Nanotechnology Characterization Laboratory

NCL News

Accelerating the transition of concepts to clinical applications

October 2011

NCL is Partnering with Companies to Salvage Failed Cancer Drugs Using Nanotechnology

It costs around a billion dollars on average to take a completely novel drug (a so-called "new molecular entity" or NME) through clinical trials and into the market in the U.S. One of the factors that makes this process so expensive is that many candidate drugs fail during clinical trials. Drugs fail or are "discontinued" for a variety of reasons, such as poor solubility, liver accumulation, toxicity, etc. A common rule of thumb is that four out of five drugs that enter clinical trials will fail, and the probability that a drug fails in the late stages of clinical trials (Phase II or Phase III) is slightly higher, on average, for cancer drugs than for most other drug types.

"Discontinued drugs" represent large sunk costs for pharmaceutical companies, since these drugs will not provide a return on the investment made in their development. Pharmaceutical companies pass the costs on to consumers by raising the prices of the drugs that do successfully complete the process.

But nanotechnology—the control of matter at dimensions between 1 and 100 nm—could offer drug developers an opportunity to reformulate discontinued drugs, improving the drug formulation so that it can successfully complete clinical trials and gain Food and Drug Administration (FDA) approval. Researchers at the Nanotechnology Characterization Lab (NCL), at the National Cancer Institute at Frederick, are using nanotechnology to "engineer around" undesirable properties of drug formulations. For example, nanotechnology is being used to increase solubility and bioavailability, enhance targeting, and provide for the controlled release of a variety of cancer therapeutics, potentially making failed drugs viable again.

Nanotechnology-based reformulations of chemotherapeutics have been particularly successful, delivering strong medicines to tumors while reducing side effects. Conventional chemotherapy usually involves chemicals that disrupt cell replication. Since the cells of many healthy tissues also replicate, chemotherapeutics can have debilitating side effects such as nausea, hair loss, and fatigue. These side effects are often so severe they limit the amount of drug that can be administered. The side effects can be reduced if the chemotherapeutic is attached to a nanoparticle, which shields the drug from healthy tissue and concentrates the drug in tumors. Such "nanotech" drugs can have increased efficacy and decreased toxicity compared to traditional chemotherapeutics.

In This Issue:

NCL is Partnering with Companies to Salvage Failed Cancer Drugs Using Nanotechnology 1
NCL Connections.....2
NCL Protocol Highlights 3
Video Protocol Demonstrations 4
Book on Nanomaterial Characterization.....4
FAQs.....4
Recent NCL Publications.....5
Upcoming Conferences.....5

NCL is partnering with the biotechnology industry (from small start-up companies to large pharmaceutical companies) to use nanotechnology to salvage failed drugs. In these partnerships, pharmaceutical companies identify candidate drugs that are compatible with nanotech reformulation. The drug structure and previously disqualifying characteristics are disclosed to NCL under confidentiality/nondisclosure agreement and NCL recommends a reformulation strategy. Once a strategy is agreed upon, the drug is supplied to NCL for reformulation and testing. When the nanotech reformulation meets an agreed-upon threshold for improvement, the improved formulation is transferred back to the pharmaceutical company for further evaluation and

Continued on page 2

The NCL is Partnering *Continued from page 1*

clinical and commercial development. The pharmaceutical company partner contributes financial support for the reformulation and testing conducted at NCL through a Cooperative Research and Development Agreement (CRADA) with the National Cancer Institute.

There are already several FDA-approved nanotechnology-based cancer therapies in clinical trials. For example, NCL has worked with Nanospectra Biosciences, Inc.'s AuroShell® and CytImmune Sciences' AurImune®. AuroShell is a gold-coated nanoshell that absorbs near-infrared laser light to thermally destroy tumors. AurImune is nano-sized gold bound to tumor necrosis factor (TNF). TNF is an exceptionally potent chemotherapeutic that was tested in clinical trials in the 1990s, but had to be discontinued due to severe adverse side effects. In a recent Phase I clinical trial of AurImune, three times what had previously been a lethal dose of TNF was given to patients with few negative side effects—illustrating how nanotech formulations can greatly reduce the toxicity of chemotherapeutics. ■

NCL Connections

NIEHS and NCL Partner to Study Nanotechnology Safety

Engineered nanomaterials (ENMs)—man-made products between one and approximately 100 nm in size—are becoming increasingly more prevalent in a host of consumer products. ENMs can commonly be found in products such as sunscreens and cosmetics (nanoscale TiO_2 and ZnO_2), household appliances and clothing (nanoscale silver), and sporting equipment (carbon nanotubes).[1] As the use of ENMs in commercial products continues to become more widespread, government labs have begun taking the initiative to probe the biological and environmental consequences of ENM exposure.

To help address some of these ENM safety concerns, the National Institute of Environmental Health Sciences (NIEHS) initiated a new $13-Million program aimed at studying ENM safety. The goal of this program is to develop an understanding of the relationship between the physicochemical characteristics of an ENM and the biological reactions to ENM exposure. As part of this initiative, NCL will be performing a thorough characterization of the physicochemical properties of selected nanomaterials. "NCL and NIEHS share common goals related to nanotechnology safety," said Dr. Scott McNeil, NCL's Director. "NIEHS and NCL are both interested in understanding how nanomaterial properties influence their biological behavior. This collaboration allows NIEHS and NCI to benefit from each other's expertise."

Some of the selected ENMs for characterization include nanoscale silver, cerium dioxide, and carbon nanotubes. Nanosilver is used in a host of commercial products, largely for its reported antimicrobial activity.

Nanosilver is used in clothing, as a lining in several types of household appliances, including washing machines and refrigerators, and is commonly used in medical applications such as wound dressings and device coatings. Nanoscale cerium dioxide has found commercial application as a diesel fuel additive (although largely only in Europe and Canada) and as a polishing agent in the semiconductor field. Carbon nanotubes likely have the broadest range of commercial applications, compared to the other selected ENMs. Carbon nanotubes have been used as light weight, ultra-strong structural materials (e.g., for sporting equipment), semiconductors, and are now being developed in the medical field as a scaffold for blood vessels and bones.

With such a diverse range of commercial applications there can be a variety of possible entry routes and ENM exposure hazards. While NCL will strictly be performing physicochemical characterization of the materials, including traits such as size, composition, purity, surface chemistry, and stability, other selected investigators from across the country will be probing the biological responses of these materials. They will assess risks such as inhalation, gastro-intestinal, and dermal exposures. The combined results of this study will be made publically available following completion of the five-year program.

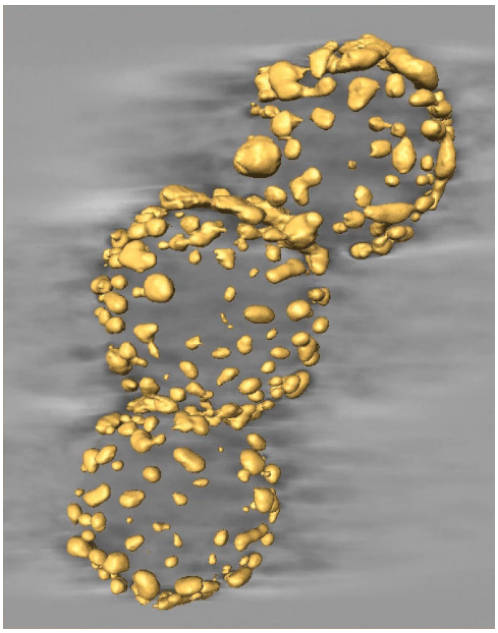
[1] http://www.nanotechproject.org/inventories/consumer/ ■

NCL Protocol Highlights: Electron Microscopy Capabilities

NCL routinely takes advantage of the many technologically advanced features of electron microscopy (EM). EM is a powerful technique that can not only provide size/size distribution information, but also three-dimensional particle morphology, elemental composition, and aggregation/agglomeration state.

Through the NCI-Frederick, NCL has access to a state-of-the art EM facility with a total of five microscopes: two Hitachi (7600 and 7650) transmission electron microscopes (TEM); an FEI Tecnai T20 TEM with cryogenic and three-dimensional tomography imaging capabilities; an FEI Tecnai T12 TEM coupled with an energy-dispersive x-ray spectrometer (EDS); and a Hitachi S3000 scanning electron microscope (SEM). The facility also houses supporting instrumentation including an FEI Vitrobot (for plunge-freezing samples for cryo-imaging), four Leica ultramicrotomes (for preparing thin-section samples), an Emitech high-resolution coater (for coating sample grids), and light microscopes with digital imaging capabilities.

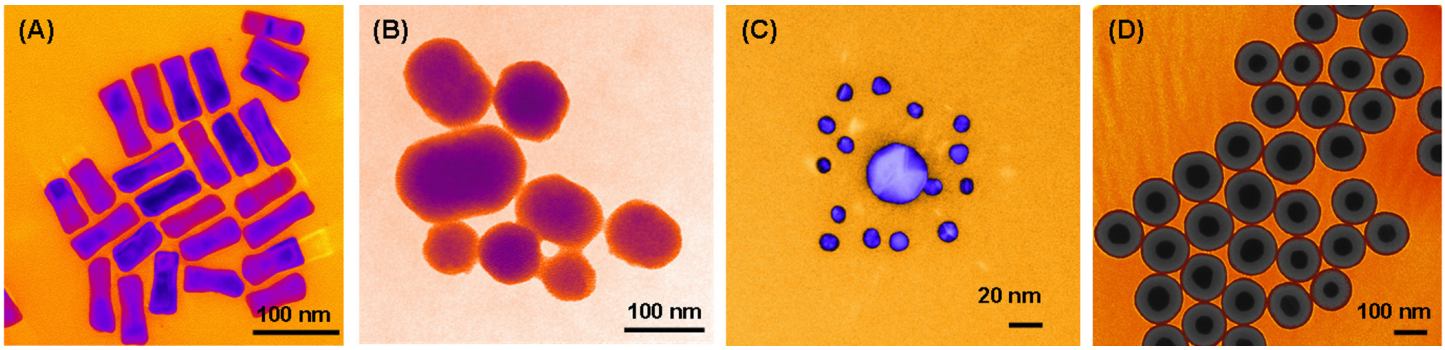
Most nanomaterial samples coming into NCL undergo a size/size distribution EM analysis. NCI instrumentation is capable of imaging particles down to a resolution of 0.3 nm. And, depending on the type of nanomaterial being analyzed, cryo-EM can be employed. Nanoparticles deemed "soft" biological particles (e.g., polymers and liposomes) use cryo-EM to allow for imaging close to their native state, without the need for staining or chemical fixation. The second most common EM analysis performed at NCL is EDS. The elemental analysis ability of EDS confirms nanoparticle composition (for example, the presence of various elements) and identifies potential impurities in the sample. Finally, NCL and NCI staff have been working together to optimize electron tomography procedures. These efforts now allow for three-dimensional surface visualization of nanoparticles down to a resolution of 5 nm. The included video highlights this remarkable capability.



This video demonstrates the three-dimensional visualization capabilities of electron tomography, which can compose images down to a resolution of 5 nm. Click on the image to see the video.

Although many sample preparation and measurement conditions must be optimized for each sample analyzed, NCL, in collaboration with the National Institute of Standards and Technology, has provided a generalized protocol for nanoparticle analysis by TEM. This protocol, PCC-7, provides details for sample grid peparation, nanomaterial sample deposition, the actual measurement process, and data analysis procedures. Full protocol details are available for download on NCL's web site: http://ncl.cancer.gov/NCL_Method_PCC-7.pdf.

For more information on NCI-Frederick's EM facility, please visit the following web site: http://atp.ncifcrf.gov/imaging-and-nanotechnology/electron-microscopy-laboratory/. ■



Nanoparticle Electron Micrographs. *NCL has utilized transmission electron microscopy to obtain size and morphology information for various types of nanoparticles. Included here are images of (A) gold nanorods, (B) mesoporous silicon nanoparticles, (C) immunolabeled gold nanoparticles, and (D) silicon-coated gold nanoparticles. (False color has been added for illustration purposes.)*

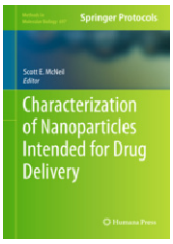
Video Protocol Demonstrations

NCL Scientist Dr. Jeff Clogston and Research Associate Sarah Skoczen have provided a demonstration of nanoparticle size measurement using dynamic light scattering (DLS) in the NCL's first video protocol. The video protocol is intended to supplement the full text version of the assay (PCC-1), and provide insight into the nuances of the technique that may not be adequately conveyed by the text version. Both the full text and video demonstrations of the DLS assay can be found on the NCL web site: http://ncl.cancer.gov/working_assay-cascade.asp

NCL encourages input from our readers regarding the video protocols; please send us your comments or suggestions. Also, if there is a protocol you would like to see portrayed in video format, please let us know (ncl@mail.nih.gov). ■

Book on Nanomaterial Characterization

NCL staff, along with other experts in the field, have collaborated to provide a book detailing nanoparticle characterization protocols. Published in early 2011, the book contains 27 chapters that cover assays for physical and chemical properties, sterility, in vitro toxicity, and in vitro immunological tests. In addition, there are chapters on the "Unique Benefits of Nanotechnology to Drug Delivery and Diagnostics" and "Challenges for Nanoparticle Characterization," written by NCL Director Dr. Scott McNeil, as well as a chapter on "Considerations When Submitting Nanotherapeutics to FDA/CDER for Regulatory Review," written by FDA Scientists Dr. Katherine Tyner and Dr. Nakissa Sadrieh. ■



Characterization of Nanoparticles Intended for Drug Delivery

Scott E. McNeil, Ph.D., editor
Methods in Molecular Biology
Humana Press, 2011

Available from Amazon.com

FAQs

Q: What kind of support can NCL provide to nanotechnology drug developers after an IND/IDE?

A: Once an IND or IDE has been submitted, some optimization or experimentation is usually necessary in response to FDA reviewer comments. NCL, provided it is within our capabilities, can continue to provide characterization assistance to help address FDA concerns.

Q: How does NCL prioritize characterization projects and experiments?

A: NCL continually performs characterization experiments and assesses project needs, beginning from the time a particle is submitted (i.e., particles submitted earlier are generally scheduled first). That being said, a minimum subset of characterization data is guaranteed to be performed in the first forty-five days (DLS, zeta potential, sterility, and endotoxin quantification). However, NCL is sensitive to the needs of its collaborators and, as possible, will adjust schedules to provide data to meet deadlines such as pre-IND meetings with the FDA.

Q: How does NCL support investigators in NCI's Alliance for Nanotechnology in Cancer?

A: As part of the Alliance for Nanotechnology in Cancer, investigators are offered an abbreviated application process; investigators will be asked to provide NCL with all pertinent synthesis, characterization, and experimental data, as well as responses to NCL's General Questionnaire. In addition, for those investigators whose nanoformulations are not yet ready for the full Assay Cascade, NCL is poised to offer Alliance investigators assistance in performing a small subset of experiments or advice on product formulation.

Q: Does NCL characterization stop once a nanotechnology product enters clinical trials?

A: No. Often times, clinical trials can unveil a previously unaddressed concern or reveal previously undetected toxicities. These preliminary clinical trial results can often lead to the reformulation of a drug developer's strategy. NCL remains committed to advancing viable nanotechnology-based drug formulations not just to clinical trials, but also to the market. NCL, provided we have the capabilities, can offer further assistance in both formulation advice and characterization assays. ■

Recent NCL Publications:

Macrophage scavenger receptor A mediates the uptake of gold colloids by macrophages in vitro.
Franca, A.; Aggarwal, P.; Barsov, E.; Kozlov, S.; Dobrovolskaia, M. A.; Gonzalez-Fernandez, A.
Nanomedicine (Lond.), Epub ahead of print, 2011.
PMID: 21675859.

Newkome-type dendron stabilized gold nanoparticles: synthesis, reactivity, and stability.
Cho, T. J.; Zangmeister, R. A.; Maccuspie, R. I.; Patri, A. K.; Hackley, V. A.
Chemistry of Materials, 2011, 23(10), 2665–2676.
PMID: 21686078.

Interlaboratory comparison of size and surface charge measurements on nanoparticles prior to biological impact assessment.
Roebben, G.; Ramirez-Garcia, S.; Hackley, V. A., Roesslein, M.; Klaessig, F.; Kestens, V.; Lynch, I.; Garner, C. M.; Rawle, A.; Elder, A.; Colvin, V. L.; Kreyling, W.; Krug, H. F.; Lewicka, Z. A.; McNeil, S. E.; Nel, A.; Patri, A. K.; Wick, P.; Wiesner, M.; Xia, T.; Oberdorster, G.; Dawson, K. A.
Journal of Nanoparticle Research, 2011, 13(7), 2675–2687.

Characterization of nanoparticles intended for drug delivery.
Methods in Molecular Biology: Humana Press; 2011,
Scott E. McNeil, editor.
ISBN: 978-1-60327-197-4

Fullerenol cytotoxicity in kidney cells is associated with cytoskeleton disruption, autophagy induction, and mitochondrial dysfunction
Johnson-Lyles, D. N.; Peifley, K.; Lockett, S.; Neun, B. W.; Hansen, M.; Clogston, J.; Stern, S. T.; McNeil, S. E.
Toxicology and Applied Pharmacology, 2010, 248(3), 249–258.
PMID: 20713077

Translational considerations for cancer nanomedicine.
Stern, S. T.; Hall, J. B.; Yu, L. L.; Wood, L. J.; Paciotti, G. F.; Tamarkin, L.; Long, S. E.; McNeil, S. E.
Journal of Controlled Release, 2010, 146(2), 164–174.
PMID: 20385183

Biological assessment of triazine dendrimers as candidate platforms for nanomedicine: toxicological profiles, solution behavior, biodistribution, and drug release and efficacy in a PEGylated, paclitaxel construct.
Lo, S. T.; Stern, S. T.; Clogston, J. D.; Zheng, J.; Adiseshaiah, P. P.; Dobrovolskaia, M.; Lim, J.; Patri, A. K.; Sun, X.; Simanek, E. E.
Molecular Pharmaceutics, 2010, 7(4), 993–1006.
PMID: 20481608

A novel nanoparticulate formulation of arsenic trioxide with enhanced therapeutic efficacy in a murine model of breast cancer.
Ahn, R.; Chen, F.; Stern, S. T.; Clogston, J. D.; Patri, A. K.; Raja, M. R.; Swindell, E. P.; Parimi, V.; Cryns, V. L.; O'Halloran, T.
Clinical Cancer Research, 2010, 16(14), 3607–3617.
PMID: 20519360

Ambiguities in applying traditional Limulus Amoebocyte Lysate tests to quantify endotoxin in nanoparticle formulations.
Dobrovolskaia, M. A.; Neun, B. W.; Clogston, J. D.; Ding, H.; Ljubimova, J.; McNeil, S. E.
Nanomedicine (Lond.), 2010, 5(4), 555–562.
PMID: 20528451

Upcoming Conferences

3rd Annual American Society for Nanomedicine
Location: Universities at Shady Grove Conference Center, Rockville, MD
Date: November 9–11, 2011
Web site: http://www.amsocnanomed.org

6th BioNanoTox International Research Conference
Location: La Quinta Inn & Suites Downtown Conference Center, Little Rock, AR
Date: November 17–18, 2011
Web site: http://sites.google.com/site/bntconference/home

2011 MRS Fall Meeting & Exhibit
Location: Hynes Convention Center, Boston, MA
Date: November 28–December 2, 2011
Web site: http://www.mrs.org/fall2011/

NCL Lessons Learned Workshop
Location: University of North Carolina Kenan-Flagler Business School, Chapel Hill, NC
Date: December 6, 2011
Contact: Zishan Haroon at zishan_haroon@med.unc.edu

Society of Toxicology Annual Meeting and ToxExpo
Location: Moscone Convention Center, San Francisco, CA
Date: March 11–15, 2012
Web site: http://www.toxicology.org/AI/MEET/AM2012/

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