is manufacturing, at the other are the lifecycle issues." Coordinating research on products such as dendrimers will provide a more complete picture of their safety profile.

Engineered nanoparticles require a standardized approach to studying their toxicity. They are generally composite in nature and can often be designed to have specific size, shape or surface properties. All of these features can affect how they are absorbed, distributed, and eliminated from the body. A question remains as to whether the properties that determine short-term acute toxicity (such as that typically studied by the NCL) will have the same or different effects for long term toxicity (an area studied by the NTP). Having the NCL and NTP compare results on toxicity studies will help resolve such questions.

According to Dr. Scott McNeil, Director of the NCL, "It is important that we all characterize nanomaterials the same way, whether we are in industry or government, NCL or NTP." To ensure that this effort is coordinated at all levels, the NCL and NTP each have representatives from the other group to advise their scientific board or executive committee.

The results of the collaboration will benefit industry and the general public concerned with increasing our understanding of nanotechnology safety. ■

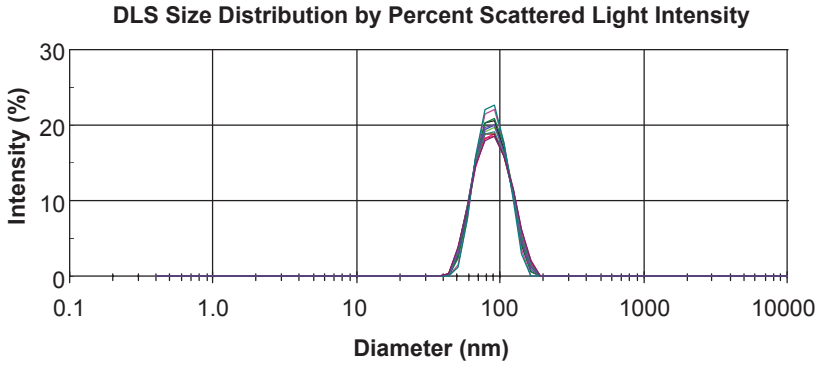
NCL Protocols

Measurement of Particle Size by Dynamic Light Scattering (DLS)

The size of a nanoparticle can have important consequences for how that particle behaves as a pharmaceutical. A small difference in diameter can determine if a particle is cleared by the liver or the kidneys. Combining data about nanoparticle size with data about biodistribution and particle interaction with the immune system will allow scientists to better design nanotechnology-based pharmaceuticals in the future.

Particle size determination is one of the first measurements in the NCL assay cascade. Results of this analysis allow NCL scientists to examine the influence of particle size on the in vitro and in vivo biological properties of the nanoparticle formulation.

Relatively few techniques are able to accurately measure the size of particles less than 100 nanometers in diameter. One technique that can provide an accurate measure of nanoparticle hydrodynamic size is dynamic light scattering (DLS). In DLS, a beam of laser light is scattered off the nanoparticle solution, and small, time-dependent fluctuations in the intensity of the scattered light are monitored with a photon detector. These fluctuations are caused by the Brownian motion of the nanoparticles; the timescale of the fluctuations is related to the timescale of the movement of the particles. The Stokes-Einstein equation relates the timescale of particle movement to the hydrodynamic diameter



DLS-Measured Size Distribution of a Nanoparticle Sample.

the particle, taking into account the viscosity of the sample solution and the temperature at which the measurement is performed.

Because DLS measures hydrodynamic diameter, it provides a fundamentally different measure of particle size than transmission electron microscopy (TEM), which measures electron diffraction. Many biological compounds are "invisible" to TEM because they don't sufficiently deflect an electron beam. These compounds can be detected and measured by DLS.

Most nanoparticle samples contain a distribution of slightly different hydrodynamic sizes as a result of imperfections in synthesis and because of the natural conformational variation of the large number of atoms involved (nanoparticle-based drugs contain thousands of atoms, many more than small-molecule drugs, which usually contain fewer than 100). Because of this distribution, it is necessary to characterize how much particle-to-particle size variability is present in the sample. The metric for this variability is the polydispersity index (pdI), which can also be measured by DLS.

DLS cannot be used to measure particle size, however, if the sample absorbs at the wavelength of the laser used in the size-measuring instrument. Also, scattered light intensity is proportional to particle diameter to the 6th power, so that larger particles scatter much more light than smaller particles. Because of this, even small amounts of dust or foreign contaminants in a sample will invalidate a DLS measurement.

FAQs

Q: How is an NCL sponsor's intellectual property safeguarded by the NCL?

A: The NCL takes the same security measures for protection of NCL sponsor intellectual property as for NCL data; every effort is made to ensure security and confidentiality. To share and safeguard research material and proprietary information, the NCL's interaction with sponsors is normally conducted under a Material Transfer Agreement (MTA). The MTA permits the collaborative exchange of materials and data between the NCL and the sponsor. In certain circumstances, NCL–sponsor interaction is conducted under a Cooperative Research and Development Agreement (CRADA).

Q: How does the FDA use NCL data when an NCL sponsor submits an Investigational New Drug (IND) filing?

A: Data derived from the NCL assay cascade are intended to be included in an investigator-led filing of an Investigational New Drug (IND) with the FDA. Since the NCL sponsor initiates and files the IND, the sponsor decides if and how NCL data are presented to the FDA in the IND filing. NCL data alone, however, will not be sufficient to meet the FDA's requirements for an IND. If the NCL's assays predict favorable in vivo safety and efficacy, NCI and the NCL anticipate the sponsor will pursue the translation of the technology into clinical applications.

Q: What information about the nanomaterials the NCL characterizes does the NCL provide to the FDA?

A: As a formal collaboration between the National Cancer Institute (NCI), the National Institute of Standards and Technology (NIST), and the Food and Drug Administration (FDA), the NCL makes an effort to maintain transparency to its governing institutions. In that spirit, all NCL characterization data are available to the FDA. The NCL and FDA work together on many aspects of nanomaterial characterization, and the FDA is informed about the properties of NCL nanomaterials as data are being generated. Characterization data that appear in NCL reports are peer-reviewed by FDA scientists who serve on the NCL's Scientific Oversight Committee (SOC). Biannually, NCL scientists formally present characterization information and data related to NCL nanomaterials to the NCL's SOC, which includes representatives from the FDA.

However, as mentioned previously, if an NCL sponsor initiates and files an IND with the FDA, the sponsor decides what NCL data are included in the IND and how the NCL information is presented.

Q: Does the NCL publish proprietary information provided to them by the sponsor?

A: Absolutely not! The NCL does not publish information provided by its sponsors without their express written permission.

In contrast, data generated by the NCL from characterization of a material may be presented in scientific and public forums if such data are deemed to benefit the cancer research

community. This public disclosure, however, pertains only to data generated at the NCL; a sponsor company's proprietary/confidential information is protected and will not be disclosed under any circumstances. Furthermore, the NCL and its sponsors agree to treat in confidence any written information about the research materials that is indicated as confidential. Before NCL characterization data are made public, the sponsor is given the opportunity to review the data to ensure they do not include proprietary information.

Q: How does the NCL safeguard nanomaterial samples during and after characterization?

A: The nanotechnology samples represent significant investments on the part of the laboratories and investigators submitting to the NCL. Many of these samples took years to develop and optimize. The NCL is cognizant of this, and is careful to retain control of the samples. NCL samples are not transferred to anyone not under the direct supervision of the NCL without advance notification to the sponsor. The samples are used only for research purposes, not for commercial purposes such as production or sale. When NCL characterization is complete, the NCL archives a sample of each submitted nanomaterial. Any remaining sample is disposed of or returned to the sponsor. ■

Upcoming Conferences and Publications

NCI Nanotechnology Alliance Investigators Meeting

Location: Chapel Hill, NC
Dates: October 16 – 18, 2007
Web site: http://www.capconcorp.com/nano2007/

Nanotechnology Safety Concerns Revisited, **by Stephan T. Stern and Scott E. McNeil, in Toxicological Sciences. Published online: 4 July 2007**

Immunological Properties of *Engineered Nanomaterials*, **by Marina A. Dobrovolskaia and Scott E. McNeil, in Nature Nanotechnology. Published online: 29 July 2007**

NIST RM8011

This fall, the National Institute of Standards and Technology (NIST) will release its new nanoparticulate reference materials (RMs) consisting of colloidal gold nanoparticles with nominal sizes of 10, 30, and 60 nanometers (nm) in suspension. Production of these RMs was supported in-part by the National Cancer Institute (NCI)'s Alliance for Nanotechnology in Cancer and Nanotechnology Characterization Laboratory (NCL) at NCI-Frederick.

The new NIST RMs are intended for use by the biomedical, Environment, Health, and Safety (EHS), and other nanotechnology communities for instrument calibration, and method qualification, and as controls for in vitro experiments used to characterize nanomaterials. These materials have been characterized extensively by NIST scientists. Each aliquot of RM will come with reference and informational values for a number of properties, including particle size determined by multiple methods, pH, conductivity, gold and citrate concentration, particle surface charge (zeta potential), optical absorption spectra, and verification of sterility.

Nanotechnology offers many advantages for traditional drug design, drug delivery, and medical diagnostics. Nanoparticles represent a wide variety of material categories, each with unique physicochemical and biological properties. These properties are often highly-dependent on synthesis, sample preparation and environmental conditions, and variations in these conditions are a major cause of inconsistency in both research and preclinical results.

NIST nanoparticulate RMs with known properties will facilitate interlaboratory comparison among researchers and drug-developers. Colloidal gold was chosen for the RMs because it is relatively inert, non-toxic, and compatible with the portfolio of instruments used for nanoparticle characterization.

The reference materials (numbered RM8011, RM8012, and RM8013 for 10, 30, and 60 nm particles, respectively) will be available for purchase (to recover production and distribution costs) on NIST's Web site: http://www.nist.gov/srm.

NIST Standard Reference Materials (SRMs) and Reference Materials (RMs).