PROGRESS AND PLANS OF NATIONAL NANOTECHNOLOGY INITIATIVE (NNI) AGENCIES

February 2019

Department of Health and Human Services (HHS)[1]

National Institutes of Health (NIH)

Summary

NIH advances creative, fundamental discoveries and translational nanotechnology research and development to ultimately enhance health, lengthen life, and reduce illness and disability through a variety of mechanisms and approaches. The NIH nanotechnology investment portfolio encompasses both basic and clinical research funded primarily through grants. Current research efforts focus on advancing new medical diagnostics, therapeutics, and vaccines; supporting nanotechnology-related environmental, health, and safety (EHS) research; developing nanotechnology information resources; and training a new generation of nanotechnology researchers. Due to the successful integration of nanotechnology-based R&D into broad areas of biomedical applications, scientists can propose ideas via non-nanotechnology-specific funding opportunity announcements (FOAs) supported by a large number of NIH institutes.

Plans and Priorities by Program Component Area (PCA)

NIH nanotechnology programs and projects awards contribute to all five NNI Program Component Areas (PCAs).[2] NIH continues to participate in two of the Nanotechnology Signature Initiatives (NSIs: Nanotechnology Knowledge Infrastructure and Sensors) through workshops, webinars, and manufacturing consortia. NIH will continue to develop strategies to enhance knowledge through greater data collection, sharing, and tool development. The NIH investment reflects the discovery and understanding of scientific principles in medical research supported throughout the NIH institutes. NIH has provided funds for nanotechnology-related proposals covering all the major diseases (e.g., cardiac, cancer, diabetes, kidney, etc.). Nanotechnology-enabled applications, devices, and systems (PCA 3) is the largest PCA in the NIH investment portfolio. Programs and projects in PCA 3 include medical devices, nanotherapeutics, drug delivery systems, and novel radiotherapeutics supported through several FOAs renewed or ongoing in fiscal year (FY) 2018–2020. NIH provides funds for resources centers in cardiac, cancer, dental, and other clinical research areas. NIH also has a long-standing practice of addressing infrastructure needs (PCA 4) through funding mechanisms. This includes information technology R&D, computing-enabled communications, and

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[2] For a complete list of NNI goals and PCAs, see: https://www.nano.gov/about-nni/what/vision-goals.

educational resources. Many NIH institutes support training through networks and centers funded to achieve their mission-specific goals.

A select number of nanotechnology-related initiatives that NIH has established, and anticipates continued support for through 2020, are presented below.

National Cancer Institute (NCI): PCAs 1, 2, and 4

NCI Alliance for Nanotechnology in Cancer[3]

The Alliance pioneers the development and deployment of nanotechnology-based diagnostics and therapeutics for cancer. In 2015, NCI announced awards to six Centers in Cancer Nanotechnology Excellence (CCNEs), seven Innovative Research in Cancer Nanotechnology (IRCNs) awards, and five Cancer Nanotechnology Training Centers (CNTCs). NCI's Alliance program will continue to execute its research and translational goals. The Innovative Research in Cancer Nanotechnology program announcement was renewed in 2017 (PAR-17-240, R01 grants)[4] with two receipt dates per year until May 21, 2020. This program announcement is focused on mechanistic studies towards revealing and enhancing fundamental understanding of mechanisms behind *in-vivo* delivery of nanoparticles and operation of *in-vitro* nanodevices. The IRCN program has had three receipt dates, with an average of 60 R01 submissions for each receipt date.

Strategic Workshop on Emerging Technologies and Interdisciplinary Team Formation for Early-Stage Researchers

NIH/NCI held this workshop on August 24–25, 2017. The goal was to bring together early-career-stage researchers working at the interfaces between technology and cancer research to identify areas with the high potential to reward interdisciplinary, collaborative research efforts that could result in transformative discoveries and inventions, and to identify the major challenges that face investigators launching independent research careers in interdisciplinary team science.

Strategic Workshop on Cancer Nanotechnology

NIH/NCI held a second workshop on December 11–12, 2017. The goal was to bring together established cancer nanotechnology investigators to evaluate the status of the field, assess progress made over the last five years, and identify emerging and promising areas to be explored by future funding opportunities. The workshop focused on identifying cancer research and clinical areas that could benefit from nanotechnology, as well as fundamental nanotechnology knowledge gaps that need to be addressed to advance clinical use.

[3] https://www.cancer.gov/nano/research/alliance

[4] https://grants.nih.gov/grants/guide/pa-files/par-17-240.html

In addition, NCI staff members within the Nanodelivery Systems and Devices Branch, Cancer Imaging Program, published 2 perspective/review papers in *ACS Nano*,[5] one workshop report in *Nature Immunology,*[6] and one review paper in *Clinical Translational Science*.[7]

Nanotechnology Research Infrastructure, Training, and Instrumentation

The NIH/NCI Cancer Nanotechnology Training Centers provide graduate and post-graduate training to researchers from diverse disciplinary backgrounds in the use of nanotechnology as an enabling tool for cancer biology and oncology research. There are currently six NCI-supported CNTCs across the Nation (Stanford University, Northwestern University, MD Anderson Cancer Center, Johns Hopkins University, the University of North Carolina at Chapel Hill, and University of California at San Diego). More details on nanotechnology projects that impact the workforce can be found on the NIH reporting system: RePORT.[8] NIH/NCI also supports the Nanotechnology Characterization Laboratory (NCL) located in the Frederick National Laboratory for Cancer Research. NCL is an intramural laboratory serving as a centralized resource to characterize nanomaterials developed by NCI-funded researchers and researchers from the broader academic, government, and industry research communities. NCL stays in close touch with the Food and Drug Administration (FDA) to be informed on regulatory aspects of nanotechnology, and benefits from the expertise of the National Institute of Standards and Technology (NIST) concerning nanomaterial standardization.

Wearable and Implantable Sensing Devices

NIH (NCI; National Institute of Biomedical Imaging and Bioengineering, NIBIB; National Institute of Mental Health, NIMH; National Institute of General Medical Sciences, NIGMS; etc.) continues to research biomedical and clinical opportunities that could benefit from the use of *in-vitro* and *in-vivo* imaging and sensing devices in FY 2020. Examples include technologies that advance basic research (mechanistic understanding of disease progression), precision medicine targets (monitoring therapeutic response), and other medical device applications. NIH representatives participating in the Sensors NSI will continue to leverage the technical developments from the Sensors NSI workshops and interagency collaborations such as the NSF-NIH Smart and Connected Health program[9] and the NITRD program's Cyber-Physical Systems interagency working group.[10] These discussions will focus on the potential for such devices to meet shared medical needs/conditions/challenges.

National Institute of Allergies and Infectious Diseases (NIAID): PCAs 2 AND 3

The Vaccine Translational Research Branch (VTRB) of the Division of AIDS (DAIDS) is a branch within NIH's extramural program that enables, facilitates, operationalizes, and translates concepts from the laboratories of vaccine developers/principal investigators (PIs) into current good manufacturing practice (cGMP)–manufactured, vialed products for clinical trials. VTRB manages and supports a broad portfolio of HIV vaccine and adjuvant products ranging from complex envelope HIV protein immunogens, adenovirus (Ad) vectors and virus-like particles (virosomes), DNA vaccines and mRNA-liquid nanoparticle (LNP) and

[5] Hartshorn et al., 2018, DOI: 10.1021/acsnano.7b05108 and Ehlerding et al., 2018, DOI: 10.1021/acsnano.7b07252

[6] *Nature Immunology* volume 18, pages 1175–1180 (2017): https://www.nature.com/articles/ni.3828.pdf

[7] Keating et al., 2018, https://doi.org/10.1111/cts.12536

[8] https://report.nih.gov/

[9] https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504739

[10] https://www.nitrd.gov/nitrdgroups/index.php?title=CPS

nanoparticle vaccines, monoclonal antibodies (Abs), and adjuvants suitable for clinical trials. In addition, VTRB is invested in capacity-building initiatives, emerging/innovative technologies, and acceleration platforms to expedite HIV vaccine manufacturing, including cell line development platforms, upstream and downstream purification approaches, and nanoparticle-based immunogen and adjuvant codelivery systems. VTRB also leads internal efforts for acquisition/procurement of GMP-quality new and/or biosimilar analogs of adjuvants to advance and support preclinical and product development requirements and meet the adjuvant demand for clinical research reagents for evaluation in human clinical trials. VTRB works closely with academic innovators, biotech, pharma, non-profits, vaccine trial networks, and contract manufacturing and contract research organizations to advance clinical HIV vaccine development. Within DAIDS, VTRB serves as a conduit for efficient clinical transition of HIV vaccines by collaborating extensively with the preclinical and clinical branches to advance vaccine concepts, coordinating with the Regulatory Affairs and Pharmaceutical Affairs branches.

Key technical accomplishments to foster the transfer of new technologies into products include the following:

- Advancing development of multiple nanotechnology-based HIV vaccine concepts, platforms, and products for feasibility, preclinical, and clinical studies.
- Fostering innovative/promising nanotechnology platforms by providing funding/seed investment to PIs and biotechnology companies to support early-stage R&D with commercial potential via the Integrated Preclinical/Clinical AIDS Vaccine Development Program (IPCAVD, U19)[11] and Small Business Innovative Research (SBIR)[12] mechanisms to meet the mission objectives of DAIDS.
- Building an armamentarium of nanoparticle-based adjuvants.

VTRB continues to fund grants that were awarded in FY 2018 through the IPCAVD program. The program is designed to enable a multidisciplinary team of investigators to complete all steps necessary from down-selection of a vaccine candidate through cGMP manufacture/testing/product release and into clinical trials.

VTRB is also managing the Regulatory and Investigational New Drug (IND) submission to FDA for membrane proximal external region (MPER) peptide-liposome nanoparticles for use in a first-in-man Phase I clinical trial to evaluate safety and immunogenicity. MPER from gp41 is a promising antigen segment of the viral envelope recognized by a number of broadly neutralizing antibodies and represents a promising vaccine target. Projects funded through this program focus on the development of lipid nanoparticles containing mRNAs for the induction of protective non-neutralizing or neutralizing antibodies. This includes developing the cGMP manufacturing processes for mRNA immunogens as well as the nanoparticle formulations for Phase 1 trials. Additional work involving influenza virosomes that display surface-elongated HIV gp41 peptides was funded to manufacture new virosome vaccine candidates in a form that can be delivered through a nasal spray.

Other ongoing activities and plans for 2019–2020 focus on building an armamentarium of particulated adjuvants for preclinical and clinical studies. This includes procuring TLR-4 liposomal adjuvant for antigen-adjuvant comparison clinical study, production of alum-based adjuvant to support various HIV immunogen/protein clinical trials, and production of polysaccharide particle adjuvant for codelivery with HIV immunogen studies.

[11] https://grants.nih.gov/grants/guide/pa-files/PAR-15-330.html

[12] https://sbir.nih.gov/

In the coming two years, VTRB will use different funding mechanisms to further advance the process development and cGMP manufacturing of native flexible linked (NFL) trimer liposome vaccine and self-assembling native-like gp140 uncleaved, prefusion-optimized (UFO) nanoparticle trimer vaccine.

Nanotechnology Workshop for Infectious Diseases

NIH/NIAID and NIH/NIBIB held a workshop on "A Convergence Research Approach to an Effective HIV Vaccine" on September 24–25, 2018. The goal of this meeting was to initiate an in-depth conversation between knowledge holders and problem solvers that could suggest new avenues to explore in HIV vaccinology. The workshop objective was to create a dynamic, interactive conversation among experts with diverse backgrounds in the hope of generating new ideas and approaches toward an efficacious HIV vaccine.

NIAID's VTRB is planning the 3rd Nanotechnology Workshop for HIV, RNA Vaccine & Adjuvant Codelivery in FY 2020. The objective is to provide impetus to innovative and enabling nanotechnologies and drug delivery systems as platforms for vaccine/immunogen delivery that can greatly accelerate next-generation prophylactic and therapeutic vaccine development. This multidisciplinary meeting will focus on discussions and exchange of ideas in diverse areas of vaccination, innovative therapies, and delivery systems for development of new-generation vaccines against prevalent and emergent infectious diseases including HIV.

NIAID Blue Ribbon Panel on Adjuvant Research

On April 23–25, 2018, NIAID hosted a Blue Ribbon Panel to discuss the institute's adjuvant and adjuvanted vaccine research portfolios. Staff from the three extramural divisions presented summaries of research activities related to adjuvant discovery, adjuvant development, the preclinical and clinical application of adjuvants to vaccines against infectious diseases, and NIAID's response to the recommendations made by a panel convened in 2010. Panel members were also provided with a draft of the strategic plan for adjuvant research and charged with identifying gaps in NIAID's approach to discovering and applying novel adjuvants to vaccines of interest to the institute. This particular strategic plan, scheduled to be released in FY 2020, will be updated based on the panel's recommendations.

National Institute of Environmental Health Sciences (NIEHS): PCA 5

Nanotechnology Environmental Health and Safety (NanoEHS)

The NIH/NIEHS research efforts are designed to gain a fundamental understanding of the molecular and pathological pathways involved in mediating responses to engineered nanomaterials (ENMs). Towards this goal, comprehensive biological response profiles are needed to gain detailed molecular understanding of the interactions between ENMs and biological systems. To continue the success achieved with a small library of ENMs, centers for nanotechnology health implications research will continue to be funded through two NIEHS funding opportunity announcements through 2021.

The Nanotechnology Health Implications Research (NHIR) consortium was established in September 2016 with nine U.S. academic institutions. The NHIR consortium is continuing its efforts to gain understanding on ENM-biological interactions using diverse *in-vitro*, tissue-on-chip, and *in-vivo* models. Over the past three years these investigators studied 26 ENMs of diverse physicochemical properties specially synthesized/commercially produced and extensively characterized by the Engineered Nanomaterial Resource and Coordination Core at Harvard University, which is a member of the NHIR consortium. The research findings are being published. Currently the focus of the NHIR consortium is to generate biological response profiles for the emerging two-dimensional and three-dimensional ENMs (graphene, graphene oxide, nanocellulose, and other anisotropic materials).

Nanotechnology Health Implications

The NIH/NIEHS National Toxicology Program (NTP) is collaborating with FDA to understand the health hazards of nanomaterials (hazard assessment) and to develop novel methods and approaches for detection of nanomaterials in FDA-regulated products. This includes physicochemical characterization and standards development processes to enable responsible development of nanotechnology. Standards are critical for the responsible development of nanotechnology-derived products from the standpoints of safety and efficacy. NIH/NIEHS/NTP is also completing an evaluation of the immune system impact of inhalation of multiwalled carbon nanotubes in rodent models to better understand the potential health effects from low-dose exposures in workers. This research complements exposure assessment of nanomaterial manufacturing facilities conducted in collaboration with the National Institute for Occupational Safety and Health (NIOSH). Work on both these projects will continue into the next fiscal year. Meanwhile, toxicokinetic studies of the *in-vivo* disposition of fullerene C60 after intratracheal or intravenous exposure were published in 2018. NTP studies on the inhalation toxicity of 10–20 nm diameter multiwalled carbon nanotubes (CASRN L-MWNT-1020) in Sprague-Dawley rats and B6C3F1/N mice have been completed, and the NTP report has been submitted for peer review.

NIH/NIEHS/NTP has been actively engaged in toxicological research in collaboration with NIOSH to understand the health hazards of nanocrystalline cellulose (NCC) and conducted *in-vitro* assessments of various NCCs to determine durability and dissolution. As part of this collaboration, NIH/NIEHS/NTP is exploring the feasibility of inhalation studies of specific well-characterized NCCs to determine if it will be possible to do *in-vivo* mammalian inhalation toxicity studies of NCC.

In mid-2016, NIH/NIEHS/NTP initiated a two-year chronic toxicity evaluation of multiwalled carbon nanotubes in rodent models to better understand the potential health effects from low-dose “lifetime” exposures. Work on this project will continue into the next fiscal year.

No significant changes are anticipated in the ongoing research program efforts at NIH/NIEHS and NIH/NIEHS/NTP in 2020. The NHIR consortium research efforts will be expanded to include additional emerging anisotropic ENMs.

National Institute of Dental and Craniofacial Research (NIDCR): PCAs 1d and 2

In 2018, NIH/NIDCR continued several strategic investments in nanotechnology-based initiatives to support its broad mission of improving dental, oral, and craniofacial (DOC) health. NIDCR leverages its investments on the significant promise of nanotechnology as an invaluable tool to produce novel structures that induce regeneration and repair of biological tissues, deliver biomolecules to tissues with pre-defined kinetics, and control tissue infection and inflammation, among other uses. Additional areas of NIDCR-supported research in nanotechnology focus on the development of oral biodevice technologies for the evaluation, monitoring, and management of oral and overall health; development of high-performing dental materials for the restoration, repair, and replacement of DOC tissues; and development of clinically relevant standards for dental materials including reference materials and quality guidance to research and product manufacturing.

Biosensors in the Oral Cavity (PCA 1d)

In FY 2016 NIDCR issued a new Request for Applications (RFA) entitled “Biosensors in the Oral Cavity” to support development of biosensors aimed to assess and monitor health and disease states in the oral cavity and in the whole body. Meritorious applications received in response to this RFA were funded in FY 2017 for

2–5 years in duration, as described above. This work is driven by the recent progress in wireless technologies, dissolvable nanotechnology-based electronics, microfabrication, and nanofabrication, as well as improved sensing and drug delivery, among other advances. This initiative supports the development of new or adaptation of existing biosensors for noninvasive, dynamic, real-time monitoring of physiological processes in the human body using the oral cavity as the sensing site. A broad range of nanotechnology tools is being used in biosensor development to address performance requirements (e.g., mechanical, chemical, and microbial) imposed by the oral environment. Biosensors developed under this initiative seek to advance precision-based medicine approaches in several clinical areas by enabling the ability to measure cortisol and melatonin levels in saliva, mapping dynamic periodontal mechanobiological activity, and tracking bone resorption in periodontitis. Development of these biosensors will include design verification and validation, as well as preclinical safety testing to facilitate the translation of the oral biosensors into clinical practice. The call for new applications under this initiative expired on October 20, 2016. NIDCR will continue supporting several research projects funded under this initiative through 2020.

Enabling Technologies to Accelerate Development of Oral Biodevices (PCA 1d)

Building on the success of the "Biosensors in the Oral Cavity" initiative, NIDCR launched this new initiative in November of 2018 under FOA PA-19-076[13]/PA-19-075[14]/RFA-DE-19-008[15]/RFA-DE-19-009.[16] The purpose of this initiative is to encourage research in transformative engineering solutions for system-level challenges that significantly improve the evaluation, monitoring, and management of oral and overall health using multifunctional oral biodevices. Projects funded under this initiative will utilize nanotechnology and nanomaterial approaches that integrate electronic, physical, and biological systems into biodevices intended for detection, diagnosis, and treatment of oral and systemic diseases. The long-term goal is to pave the way for acceleration of technical development and clinical translation of innovative oral biodevices that are highly sensitive and specific for maintaining wellness and timely management of disease and disease risks. These novel integrated oral biodevice systems would help facilitate incorporation of precision-medicine-based approaches into clinical practice. NIDCR expects to fund several research projects under this initiative in FY 2019. This initiative is aligned with NIDCR's strategic plan and is one of five goal areas in NIDCR's 2030 strategic vision. NIDCR is committed to continue supporting research projects to be funded under this initiative at least until FY 2021.

Design and Development of Novel Dental Composite Restorative Systems (PCA 2)

FY 2018 marked the final year of NIDCR's five-year award supporting the "Design and Development of Novel Dental Composite Restorative Systems" Cooperative Agreement Program. This program supported six different projects led by prominent U.S.-based research groups. The goal of the program was to enable development of novel dental composite materials, including nanomaterial-based composites, that demonstrate superiority in material properties and durability in the oral environment compared to currently utilized dental composites. Following interactions with FDA and industry experts organized by NIDCR in 2017, grantees made significant progress to optimize the nanoscale properties of their candidate dental composites by implementing strategic feedback on product development and regulatory requirements. The newly developed dental materials have shown unique features such as self-healing and anti-microbial

[13] https://grants.nih.gov/grants/guide/pa-files/PA-19-076.html

[14] https://grants.nih.gov/grants/guide/pa-files/PA-19-075.html

[15] https://grants.nih.gov/grants/guide/rfa-files/RFA-DE-19-008.html

[16] https://grants.nih.gov/grants/guide/rfa-files/RFA-DE-19-009.html

properties. Additionally, investigators have demonstrated improvements in mechanical performance, biocompatibility, ease of clinical handling, and durability of candidate dental resins combined with nanoparticle-based materials. In this way, grantees were enabled to accelerate product development activities in 2018, and several groups reported pursuing commercialization strategies via partnerships with industry, product licensing, or via startup ventures. Several patents have been issued to date stemming from this program, and future efforts will build on current success towards the clinical translation of novel dental materials with superior longevity and durability. NIDCR's support towards development of new nanomaterials-based dental composites will continue at least until FY 2021 as part of a new initiative on "Advancing Imaging, Device Production, and Clinical Capabilities in Digital Dentistry" (PA-19-021[17]/PA-19-022[18]).

Dental, Oral and Craniofacial Tissue Regeneration Consortium (DOCTRC-PCA 2)

Beginning in FY 2015 and continued in FY 2018, NIDCR has been supporting a three-stage effort called Dental, Oral and Craniofacial Tissue Regeneration Consortium (DOCTRC**)**, which will extend over an additional six years. DOCTRC represents an excellent example of longstanding support by NIDCR of nanotechnology development. The consortium is facilitating the advancement of promising technologies, including nanotechnology-based approaches for regeneration and reconstruction of DOC tissues, to Phase 1 clinical trials. Several dozen individual projects are currently supported by the DOCTRC, thus facilitating introduction of nanotechnology into clinical practice. Examples of such products/devices include tissue-regeneration-enhancing scaffolds and drug and cell delivery systems, among others.

Autotherapies: Enhancing Our Innate Healing Capacity (PCA 2)

In FY 2018 NIDCR organized and held a symposium and a workshop titled "Autotherapies: Enhancing Our Innate Healing Capacity." Autotherapies are treatments based on the capacity of the body for endogenous healing and regeneration. For example, immunotherapy harnesses the body's immune cells to fight cancer and is now in clinical use. In the DOC region, autotherapies could be used to selectively signal the body to repair and regenerate tissue, trigger immune responses, and restore a natural microbial balance. Among the multidisciplinary group of investigators presenting at the symposium and the workshop were developmental and stem cell biologists, immunologists, and bioengineers. Many of the potential autotherapy strategies discussed at these meetings were nanotechnology-based strategies, including nanotechnology-based scaffolds that can promoted endogenous tissue healing and regeneration and nanotechnology-based biomolecule delivery methodologies.

Interagency Agreement (IAA) between NIDCR and the National Institute of Standards and Technology (NIST-PCA 2)

An ongoing multi-year interagency agreement between NIDCR and NIST supports development of performance-based, clinically-relevant standards for dental materials, including nanomaterials, for applications in the oral environment. Additionally, the IAA supports design of new analytical instrumentation for characterization of nanomaterial-based dental composite restorative systems. In this

[17] https://grants.nih.gov/grants/guide/pa-files/PA-19-021.html

[18] https://grants.nih.gov/grants/guide/pa-files/PA-19-022.html

collaboration, NIST has been developing measurement strategies, standard methods, and reference materials, and NIDCR has been providing funding support and expert scientific and clinical advice. NIDCR also provided oversight of the reproducibility and rigor for the conducted research.

Advances made under this NIDCR-NIST IAA have resulted in the development of the NIST Standard Reference Instrument (SRI) 6005 Polymerization Stress Tensometer (SRI 6500 PST) described in the NNI Supplement to the President's 2019 Budget.[19] This instrument allows inter-comparability testing during development of nanomaterial-based dental composites. The SRI 6500 PST instrument is commercially available, and several units have already been acquired by various national and international stakeholders involved in dental materials research. Future work will build on the application of the standard reference instrument to establish measurement capabilities and standards that enable technology development and clinical translation of dental materials. Broader research applications of the SRI 6005 PST instrument are currently enabling the optimization of nanoscale properties, photo-curing protocols, and clinically relevant parameters in the design of new dental composite materials. It is expected that this new instrument will find broad application across the dental materials research community. The NIST team is working towards International Organization for Standardization certification of the SRI and is developing protocols for determining key polymerization parameters for photopolymerized dental materials.

Other accomplishments under the NIDCR-NIST IAA in FY 2018 include advances towards the development of a nanoscale carbonated apatite material mimicking human dental enamel that may find use as a Standard Reference Material. Using bulk carbonate-containing hydroxyapatite (BCH) as a self-hardening calcium phosphate cement, researchers were able to successfully produce BCH materials with sodium and carbonate contents similar to those reported in the literature for human enamel. Additionally, NIST researchers have reported successful preparation of nanoscale carbonated hydroxyapatite samples using a high-pressure method to prototype an enamel-like material with comparable composition, porosity, and mechanical properties to those of human enamel. Upon further refinement and validation of production methods, this enamel-mimicking material may find multiple applications to drive scientific research and quality manufacturing of nanomaterial-based dental composites. In the long term, the researchers aim to develop and produce a portfolio of biologically relevant calcium phosphate reference materials that mimic the nanostructure and mechanical properties of biologic tissues of interest.

Other accomplishments in FY 2018 include systematic evaluations of photocuring protocols (typical vs. pulse-delay) associated with key polymerization properties for photopolymerized nanomaterial-based dental composites. Optimization of photocuring protocols used for the photopolymerization of nanomaterial-based dental composites has a potential to improve overall material performance to enhance clinical outcomes and provide informed guidance on best practices to manufacturers and clinicians. NIDCR's efforts to promote development of clinically-relevant standards for dental materials will continue through FY 2020.

Forum on Regenerative Medicine

In 2019, NIDCR is continuing its participation in the National Academy of Medicine (NAM) effort titled "Forum on Regenerative Medicine." From FY 2016 to FY 2018 NIDCR served as a lead NIH institute of the five NIH institutes participating in this effort**.** FDA, NIST, the Veterans Administration, and other regenerative medicine stakeholders from academia, industry, patient advocacy groups, and private foundations are also participating in this forum. Many of the regenerative medicine strategies that are currently being developed

[19] https://www.nano.gov/sites/default/files/pub_resource/NNI-FY19-Budget-Supplement.pdf, p. 26.

involve nanotechnology, and the forum aims to engage the different participating stakeholders in a dialogue about challenges, opportunities, and ethical aspects of translating regenerative medicine strategies to the clinic. In October 2018, NIDCR, along with other forum partners, participated in the forum workshop titled "Exploring Sources of Variability Related to Clinical Translation of Regenerative Engineering Products." In FY 2019, NIDCR will play a prominent role on the forum by promoting efforts to define the goals and objectives of the next series of forum activities. NIDCR participation in the NAM Regenerative Medicine Forum will continue at least through FY 2019–2020.

Armed Forces Institute of Regenerative Medicine (AFIRM)

From its inception in 2008 and continuing in FY 2018, NIDCR, in collaboration with two other NIH institutes, continued its support and programmatic guidance for the Armed Forces Institute of Regenerative Medicine.[20] AFIRM is a Department of Defense (DOD)-led interdisciplinary multi-institutional consortium that supports a network of academic institutions, Federal laboratories, and commercial partners to develop life-saving tissue/organ regeneration strategies for wounded warriors. AFIRM investigators have been utilizing numerous nanotechnology strategies. The AFIRM research focus areas include projects relevant to the NIDCR mission and are geared toward craniofacial regeneration and reconstruction. The current phase of the AFIRM program will sunset in FY 2019. At this time, it is not known if this program will be extended.

Microphysiological Systems (MPS) for Disease Modeling and Efficacy Testing

In FY 2017, FY 2018, and continued in FY 2019, NIDCR has been participating in a trans-NIH effort led by the National Center for Advancing Translational Sciences entitled "The Microphysiological Systems (MPS) for Disease Modeling and Efficacy Testing," which will continue until FY 2022. This initiative supports studies to develop micro- and nanoscale *in-vitro* microphysiological system platforms (also known as tissue chips), and to validate these platforms for their ability to mimic physiological functions of human tissues and organs. This nanomedicine effort, executed in partnership with DOD, FDA, and pharmaceutical industry partners, supports studies to demonstrate the functional utility of the tissue chips for disease modeling, to understand disease mechanisms and to identify novel therapeutic targets and treatments to inform design of clinical trials. Technologies developed under this initiative utilize multidisciplinary approaches combining an array of nanotechnology-based strategies across fields of bioengineering, biology, microfluidics, materials science, "omic" sciences, and clinical science. NIDCR supports meritorious applications for development of tissue chip platforms relevant to the institute's mission areas.

Key Technical Accomplishments

National Cancer Institute

The following are illustrations of two key technical accomplishments from the NCI Alliance for Nanotechnology in Cancer program that support PCA 1 and PCA 2, respectively.

Silica Nanoparticles for Intraoperative Sentinel Lymph Node Mapping and Radiotherapy

Investigators: Michelle Bradbury, MD, PhD, and Ulrich Wiesner, PhD, Sloan-Kettering Institute for Cancer Research and Cornell University CCNE

Innovation: Clinically-translatable versatile platform for accurate sentinel lymph node (SLN) dissection, targeted radiotherapies, and small molecule delivery. Researchers at the Memorial Sloan-Kettering Institute

[20] https://www.afirm.mil/

for Cancer Research and Cornell University have developed Cornell-dots (or c-dots) that have been already cleared by the FDA for clinical trials. C-dots are silica spheres, less than 8 nanometers in diameter, that contain fluorescent dye molecules and exhibit spectrally distinct optical properties. C-dots allow detection of the presence of SLNs and can be used in real time during the surgery to guide the surgeon. The group further modified C-dots to also target tumor cells for real-time visualization of SLN micrometastases using clinically portable optical imaging cameras (Figure 1). Such enhanced capabilities in intraoperative optical imaging for SLN mapping can provide improved accuracy in resection of tumor-bearing SLNs and significant improvement in melanoma treatment outcomes. C-dots are also being used in pre-clinical therapeutic studies. C-dots were radiolabeled with lutetium (Lu-177, beta particle emitting) or actinium (Ac-225, alpha particle emitting) for targeted melanoma radiotherapy. These constructs also used a targeting ligand towards melanocortin-1 receptor (MC1R), which is over-expressed in melanoma.

Clinical/commercial outlet: FDA approval: (1) to initiate advanced phase (Phase 2) clinical trials for SLN mapping in melanoma patients; (2) for a new ^{89}Zr-labeled, integrin-targeted C-dot product for use in a "pre-therapeutic" P1 clinical trial in malignant brain tumor patients; and (3) for breakthrough designation in progress to accelerate product approval for image-guided fluorescent SLN mapping.

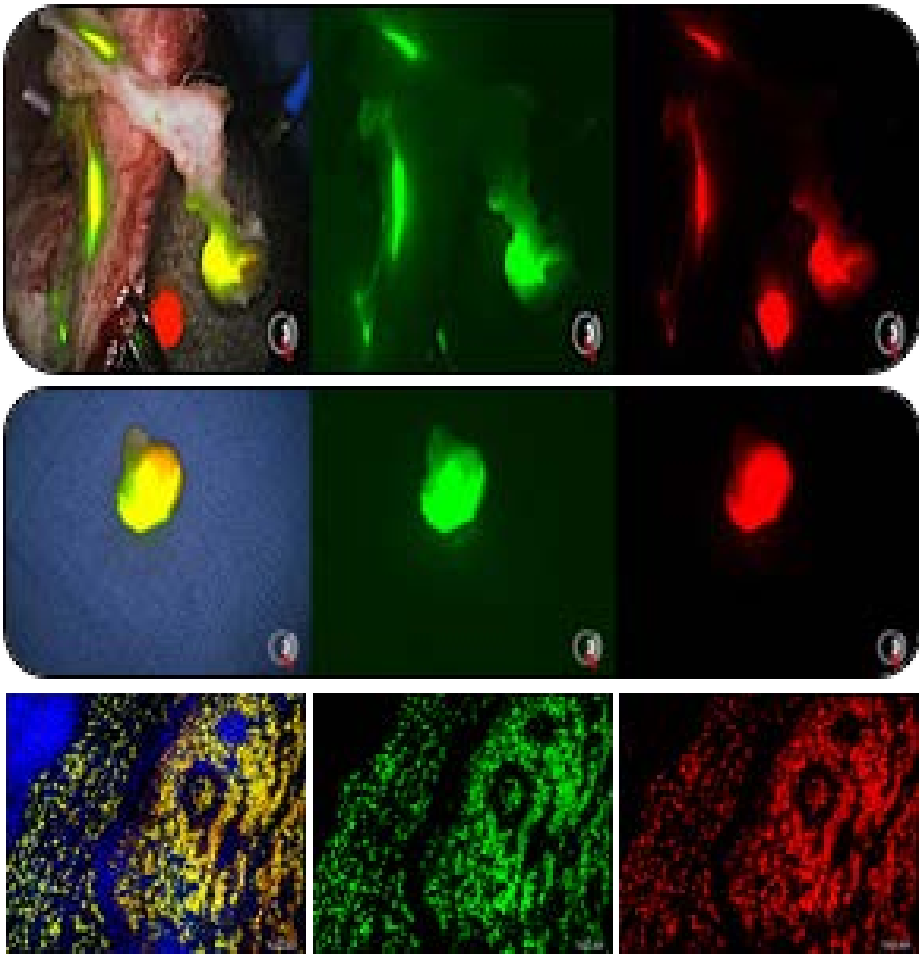

Figure 1. Green and red dyes indicate unique cancer markers in a large-animal model (top row), in tumors that have been excised (middle row), and in tissue samples of the excised tumors (bottom row). The images in yellow show where the green and red dyes overlap. Image credit: Michelle Bradbury, Memorial Sloan Kettering Cancer Center, and Ulrich Wiesner, Cornell University.

A Comprehensive Treatment of Multiple Myeloma through Tumor Microenvironment Targeting

Investigators: Kareem Azab, PhD, and John DiPersio, MD, Washington University CCNE.

Innovation: Reduce drug resistance to multiple myeloma (MM) treatments using multi-targeted nanoparticles. Researchers at the Washington University in St. Louis have developed MM tumor cell-targeting nanoparticles (liposomes) that were loaded with bortezomib to improve their therapeutic index and reduce side effects in normal tissues. These liposomes were also loaded with a ROCK inhibitor that blocks integrin-mediated cell-cell interaction between MM cells and other cells in the surrounding tumor microenvironment (TME), to overcome the TME-mediated resistance. Furthermore, these liposomes were decorated with PSGL1, a ligand that binds to endothelial and stromal cells, to facilitate the release the MM cells from the TME into the blood circulation, where they are readily exposed to chemotherapeutic drugs (Figure 2). Combination of the three strategies proved more effective in suppressing drug resistance and increasing treatment efficacy in MM (*in vitro* and *in vivo*) compared to treatment with chemotherapeutic drugs alone.

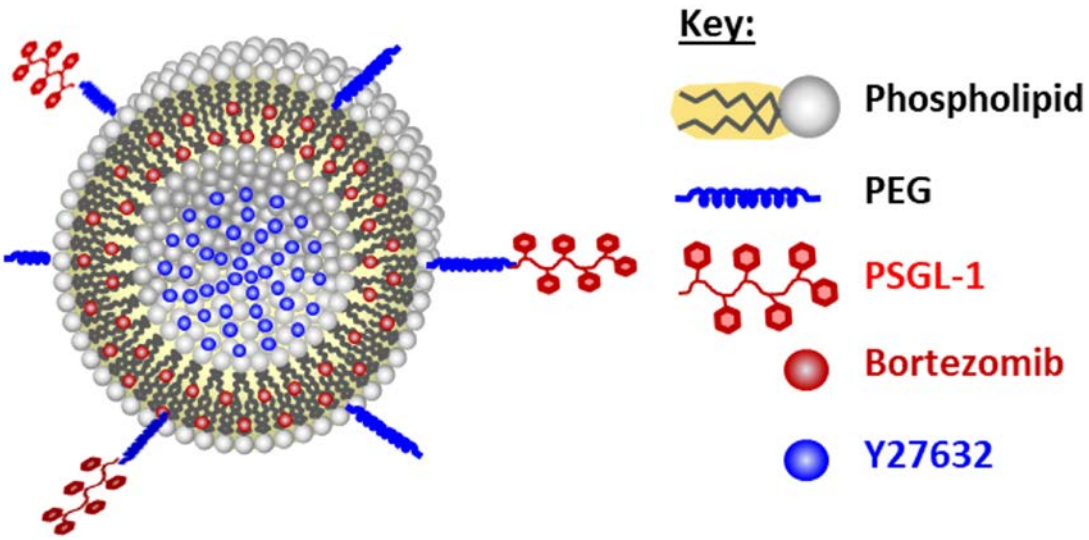

Figure 2. Design of multi-target nanoparticle against multiple myeloma.
Image credit: Kareem Azab, Washington University.

National Heart, Lung, and Blood Institute (NHLBI)

Although NHLBI does not have a program or special initiative in nanotechnology, there are some interesting scientific accomplishments to note in this area:

Organ Transplant Acceptance

Inducing graft acceptance without the need for chronic immunosuppression would greatly benefit patients receiving organs. Investigators from the Icahn School of Medicine at Mount Sinai demonstrated a key role for local macrophage activation in allograft rejection in an experimental mouse transplantation model. Blocking macrophage activation with a high-density lipoprotein-based nanobiologic targeting mammalian target of rapamycin (mTOR) prevented CD8+ T cell-mediated immunity and tolerogenic regulatory T cell

expansion. Adding a second nanobiologic targeting CD40-TRAF6 to inhibit co-stimulation resulted in a synergistic enhancement, leading to indefinite allograft survival.[21]

Cargo Delivery

Investigators at the University of Pennsylvania and colleagues have developed a method to enhance delivery of nanocarriers to organs by adsorbing the particles onto red blood cells (RBCs). Selective intravascular injection of the RBCs then leads to transfer of the absorbed nanocarriers into the first downstream organ. For example, intravenous injection results in a 40-fold increase in nanocarrier delivery to the lungs, the first downstream organ, relative to injection of free nanocarrier, while injection of RBCs into the carotid artery results in a 10-fold increase in delivery to the brain relative to free nanocarrier. This technology has the potential to enhance drug delivery in diseases such as acute lung injury and stroke while reducing off-target toxicity.[22]

Optimal delivery of nanoparticle cargoes may require targeting to subcellular features such as caveolae, cell membrane invaginations. However, the geometry of caveolae may limit access to larger nanoparticles due to steric effects. The use of deformable nanoparticles was found to allow access of larger nanoparticles, which can carry higher payloads, to a caveolae marker on mouse lungs, plasmalemma vesicle-associated protein (PLVAP). Rigid nanoparticles of similar size could not access the mouse lung PVLAP, indicating a role for mechanical deformability in targeting high-payload drug delivery vehicles to sterically-obscured targets.[23]

Genome Editing/Gene Therapy

Genetic diseases can be detected *in utero*, but the lack of treatment options leads to significant morbidity and mortality. To address this problem, researchers at Yale University demonstrated that polymeric nanoparticles could be safely administered to fetal mouse tissues via intravenous and intra-amniotic delivery at selected gestational ages. *In-utero* introduction of nanoparticles containing peptide nucleic acids and donor DNAs corrected a disease-causing mutation in the β-globin gene in a mouse model of human β-thalassemia, normalizing symptoms and improving survival, with no detected off-target mutations. Development of this technology could lead to a safe and versatile method of fetal gene editing for human monogenic disorders.[24]

Lipid nanoparticle-mediated delivery of mRNA has the potential to provide effective treatment for a spectrum of diseases, but efficacy is limited by the predominant hepatic uptake of LNPs. Conjugation of mRNA LNPs with antibodies specific to the vascular adhesion molecule PECAM-1 resulted in a 200-fold inhibition of hepatic uptake and a 25-fold increase in mRNA uptake and protein expression in lungs relative to non-targeted LNPs. The technology should be generalizable to enable targeting to other organs while suppressing hepatic uptake.[25]

[21] https://www.ncbi.nlm.nih.gov/pubmed/30413362

[22] https://www.ncbi.nlm.nih.gov/pubmed/29992966

[23] https://www.ncbi.nlm.nih.gov/pubmed/29956381

[24] https://www.ncbi.nlm.nih.gov/pubmed/29946143

[25] https://www.ncbi.nlm.nih.gov/pubmed/30336167