Emerging Trends in Micro- and Nanoscale Technologies in Medicine: From Basic Discoveries to Translation

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Emerging Trends in Micro- and Nanoscale Technologies in Medicine: From Basic Discoveries to Translation

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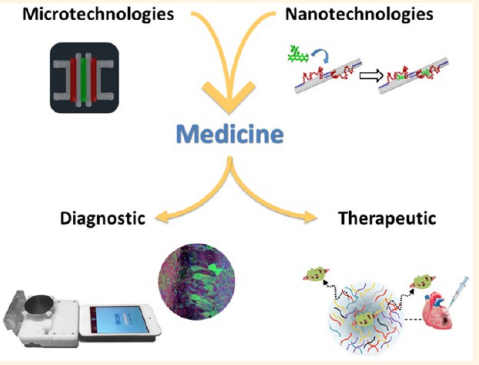
ABSTRACT: We discuss the state of the art and innovative micro- and nanoscale technologies that are finding niches and opening up new opportunities in medicine, particularly in diagnostic and therapeutic applications. We take the design of point-of-care applications and the capture of circulating tumor cells as illustrative examples of the integration of micro- and nanotechnologies into solutions of diagnostic challenges. We describe several novel nanotechnologies that enable imaging cellular structures and molecular events. In therapeutics, we describe the utilization of micro- and nanotechnologies in applications including drug delivery, tissue engineering, and pharmaceutical development/testing. In addition, we discuss relevant challenges that micro- and nanotechnologies face in achieving cost-effective and widespread clinical implementation as well as forecasted applications of micro- and nanotechnologies in medicine.



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Many medical problems remain unresolved, as evidenced by the millions of people who continue to succumb to disease. Chronic diseases such as diabetes,[1,2] cancer,[3] and vascular diseases[4] are epidemics today in both the developed and underdeveloped worlds. Obesity trends continue unabated, worsening the overall health of patients without any signs of control, despite massive worldwide research efforts. Infectious diseases such as malaria, tuberculosis, and human immunodeficiency virus (HIV) also remain major health concerns, affecting millions of people, primarily in underdeveloped countries[5] and in underprivileged sectors of modern societies.

Frequently, the solutions to these complex public health problems require the integration of multiple technological elements. Micro- and nanotechnologies are now coming together to offer new possibilities for addressing diagnostics, prevention, and treatment of these current health threats.[6,7] In this Nano Focus, we describe and explore the use of micro- and nanotechnologies in diagnostic and therapeutic applications. This article aims to provide a current landscape of this field as well as to illustrate relevant needs, opportunities, trends, and challenges.

We have divided our discussion into three sections. First, we discuss the integration of micro- and nanotechnologies into the development of better diagnostic platforms. Second, we provide several examples of how micro- and nanotechnologies add to the therapeutic toolbox to combat some of the major health concerns of modern societies. Finally, we discuss some of the translational considerations and challenges relevant to the clinical application of micro- and nanotechnologies, and we briefly offer prospective comments, highlighting bioinspiration and integration as some of the current trends in the field.

DIAGNOSTICS AND SCREENING APPLICATIONS OF MICRO- AND NANOTECHNOLOGIES

Accurate and early diagnosis of disease remains one of the greatest challenges of modern medicine. The rapid increase in healthcare costs—in the United States and in many other developed countries—is a powerful driving force that directs attention towards applications related to diagnosis and prevention rather than costly treatment of end-stage disease. Diagnosis frequently involves massive screening strategies, which, in turn, open the possibility of using diverse micro- and nanotechnologies such as microfluidic platforms, microfabrication techniques, micro- and nanoparticles (NPs), and micro- and nanosensing platforms, among others.

In this section, we focus on two application areas to illustrate the combined use of micro- and nanotechnologies to provide smart diagnostic solutions to highly relevant global health problems: the identification and separation of circulating tumor cells (CTC) and the cost-efficient diagnosis of infectious diseases. In addition, we briefly review several recently developed technologies that enable the study of micro- or nano processes and structures, thereby creating new venues for diagnostics. For example, expansion microscopy, a novel form of microscopy that relies on the physical expansion of samples, opens up possibilities for economical, high-resolution microscopy. We also describe the use of new nanosensors for real-time monitoring of events at the molecular level.

Micro- and Nanotechnologies for Cancer Diagnostics. More lives could be saved by early detection of cancer than by any form of treatment at advanced stages. In this respect, the detection (and isolation) of CTCs is a major breakthrough that will enable new and more effective treatment strategies against cancer.

The combination of micro- and nanotechnologies has proven useful for the identification and isolation of CTCs in the patient's blood.[8] Circulating tumor cells, which are viable cells derived from tumors, are hypothesized to represent the origin of metastatic disease. These cells are present in extremely low numbers in blood; therefore, their capture is highly challenging, and many microfluidic strategies have been explored to improve the efficacy of CTC isolation. Pioneering work on this subject by Nagrath *et al.*[9] has demonstrated the feasibility of capturing CTCs using a micropost CTC-Chip,[9] where blood is circulated by laminar flow through an array of microposts coated with antibodies against the epithelial cell adhesion molecule (EpCAM). The herringbone CTC-Chip (HbCTC-Chip) system uses herringbone-shaped grooves to originate eddies for efficient guidance of cells toward the anti-EpCAM surfaces.[10] The HbCTC-chip was able to capture individual CTCs as well as the CTC microclusters[11] that may be key to the hematogenous dissemination of cancer. Ozkumur *et al.*[12] demonstrated the use of a tumor antigen-independent (or marker-free) microfluidic chip, based on an inertial focusing strategy, for isolating CTCs (CTC-iChip). This CTC-iChip enables the cells to align in nearly single rows, from which their trajectories are accurately deflected using a low intensity magnetic field (Figure 1A). Tseng and co-workers used "nano-velcro" to capture CTCs efficiently.[13] These microfluidic systems have been used to separate CTCs from the bloodstream of pancreatic, breast, prostate, colon, melanoma, and lung cancer patients. Also, they have effectively enabled the isolation of CTCs from patients with metastatic tumors for further analyses of these cells. For example, androgen receptor expression signals can be evaluated in the captured CTCs before and after therapeutic interventions.[14]

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This use of microfluidics can be generalized. An increasing body of evidence suggests that CTCs are relevant cancer biomarkers. Using microfluidic tools, these markers can be collected, concentrated, and analyzed to provide information concerning the prognosis of a patient. The effect of therapeutic intervention can be assessed, almost in real time, by analyzing the concentrations of CTCs and their gene expression and mutation profiles before and after a particular therapy. Indeed, continuous monitoring of the number and characteristics of CTCs in a patient could be an effective strategy for assessing disease progression.[15] The capture and concentration of CTCs also enables the performance of fundamental studies on the biology of cancer and on the mechanistic nature of metastasis, as has been done for cancer cell lines.[16]

Micro- and Nanotechnologies for Point-of-Care Diagnostic Applications. The detection of pathogens at the point-of-care (POC), particularly in underprivileged areas, continues to be one of the major challenges of modern medicine. The 2009 influenza pandemic, epicentered in Mexico, and the recent Ebola epidemic event in West Africa have also emphasized the critical need for cost-effective POC diagnostic platforms.[17−19]

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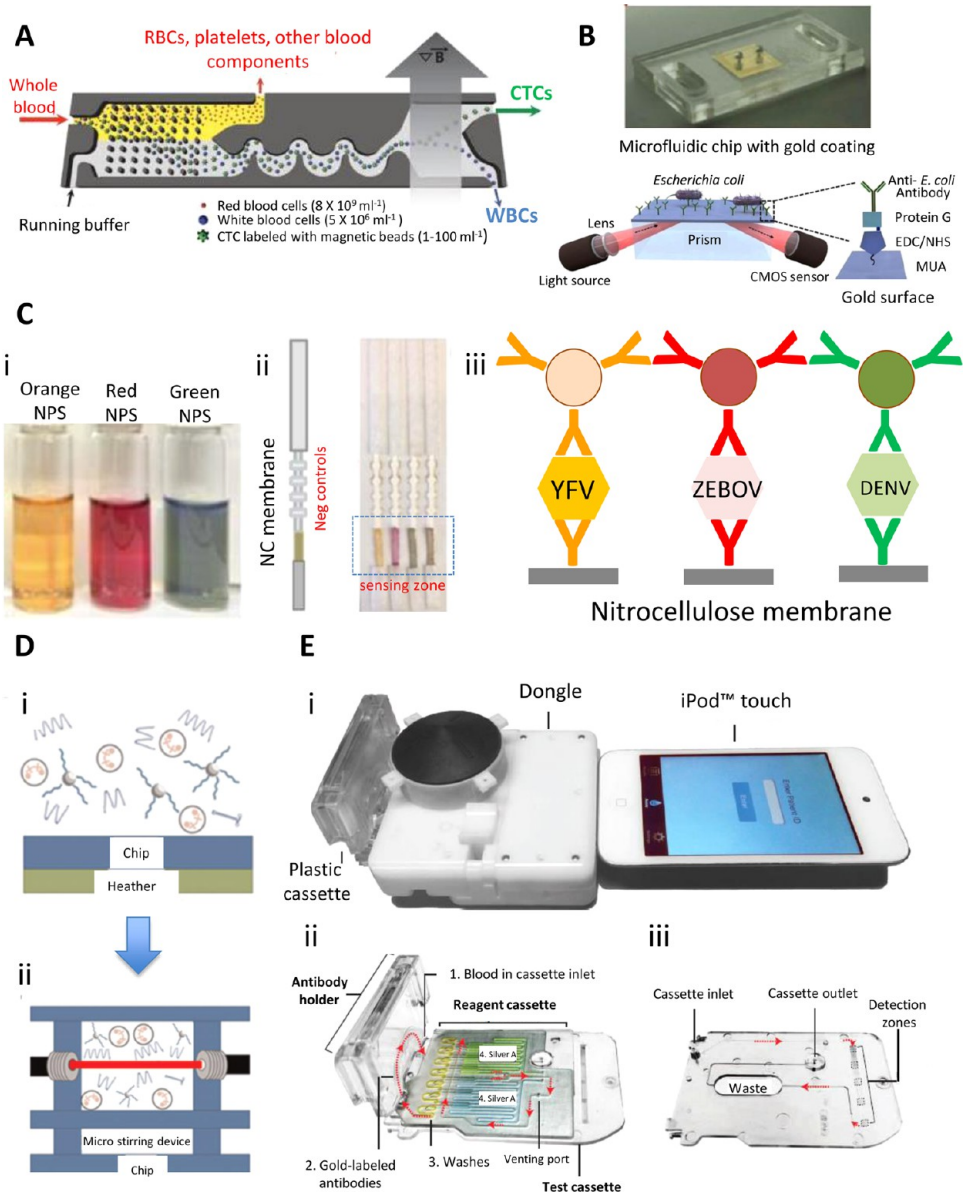

Figure 1. Micro- and nanotechnologies for cancer cell isolation and POC applications. (A) The CTC-iChip system: Three microfluidic components of the CTC-iChip are shown schematically. The inputs are whole blood premixed with immunomagnetic beads and buffer. Beads can be functionalized with antibodies for selective capture of CTC cells. Reprinted with permission from ref 12. Copyright 2013, American Association for the Advancement of Science. (B) Microfluidic chip with gold coating for the specific detection of *Escherichia coli* by plasmon resonance. The gold surface of the microchip is functionalized with specific antibodies against *E. coli* (attached to the gold surface through a bridge of molecules, including Protein G. The degree of bacterial attachment is determined by a change in the refractive index. Reprinted with permission from ref 24. Copyright 2015, Macmillan Publishers Ltd. (C) Detection of three different viral proteins (yellow fever virus NS1, Zaire Ebola virus GP, and Dengue NS1) using a paper-based microfluidic system and gold NPs loaded with specific antibodies: (i) specific antibodies against dengue, Ebola, and yellow fever viruses were immobilized on nitrocellulose strips; (ii) gold NPs of three different colors were functionalized with specific antibodies against each of the viruses; and (iii) the functionalized NPs are captured only if coupled with their target virus. Reprinted with permission from ref 29. Copyright 2015, Royal Chemical Society. (D) Microfluidic system for the specific detection and amplification of viral RNA by RT-LAMP: (i) The sample is treated with a lysis buffer, the target viral RNA is hybridized using magnetic particles functionalized with virus-specific primers, and then, the targeted viral RNA sequence undergoes selective RT-LAMP amplification. Reprinted with permission from ref 32. Copyright 2015, Elsevier. (ii) Optical fibers are used to sense the magnesium pyrophosphate precipitate. (E) A smartphone dongle for the simultaneous immunodetection of HIV and syphilis. Image of the dongle with three different cassettes: (i) a microfluidic cassette connected to an iPod Touch; (ii) a reagent cassette (top layer) containing prestored reagents (numbered in the order they flow through the test cassette) and a test cassette (bottom layer) containing different detection zones; and (iii) flow sequence through the test cassette. Reprinted with permission from ref 35. Copyright 2015, American Association for the Advancement of Science.

The design and characterization of POC microfluidic devices have therefore become an important trend in biomedicine today.[20−22] The integration of micro- and nanocomponents now enables the design of simpler and more cost-effective POC systems than those currently in use. Recent examples include the integration of magnetic NPs, high affinity antibodies, and quantum dots for the simultaneous capture and sensing of viruses (*i.e.*, Enterovirus 71 and Coxsackievirus B3) from throat

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swabs;[23] the use of a surface plasmon resonance microfluidic platform that quantifies bacteria (*i.e.*, *Escherichia coli* and *Staphylococcus aureus*) using a combination of proteins (*e.g.*, Protein G and specific antibodies) attached to gold surfaces as the active surface for capture (Figure 1B);[24] integration of smartphone[25−27] and Google Glass[28] systems with diagnostics; and the integrated use of paper-based microfluidics, specific antibodies and gold NPs of different colors for simultaneous detection of the presence of three different viral agents (Figure 1C).[29]

In addition to immunoassays on chips, the detection of nucleic acids from pathogens is now also possible on microfluidic platforms. The polymerase chain reaction (PCR) and the more recently introduced reverse transcription loop-mediated isothermal amplification (RT-LAMP) protocols[30−32] have been miniaturized. The RNA from pathogens can be identified and amplified with different detection strategies, including colorimetric and fluorescence techniques, immuno-chromatography on paper,[33] and optical fiber sensing of turbidity caused by the side products of the LAMP-reaction (Figure 1D),[32] among others.

The integration of diagnostic microdevices with smartphones is another emerging trend in POC diagnostics. Microscopies and spectroscopies can be integrated with smartphone cameras or with Google Glass. The power source, processing, and communications capabilities of smartphones can be used to analyze and to report data in and from the field.[19,25,27,28,34] Another recent example is the coupling of an enzyme-linked immunosorbent assay (ELISA) dongle with a smartphone for accurate performance of a triplex immunoassay for HIV, syphilis, and active syphilis infection (Figure 1E).[35] Healthcare workers in Rwanda obtained on-site diagnostic results comparable to those obtained by conventional laboratory ELISAs (sensitivity between 92 and 100%; specificity between 79% and 100%) using this triplex assay in only 15 min.

Emerging Micro- and Nanoimaging Platforms for Diagnosis. Imaging biological samples with nanoscale precision is important for resolving the location and identity of genes, RNAs, proteins, and complexes of multiple such entities in cells and throughout organs, cancers, and brain circuits. The resulting molecular maps not only inform us as to the fundamental building blocks of life and how they are organized to support biological computation but also can present clinically relevant maps of biomarkers or pathological changes that may indicate novel therapeutic targets. The diffraction limit, however, prevents the use of light microscopy for accurate reporting at resolutions below *ca.* 200 nm, which means that identifying and localizing single biomolecules or clusters of biomolecules remains out of reach for many biological and clinical investigations. Pioneering advances using super-resolution microscopy platforms such as stochastic optical reconstruction microscopy,[36] stimulated emission depletion microscopy,[37] and photoactivated localization microscopy[38] have enabled imaging with resolutions below the diffraction limit; however, these technologies are slow, require expensive equipment, and/or struggle in the context of imaging large three-dimensional (3D) samples. A recent study[39] reported the physical magnification of a biological sample by embedding it in a dense, swellable polymer having a mesh size in the few nanometer range (Figure 2Ai,B). The addition of water to swell the polymer resulted in the entire polymer−sample composite increasing in size (Figure 2Aii,C). Key molecules within the sample (*e.g.*, fluorophores tagged to proteins of interest *via* targeted antibodies) were transferred to the polymer, and the sample was then mechanically homogenized by destroying its structural molecules (Figure 2D−F), thereby enabling isotropic expansion. This technology, called expansion microscopy (ExM), where the sample itself is enlarged, is a stark departure from the dominant strategy of light microscopy, which relies on lenses to magnify images of samples. Although the principle of embedding biological specimens in a hydrogel was developed decades ago,[40] the use of a swellable hydrogel to increase the size of a biological specimen for resolution improvement is a new development.

The first version of ExM[39] involved synthesizing the swellable polymer (sodium polyacrylate) within and throughout fixed cells or tissues and then anchoring fluorophores to the polymer at sites of antibody binding to proteins of interest (Figure 2D−F). Proteinase K treatment then resulted in the destruction of the endogenous protein structures within the sample. Addition of water resulted in ∼4.5× linear expansion in all directions or ∼100× volumetric expansion of the sample. Thus, at 300 nm, a diffraction-limited lens would have its effective resolution improved to 300/4.5−70 nm. This indicates that a sample prepared with standard primary antibodies and conventional fluorophores, processed with ExM, could be imaged on a conventional diffraction limited microscope with nanoscale precision. This advance is important because conventional diffraction-limited microscopes can scan rapidly; ExM therefore enables a "best of both worlds" imaging modality, where samples can be imaged with the voxel sizes of super-resolution method, but at the voxel acquisition rates of fast diffraction-limited microscopy technique. The first paper on ExM presented three-color imaging of a volume of a mouse hippocampus with ∼70 nm lateral resolution, using a commercial confocal microscope, in about a day (Figure 2G). Many investigators are now applying ExM for imaging microbes, cancers, brain circuits, developing embryos, and large numbers of other samples.[29,39,41−46] In addition, the technology itself is constantly being improved and refined. For example, a simple expansion microscopy protocol has been developed in which proteins (including conventional antibodies and genetically encoded fluorophores) are directly anchored to the swellable polymer, and then the sample is expanded;[43] furthermore, a new and simple chemistry was recently developed enabling expansion microscopy to be applied to RNA.[46]

Expansion microscopy therefore enables a "best of both worlds" imaging modality, where samples can be imaged with the voxel sizes of a super-resolution method, but at the voxel acquisition rates of a fast diffraction-limited microscopy technique.

Expansion microscopy offers many potential advantages. The expansion factor is the same for axial as well as lateral directions, enabling improved magnification in all directions. Expanded samples, since they are ∼99% water, are completely transparent in the visible range, facilitating fast imaging by lightsheet microscopy and other high-speed 3D imaging methods.

The additional room created around anchored biomolecules or labels also suggests the possibility of running complex biochemical reactions in expanded samples, taking advantage of the ability to set the chemical environment precisely around anchored biomolecules or labels for precision biochemical analyses. Already a basic demonstration, the performance of

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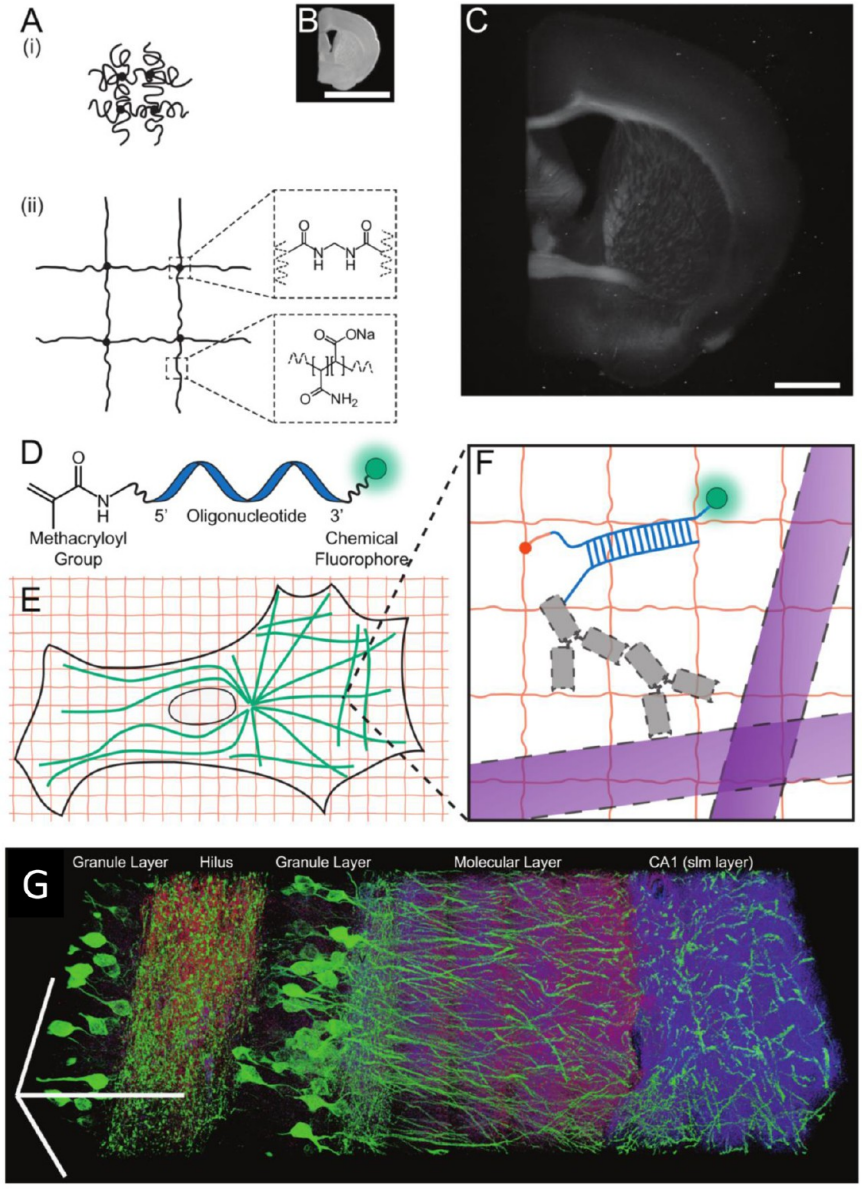

Figure 2. Expansion microscopy (ExM). (A) Polyelectrolyte network (i) in collapsed stage, showing the polymer chain (line) and the cross-linker (dot); and (ii) expanded network after H_2O addition. (B) Pre-ExM and (C) post-ExM image of a fixed mouse brain slice. Scale bars: (B, C) 5 mm. (D) Schematic of a label anchored to the gel, the polymer network (orange), and the cell cytoskeleton (green). (F) The label (D), hybridized to the oligo-bearing secondary antibody (gray) and anchored *via* the primary antibody (gray) to microtubules (purple), is bound into the gel (orange lines) *via* the methacryloyl group (orange dot) and remains after proteolysis (dotted lines). (G) A portion of hippocampus showing neurons (expressing Thy1-YFP, shown in green) and synapses (marked with anti-Bassoon (blue) and anti-Homer1 (red)). Scale bars: (G) 100 μm in each dimension. Schematics are not to scale. Adapted with permission from ref 39. Copyright 2015, American Association for the Advancement of Science.

the hybridization chain reaction to amplify RNA fluorescence *in situ* hybridization signals in expanded tissue samples has highlighted the promise of this direction.[46]

Biosensing has become a key component of modern diagnostic technologies. The use of state-of-the-art microfabrication platforms and nanotechnology has enabled precise sensing of chemical and electrochemical events that occur at various biological scales, including tissues, cells, and even molecules.

Nanomaterials are useful for a wide range of sensing applications aiming for detection at the molecular level.[47] The development of new compounds that can detect and recognize analytes in complex environments at the nanoscale has been enabled by novel nanotechnology tools.[48−52] Traditional molecular recognition systems, such as antibodies and aptamers, suffer from relatively low chemical and thermal stability, whereas synthetic approaches, such as molecular imprinting techniques, are limited to low-molecular-weight targets.[53] The quest for novel, nanoscale, synthetic, nonbiological antibody analogs has seen notable progress. For example, the work of Zhang *et al.*[54] introduced the concept of corona phase molecular recognition (CoPhMoRe): A heteropolymer adopts a structured configuration when adsorbed onto the surface of a fluorescent NP, generating a corona phase around it, so that the complex can specifically and selectively recognize a target analyte. Utilizing a NP sensor with an optical response to the binding of a target is advantageous for cases in which high spatial resolution, in addition to temporal information, is required. Single-walled carbon nanotubes (SWCNT) are preferably used as the underlying NPs,

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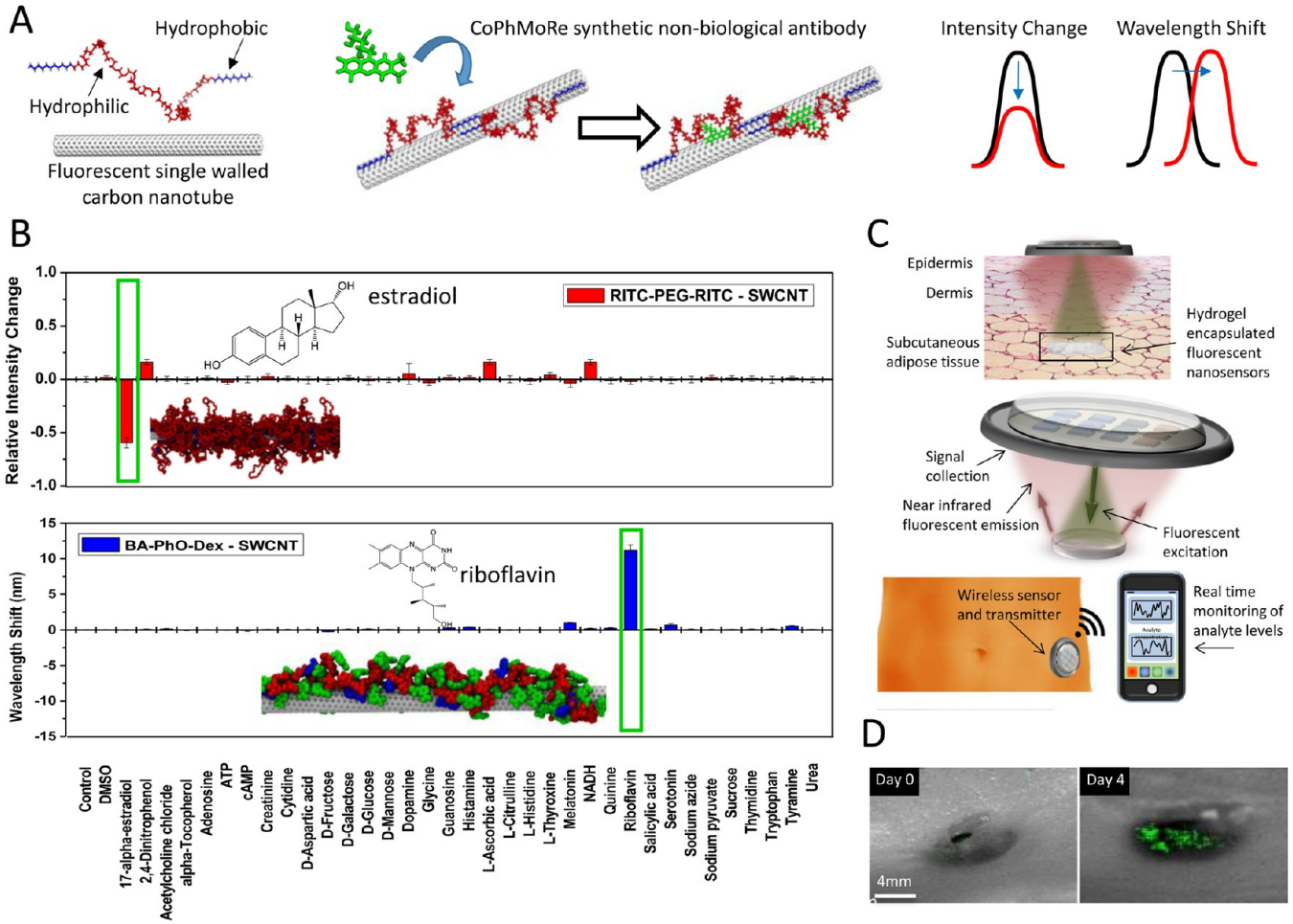

Figure 3. Corona phase molecular recognition (CoPhMoRe) strategy. (A) A heteropolymer with hydrophobic and hydrophilic segments is adsorbed onto the surface of fluorescent single-walled carbon nanotubes. The corona phase enables the recognition of a target analyte, resulting in an intensity change or a wavelength shift of the emission signal. (B) Top: Rhodamine isothiocyanate-difunctionalized poly(ethylene glycol) (RITC-PEG-RITC) corona enables the selective identification of estradiol. Bottom: Boronic acid substituted phenoxy-dextran (BA-PhO-Dex) corona enables the specific recognition of riboflavin. Adapted with permission from ref 54. Copyright 2014, IEEE. (C) Illustration of a hydrogel encapsulating fluorescent nanosensors implanted in subcutaneous adipose tissue. An external detector excites and detects the fluorescence signal and sends the information to any electronic device. Adapted with permission from ref 64. Copyright 2015, John Wiley & Sons. (D) *In vivo* fluorescence image of a hydrogel encapsulating NO nanosensors immediately after implantation (day 0), where high NO levels resulting from local inflammation quench the fluorescence signal. The signal recovers 4 days following the procedure as the incision heals and NO levels decrease. Adapted with permission from ref 66. Copyright 2013, Macmillan Publishers Ltd.

since their fluorescence can serve as an optical reporter of a binding event, manifested as a modulation of the emission intensity or a wavelength shift (Figure 3A).[55] Moreover, SWCNTs fluoresce in the near-infrared part of the spectrum, which aligns with the tissue transparency window and enables deep-tissue sensing and detection *in vivo*.[56]

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Discovery of novel corona phases for molecular recognition is achieved by high-throughput screening of wrapping polymer libraries against analyte panels, where the fluorescence signal enables rapid detection of selective phases. The inverse problem of a rational design of a corona phase that would detect a specific analyte of interest was recently addressed by a mathematical model for helically wrapped SWCNT complexes.[57] This advance, in turn, accelerated CoPhMoRe discovery by substituting the combinatorial process involved in extensive library screenings. Corona phase molecular recognition was successfully demonstrated (1) with a rhodamine isothiocyanate-difunctionalized poly(ethylene glycol) (RITC-PEG-RITC) corona for the selective recognition of estradiol; (2) with Fmoc L-phenylalanine-functionalized PEG brush corona for the selective recognition of L-thyroxine; (3) with boronic acid substituted phenoxy-dextran (BA-PhO-Dex) corona for the selective recognition of riboflavin (Figure 3B);[54] (4) with a single-stranded DNA corona[58] for the detection of neurotransmitters;[59,60] (5) with a phospholipid-PEG derivative corona for the detection of the protein fibrinogen;[61] and (6) with a peptide corona for the detection of nitroaromatics.[62]

In vivo diagnostics and sensing can be accomplished by utilizing a biocompatible hydrogel to encapsulate the fluorescent nanosensors[63] and then implanting the encapsulated material subcutaneously to detect analyte concentrations in its vicinity (Figure 3C).[64,65] In this scheme, the fluorescent nanosensor is

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excited and detected by an external detector placed on top of the implant location, where the information can then be transmitted to an electronic device. *In vivo* detection of nitric oxide (NO) was demonstrated using single-stranded DNA wrapped SWCNTs encapsulated within an alginate hydrogel and subcutaneously implanted onto the back of a mouse.[66] Elevated NO levels, resulting from local inflammation at the incision site shortly after the implantation procedure, quenched the fluorescent emission signal, but the signal later recovered as the incision healed (Figure 3D). *In vivo* detection of molecular targets was also demonstrated in living plants using fluorescent SWCNT sensors that were infiltrated directly into leaf tissues.[62,67]

These recent achievements demonstrate the enormous potential of fluorescent nanosensors for clinical applications requiring continuous *in vivo* monitoring of important biomarkers. The optical nature of the detection scheme can provide real-time readout with high spatial and temporal resolution. These platforms hold great promise as alternatives to conventional natural recognitions elements, both for diagnostics and for treatment purposes, to improve patient care.

MICRO- AND NANOTECHNOLOGIES FOR THERAPEUTIC APPLICATIONS

Next, we briefly examine emerging contributions of micro- and nanotechnologies in solving major therapeutic challenges.[68] We have selected topics that we consider revealing and relevant. We discuss the design of therapeutic cancer vaccines based on materials with micro- and nanoscale features that elicit powerful immune responses, the use of NP carriers for targeted drug release, the integration of micro- and nanotechnologies in tissue engineering applications, and the design of nonbiofouling surfaces with micro and nanofeatures for implants and surgical devices. We also discuss the use of lab-on-chip systems for drug testing/development with particular emphasis on the recent advances on the fabrication of vascularized tissues for drug testing/screening.

Therapeutic Cancer Vaccines. A major challenge in cancer immunotherapy is the induction of robust cytotoxic T lymphocyte responses, particularly in the face of immunosuppressive signals arising from tumors. However, appropriately designed biomaterials may create a new physical microenvironment within cancer patients to initiate this process. The magnitude and type of an immune response are regulated by antigen-presenting cells; among these, the dendritic cells (DCs) may be the most important. Efforts are thus focusing on the recruitment and manipulation of DCs to control the type and magnitude of immune responses to cancer antigens. Highly porous materials are desirable for housing large numbers of DCs while they are loaded with antigens and activated as well as enabling trafficking of DCs into and out of the device; specific ranges of porosity and pore size were found to maximize DC migration and recruitment in response to chemotactic agents.[69,70] A variety of chemotactic agents can be utilized to recruit DCs, with granulocyte-macrophage colony-stimulating factor being particularly effective.[71] Delivery of cytosine-guanine oligonucleotide, a potent adjuvant and ligand for toll-like receptor 9, in NP form effectively activates the DCs housed within biomaterial scaffolds.[72] Porous poly(lactide-*co*-glycolide) scaffolds with relevant microscale features that provide these functionalities have been demonstrated to generate highly potent, antigen-specific, cytotoxic T lymphocyte responses capable of inducing complete regression of established melanoma in preclinical models.[73] This approach has demonstrated further utility in other models of cancer, including lung cancer.[74] This strategy is currently in a physician-sponsored Phase I clinical trial for Stage IV melanoma patients (https://clinicaltrials.gov/; #NCT01753089).

Current efforts aim to simplify treatment with biomaterial-based therapeutic cancer vaccines by focusing on creating materials that can be delivered into the body in a minimally invasive manner. For example, cryogels are being fabricated from alginate and gelatin, as they enable minimally invasive delivery *via* a syringe and needle.[75,76] These gels have a microporous structure, with interconnected pores that enable the recruitment and establishment of large numbers of DCs. These scaffolds are highly elastic and deformable, leading to shape memory properties that enable them to undergo the necessary deformation for injection through a needle and then to resume their original size and shape once inside the tissue. The utility of these materials in therapeutic vaccination has recently been demonstrated.[77] Another system takes a distinct approach: Rather than fabricating a 3D porous biomaterial and then addressing the challenge of how to introduce it into the body, micro- and NPs that can readily traverse a needle are fabricated instead. After delivery, they form a 3D porous structure *in situ*.[78] Most of the work conducted to date with these biomaterial systems has focused on generating cellular immunity, but some data also suggest that this strategy can generate potent antibody responses.[78] So, while biomaterial-based vaccines have been developed for therapeutic cancer treatments, this concept may be broadly useful to treat other diseases involving immune dysfunction. These technologies may be useful in the future to generate immune responses in the treatment of chronic infectious diseases as well as to ameliorate pathologic immune responses in the context of autoimmune diseases and organ transplantation.

Nanoparticle Carriers for Targeted Drug Delivery. The design of functionalized nanocarriers (NCs) for drug-delivery applications has received a great deal of attention in the past decade.[79,80] Nanoparticles can be internalized by cells and thus are good prospects as drug carriers. They can be distributed systemically *via* intravenous injection and locally delivered into a particular tissue. Many functionalization strategies have been used to favor homing of these particles to particular tissues. The use of functionalized NCs has been proposed for different therapeutic purposes including the treatment of cancer and central neural system disorders, cardiac tissue repair, and thrombolysis, among others.[81−84]

Nanoparticle-based therapies continue on the path to clinical implementation. Next, we review some of the technical challenges that nanobased technologies face in fulfilling their promising potential in the clinical setting. The case of cancer NCs is an illustrative example; research and development of NCs for cancer treatment are among one of the most active fields in nanomedicine. Nanocarriers may have great impact on patient compliance by increasing drug efficacy and reducing systemic toxicity. Nevertheless, current NCs seem far from fulfilling their therapeutic potential, as an enormous gap exists between the numbers of NCs that are currently under preclinical and clinical evaluation and the numbers of NCs with Food and Drug Administration (FDA) approval as anticancer drugs. Doxil and DaunoXome were the first nanoscale liposomal anthracycline drugs approved by the FDA, in 1995 and 1996, respectively.[85,86] Only a few liposome-based anticancer formulations, and no polymeric nanoscale delivery systems, have been approved by the regulatory agencies since then. This relatively low number of approvals may have several explanations. Systemic administration

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is not an easy strategy for delivery, and intracellular uptake of NCs continues to be a challenge. In addition, the animal models used for nanomedicine testing require urgent refining, new techniques and approaches are needed for proper assessment of the *in vivo* stability of NCs, and scaling up NC manufacturing is challenging. Lastly, the FDA has required extensive biodistrbution and toxicity studies for NCs due to their ability to penetrate tissues and cells not possible with micron-carriers, lengthening approval processes.

The use of functionalized NCs has been proposed for different therapeutic purposes including the treatment of cancer and central neural system disorders, cardiac tissue repair, and thrombolysis, among others.

Systemic administration of NCs could affect the loaded drug and its effectiveness. In addition, several considerations must be taken into account when NCs are administered systemically. Importantly, NCs undergo vast dilution in the bloodstream[81] when administered systemically, and their pharmacokinetics and biodistribution are determined by several factors, including their absorption, distribution, metabolism, and excretion; the materials from which they are made; their surface charge; and their curvature. In general, NCs can easily be tagged with proteins to alert the immune system.[87−94] Moreover, how these carriers penetrate tumors and by which mode of action (MoA) they release their payload are additional factors that can determine the effectiveness of a NC treatment and also relate to the particular characteristics of a tumor (*i.e.*, size, architecture, and physiological state).[87,95,96] The particular tumor biology must be understood in order to customize the appropriate NC system. Tumor heterogeneity with different cell milieus adds complexity to the design of NCs.

The MoA of NCs can be divided into passive tissue targeting or active cellular targeting strategies.[81,88,97] The passive-tissue-targeting MoA relies on the "leaky" nature of blood vessels next to the tumor and ineffective lymphatic drainage, known as the enhanced permeability and retention (EPR) effect, which permits selective penetration of NCs into the tumor vicinity. However, even in highly vascularized tumors, sole reliance on the EPR effect is typically not sufficient. In many cases, the released drug will not reach the inner tumor layers where cancer stem cells (CSCs) or tumor-initiating cells are often located (*i.e.*, in the necrotic inner core or within deeper embedded CSC niches).[98] The development of new methods for enhancing tumor vascular permeability will contribute to efficient penetration of the NCs into the tumor microenvironment, thereby increasing treatment effectiveness. Some efforts have been made to develop strategies to probe the EPR in a particular patient *via* positron emission tomography-computed tomography (PET/CT) or imaging modalities such as magnetic resonance imaging (MRI).

These strategies are based on pre-injecting patients with iron oxide NPs (in the case of MRI) or by injecting size-defined nanoliposomes containing ^{64}Cu (in the case of PET/CT) to enable quantification of the EPR dimensions and to provide better fits for patients with the appropriate NC modalities. Nanocarriers with active targeting capabilities (*i.e.*, those which present targeting molecules, such as natural ligands, peptides, antibodies, and nucleic acids, on their surface) enable high-affinity interactions with abundant tumor cells surface receptors, thereby increasing binding and cellular uptake into the tumor cells and reducing off target effects or effects on bystander cells.[81,88,91,92,99−102] A step forward is the delivery of more than one active ingredient from the same carrier at different times (staged delivery) by using multimaterial and/or multilayered NPs. Different strategies for staged delivery have been reported. Anticancer drugs and small interfering RNA (siRNA, to knockout the expression of an efflux transporter implicated in drug resistance) have been codelivered using mesoporous silica particles.[103] Deng *et al.* developed layer-by-layer (LbL) NPs by the sequential deposition of polymers with opposite charges; this strategy enables the sequential entrapment and release of multiple agents (*i.e.*, an inner core containing an anticancer drug, an intermediate layer of siRNA, and an outer shell engineered for specific tumor targeting).[104] Many variations of the LbL strategy have been used recently to target cancerous cells specifically and to deliver nucleic acids and anticancer drugs effectively from surfaces loaded with NPs and engineered spherical NCs.[105−107]

Resourcing in experimental therapies (*i.e.*, nanoembolization),[108] interventional radiologists can deliver cancer-drug NCs directly into a tumor site *via* catheters. This procedure can extend the survival of terminal cancer patients by several months. However, improving the clinical success of the systemic application of drug-loaded NCs has proven to be a challenging task. Changing the prevailing way of treating cancer and moving from a short-term "tumor-shrinking effect" to a long-term destruction requires massive changes, modifications, and multidiscipline attitudes in order to deal successfully with a complex disease. In addition, the treatment of more aggressive tumor-cell populations consisting of drug-resistant cells demands smarter and more sophisticated drug NC platforms.

Nano- and Microtechnologies for Tissue Engineering. Various types of NPs have been used in tissue engineering scenarios for different purposes. Two important fronts of applications are improvements in the osteogenicity, antimicrobial activity, conductivity, and/or mechanical properties of construct and scaffolds and the delivery of genes or drugs to induce tissue repair, promote osteogenesis, or enhance osteointegration, among other aims.

The use of decellularized matrices has enabled great progress on the development of active biomaterials for tissue engineering applications,[109,110] 3D-printed scaffolds,[111−113] and novel chemistries for improved bioactive and bioresorbable materials.[113] However, many of these matrices lack the desirable nanoscale features present in natural tissues, specifically in bone. Bone structure spans several scales, to impart high strength, durability, and flexibility for its weight and size.[114] Hydroxyapatite (HA) is a popular choice as a bone biomaterial due to its chemical similarity to natural bone crystals, but, as has been well documented, micro- and macroscale HA lacks the mechanical properties necessary for physiological use and, consequently, for adequate bone cell differentiation from mesenchymal stem cells (MSCs) and osteo-regeneration.[114−116] Titanium has been the gold-standard material for large bone implants, including replacements in the hip and knee, largely due to its close-to-ideal mechanical strength and moderate biocompatibility. In either case, bone implants containing HA or titanium (or any current orthopedic material, for that matter) continue to require revision surgery due to infection, implant loosening, and poor bone integration. Many implants are roughened with a porous coating to improve bone cell integration, but this largely affects the micro- and macroscale, and these surfaces are still prone to

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infection. Several coating types and processes have been explored to improve titanium biocompatibility and osseointegration while releasing antibiotics to ward off bacteria.[117,118] However, these approaches have also failed, as bacteria increasingly develop resistance to any antibiotic developed. In fact, the World Health Organization has recently predicted more deaths due to antibiotic resistant bacteria than cancer over the next decade. New approaches are needed that can simultaneously reduce infection while increasing bone growth. Nanotechnology may provide the answer.

Nanoscale texturing has been shown to improve osseointegration as well as to reduce bacterial adhesion on a number of different materials.[119−121] In addition, the combined actions of HA coatings on titanium implants may provide the biocompatibility and osteoconductivity of HA while imparting the mechanical strength of titanium. Further improvements in bone cell functions have been demonstrated with nanoscale hydroxyapatite (nano-HA), which is significantly more bioactive than traditional micro-HA. Incorporation of nano-HA into common bone implant materials, such as titanium,[111,115] to create nanocomposites has recently demonstrated substantial improvement in osteoconductivity and, more recently, infection reduction. Importantly, this was accomplished without introducing antibiotics or any new chemistry that is not already FDA approved into/onto the medical device surface. Such materials[116] just received FDA approval for human implantation. Coating titanium with nano-HA using electrophoretic deposition (EPD) retains the nanoscale properties of HA for reducing bacterial functions, especially when compared to the traditional plasma-sprayed HA where the retention of nanoscale HA geometries is difficult (Figure 4).[116] The nano-HA coatings are also osteogenic and increase osteoblast density.[122] Thus, nanomaterials can be used to reduce bacterial infections without the use of antibiotics, while retaining improved bone cell functions; these properties are critical for combating the growth in medical-device-related infections, which is currently a global concern.

Gold[123,124] and silver NPs, carbon nanotubes, graphene-based nanomaterials,[84,125] and polypyrrole NPs[126] have been widely used in cell-laden hydrogel matrices for different purposes, including imparting conductivity, improving mechanical properties, or inducing cell alignment or osteogenesis on tissue-engineering constructs (Figure 5).[123,127,128] Baei *et al.* embedded gold NPs in chitosan hydrogels to obtain conductive gels (0.13 $S \cdot m^{-1}$) that supported the active metabolism, migration,

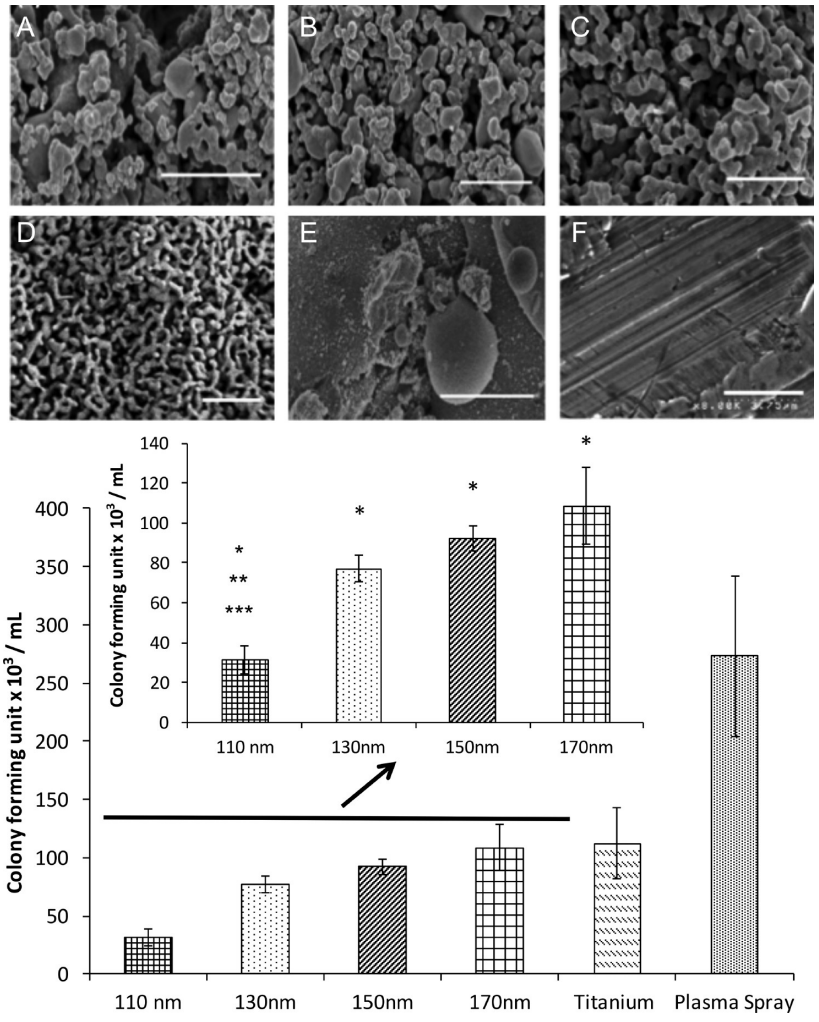

Figure 4. Nanoparticle deposition creates antibacterial surfaces. Electrophoretically deposited nano-HA using 170, 150, 130, and 110 nm HA nanocrystals (A−D, respectively) compared to plasma sprayed (E) and plain titanium (F). Samples A−D all had significantly fewer (* $p < 0.01$) colony-forming units adherent to the surface when compared to plasma sprayed samples, with sample D (with 110 nm nano-HA) having fewer than both plain titanium ($p < 0.01$) and 170 nm coated samples (***$p < 0.01$). Adapted with permission from ref 116. Copyright 2015, Royal Society of Chemistry.**

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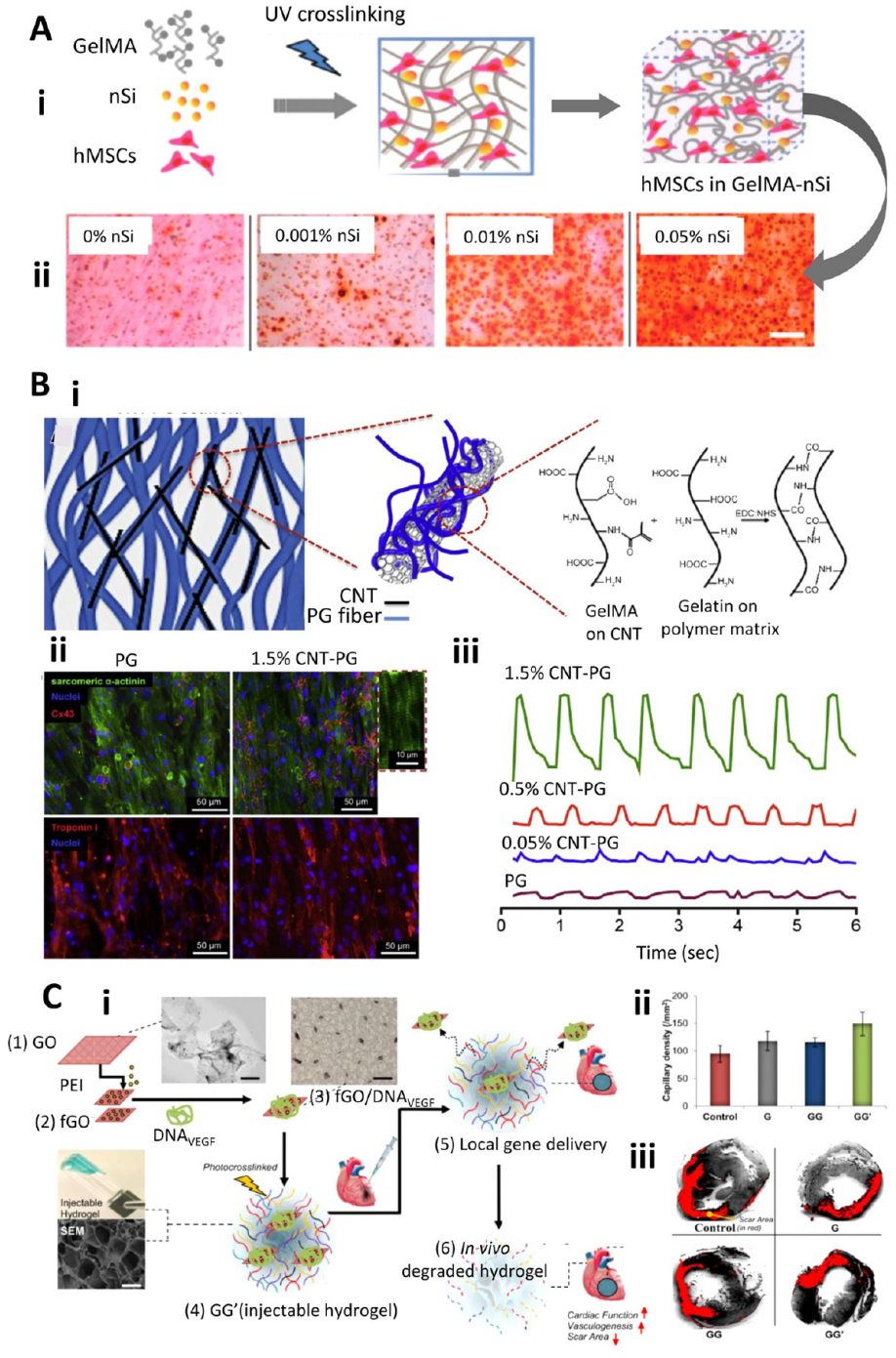

Figure 5. Nanotechnologies in tissue engineering applications. (A) Silicate NPs embedded in a GelMA hydrogel (nSi-GelMA) promote osteodifferentiation. (i) Scheme of the system; (ii) a system composed of nSi particles; (iii) alizarin red staining suggests a higher degree of differentiation in gels loaded with a higher percentage of nSi particles. Reprinted with permission from ref 127. Copyright 2016, Royal Society of Chemistry. (B) cNTs enhance synchronous beating of cardiomyocytes culture in PG fibrous scaffolds. (i) Schematic description of the cNT-scaffold system; (ii) comparison of cardiomyocyte alignment and proliferation in pristine PG fibers and cNT-PG fibers; images of cardiomyocyte stained for sarcomeric α-actinin (green), Cx43 (red), and DAPI (blue); and (iii) the patterns of spontaneous contraction of cardiomyocytes cultured on PG-CNT scaffolds at different CNT concentration as recorded at day 7. Reprinted with permission from ref 128. Copyright 2014, Elsevier. (C) Injectable gel loaded with NPs for cardiac transfection. (i) Formulation and subsequent injection of a nano-based bioactive hydrogel to treat acute myocardial infarction in a rat model. (1) GO nanosheets were decorated with branched PEI to form cationic fGO; (2) The fGO surface was functionalized with anionic plasmids (DNAVEGF). (3) The bioactive fGO/DNAVEGF hybrids were suspended in a prepolymer of GelMA and UV cross-linked to produce (4) an injectable fGO/DNAVEGF loaded GelMA hydrogel (GG0). (5) The hydrogel is injected intramyocardially into a rat heart with acute intramyocardial infarction for local gene delivery of fGO/DNAVEGF nanocomplexes. (6) The delivery of this nanobioactive hydrogel induces myocardial vasculogenesis that leads to improved cardiac function and reduced scar formation. This treatment (iii) promotes a higher capillary density in an infarcted rat model, and (iii) reduces scar area. Reprinted from ref 131. Copyright 2014, American Chemical Society.

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and proliferation of MSCs and enhanced the expression of cardiac markers over the effect observed with pristine chitosan gels.[123] Similarly, Shin *et al.* added reduced graphene oxide (rGO) NPs to improve the mechanical properties and conductivity of gelatin methacryloyl (GelMA) hydrogels.[125] Cardiomyocytes cultured in these GelMA–rGO scaffolds exhibited higher viabilities and proliferation rates, stronger contractibilities, and faster spontaneous beating rates than those cultured in pristine GelMA. Hydrogels loaded with conductive polypyrrole NPs, in the form of cardiac patches used to treat infarcted rats,[126] significantly reduced the size of the infarcted area (42.6%) in treated animals. The immune response of rGO can be controlled by functionalization.[129]

Another front in the application of nanotechnology for tissue engineering is the use of NPs as vehicles to deliver genes for tissue reprograming or repair.[130,131] Zhu *et al.* used hollow mesoporous organosilica NPs functionalized with branched polyethylenimine (PEI) to transfect bone marrow mesenchymal stem cells (BMMSCs) with a hepatocyte growth factor gene.[130] Transfected BMMSCs were then implanted into an infarcted rat model. Treated animals exhibited a reduced infarct scar size, greater angiogenesis, relief of interstitial fibrosis in the infarcted area, and a significant improvement in overall heart function. Paul *et al.* used GO nanoplatelets functionalized with PEI to deliver vascular endothelial growth factor (VEGF)-encoding genes into cardiomyocytes to improve cardiac function in an infarcted rat model (Figure 5C). An injectable GelMA hydrogel loaded with the transfective NPs was developed and injected into the hearts of infarcted rats.[131] Animals that received this treatment showed significantly higher capillary densities and significantly smaller scar sizes when compared to animals treated with gels loaded with VEGF-DNA (without GO nanoplatelets) or to untreated controls.

Nanoparticles have been frequently used as drug-delivery carriers from scaffolds in different tissue engineering applications. Shin *et al.* developed scaffolds based on a gelatin-derived hydrogel (gelatin methacryloyl or GelMA) containing NPs loaded with kartogenin, a small molecule known to induce the differentiation of MSCs into chondrocytes.[125] When implanted in a murine model, endogenous MSCs were actively recruited from the scaffold, and the regenerated tissue exhibited similarities to hyaline cartilage in terms of histology, presence of cartilage biomarkers, and mechanical performance. Qiu *et al.* used amine functionalized silica NPs to deliver dexamethasone (DEX) from nanofibrous scaffolds of poly(L-lactic acid)/poly(ε-caprolactone).[132] The DEX-loaded NPs were inserted into the scaffold by EPD. These scaffolds promoted osteogenesis in both *in vitro* and *in vivo* experiments.

Novel Nonbiofouling Surfaces for Implants and Surgical Devices. The integration of micro- and nanotechnologies is also changing the design approaches for fabricating new materials and surfaces for medical applications. Physical and chemical cues, as well as the micro- and nanoscale architectures, can greatly influence cell behavior, including cell adhesion and alignment, proliferation, and even metabolic activity.

The development and use of antifouling surfaces with antiadhesive properties are an example of the integration of micro- and nanotechnologies to address a medical challenge. In addition to performing sophisticated diagnostic and therapeutic tasks, biosensors, surgical devices, implants, drug-delivery systems, and tissue engineering equipment must also have surfaces that can be kept free of bacteria, blood, and other sticky substances. Fouling by these materials clogs tubes, distorts readings, obscures camera screens, and is widely responsible for infections, but efforts using various chemical and structural coatings have failed to provide sustainable solutions to end fouling. Nevertheless, living systems appear to handle potential fouling issues by having surfaces as diverse as cilia, spiny skin, tear films, and mucus. The only common denominator seems to be that the surfaces are alive—continuously changing, moving, and renewing.

> Surface design has increasingly drawn inspiration from the varied surfaces found in living systems, with the aim of building a variety of self-powered, dynamic behaviors into materials.

Nano- and microstructure arrays, composed of flexible polymeric and/or high-aspect-ratio features that are set in motion by responsive hydrogels or direct stimuli, have been designed to generate changing patterns, waves, and oscillations[133−135] that are capable of blocking settlement or extruding particles on demand. More recently, the continuous molecular turnover and defect-free smoothness of liquid surfaces has emerged as a highly promising way to resist biofouling over all scales, from proteins to cells to biofilms.[136,137] The fluidic interface is created by infusing a nano/microstructured surface or porous polymer with a chemically compatible liquid. The resulting material shows an effective antibiofouling performance across a range of biomedical environments, microfluidic tubes, and other microscale devices.

These strategies for utilization of liquid surfaces and responsive, self-driven microscale dynamics are now coming together to enhance effectiveness and longevity of biomaterials as well as to integrate fouling resistance with numerous other functions. Microvascular networks engineered into liquid-infused materials enable spontaneous liquid flow that continuously replenishes the interface and actively releases any bacteria that do settle.[138] Feedback between liquid-infused dynamic polymers and embedded liquid nanodroplets actively restores the repellent liquid surface even after wholesale removal, while simultaneously enabling a range of finely self-modulated secretion functions.[139] Elastic nanostructured materials infused with liquid create a new kind of adaptive surface that can be reconfigured between a flat liquid interface and a gradation of stretching-induced liquid-coated nanotopographies, enabling systems to function as structured surfaces in the stretched state and easily release fouling upon relaxation.[140] Liquid-filled nanopores have further yielded a novel pore-gating mechanism that reversibly switches between a flat repellent interface and a pressure-induced, fouling-resistant, liquid-lined, open microchannel, combining antifouling behavior with controlled separations and selective transport of multiphase substances for analysis, delivery, or 3D printing of biomaterials.[141] There is growing insight into the principles and mechanisms acting at the nanoscale in these diverse surfaces and greater understanding of their interactions with biological organisms and substances. This new knowledge is generating research directions that offer unprecedented hope that emerging biomedical technologies, fueled by the advances in nanoscience, can be seamlessly fused with designs that minimize the fouling-induced impairments and infections common in present-day medical devices.[142,143]

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Micro- and Nanotechnologies for Drug Development. The development of new pharmaceutical compounds—from the filing of the first patent of the active ingredient to the ultimate commercialization—is a long and costly process. Thus, any technology capable of reducing the cost and time invested in pharma development is highly attractive to the pharmaceutical industry and their stakeholders.[144−146] Along this long path of pharma development, microtechnologies have found diverse application niches, including the screening of process conditions for pharma[147] and biopharma manufacturing,[148] development of new cell lines,[149] and drug testing. In this regard, organ-on-chip systems promise to be cost-effective screening/testing platforms for drug development that can eventually minimize the need for (or extent of) preclinical trials using animal models. The translatable pharmacological data (*i.e.*, effectiveness and cytotoxicity) derived from the use of human cells in organ-on-chip platforms might be more relevant than that obtained from animal models and might result in preclinical trials with a higher predictive value.[145] Organ-on-chip platforms can also be to study fundamental physiological behaviors, specific pathologies, the effects of pollutants or pathogens,[150] or the effect of nanomedicines.[146,151] Microfluidic systems serve as powerful tools to accelerate the development[152] and clinical translation of nanomedicines.[153]

The operation of microfluidic platforms for biomedical research demands a high degree of automation and online monitoring, which creates a need for highly precise online biosensing. Here, nanotechnologies are starting to play an important role by enabling the real-time monitoring[154] of biological processes occurring within a microfluidic bioreactor or a tissue engineering construct.[147,155−157] One recent example is the development of a biosensing system based on platinum nanopetal[158] sensors that are capable of sensing potassium ions in real time and determinine the extent of cell death in a microreactor.[147] Another is the coupling of a NP-based detection system and laser-induced fluorescence into a microfluidic immunosensing device for cancer biomarker determination.[156]

Integrating vasculature into organ-on-chip systems is another major challenge in this field. The critical importance of vascularization to the function of most tissues has long been recognized, yet developing a perfusable microvascular network within an on-chip tissue model has proved challenging. Most organs in multiorgan-on-chip systems are connected by microfluidic channels or tubing. The development of vascularization to interconnect these organs is an aspiration that appears to be in reach. To that end, different strategies have been explored, including the fabrication of networks embedded in hydrogel blocks that can later be lined with vascular cells[158] and the development of microvasculature generated by cells seeded at the border of a microgel[159,160] or loaded uniformly within the hydrogel.[161−163] The vasculogenesis approach has proven to be highly effective at developing perfusable vascular-like networks within hydrogels in microfluidic systems. Microvasculature can be developed from endothelial cells, either using the axenic cell line or in coculture. The use of fibrin-based extracellular matrix and a coculture of endothelial cells with normal lung fibroblasts seeded in separate chambers have shown the best results (Figure 6A).[161] Recent work has translated this concept to millimeter-scale using cocultures of fibroblasts and endothelial cells, resulting in microvascularized perfusable constructs with potential use for organ-on-chip applications (see also Wang *et al.*, Figure 6B).[164] These new results open up the opportunity to produce a new generation of tissue models for drug screening that incorporate the organ or tissue specific cells[165] as well as the perfusion necessary for long-term maintenance of these cells. Moreover, signaling between vascular cells and other tissue-specific cells help to produce a more realistic microenvironment for the tissue model.

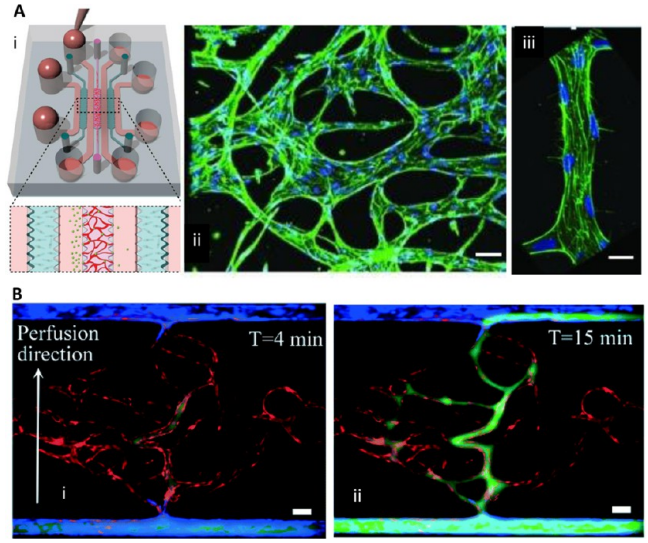

Figure 6. Microfluidic systems to develop microvasculature. (A) Development of microvasculature on a chip. (i) A microfluidic system having three gel regions, two for fibroblasts (green) and one for endothelial cells (red). Central gel region is 1 mm wide from the outside of the posts. Reprinted with permission from ref 167. Copyright 2017, Naure Publishing Group. (ii) Confocal image of a microvascular network (green) that is perfusable from one medium channel to the other across the red gel region in (a). Scale bar = 10 μm. (iii) High-magnification confocal image of a single vessel in the network of (ii), showing the endothelial cells (green) and nuclei (blue). Scale bar = 10 μm. Reprinted with permission from ref 163. Copyright 2014, Mary Ann Liebert, Inc. publishers. (B) Perfusion and permeability testing of a vascularization bridge built between two chambers. Dextran perfusion in the absence of nonphysiological leakage at (i) 4 and (ii) 15 min of continuous perfusion with a fluorescent (green) dextran solution. Reprinted with permission from ref 164. Copyright 2016, Royal Society of Chemistry.

Another important issue is the transport of drugs across the endothelial barrier. In many tissues, especially the brain, the endothelium presents a major impediment to drug delivery, and various delivery methods have been proposed to enhance transport across the blood−brain barrier. Significant advances have been made in the development of vascularized networks in microfluidic chambers (see Figure 6B). Wang *et al.* described a system consisting of two connected microfluidic chambers with a vascularization network developed *in situ* and capable of continuous perfusion from one chamber to the other.[164] Transmigration of immune cells or tumor cells can also be modeled using these new systems.[166,167]

MICRO- AND NANOTECHNOLOGY TRANSLATIONAL CONSIDERATIONS AND CHALLENGES

Translational research—when compounds with new drug targets or formulations are brought to humans for testing—encompasses a broad range of complexities, including scientific, financial, regulatory, and ethical issues. Perhaps the most significant issue concerns the participation of human subjects and their safety, which mandates a range of rules covering processes from recruitment to monitoring and reporting.

Translation to the first clinical trial (Phase 1) and following to the later stages (Phases 2 and 3) presents a unique risk and cost

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Table 1. Global Nanomedicine Market by Therapeutic Area through 2019 Estimates (US billions)[170]

therapeutic area	2012	2013	2014	2019	compound annual growth rate (%) 2014–2019
anticancer	59.1	65.8	75.4	140.1	13.2
central nervous system	36.7	43.9	50.3	103.0	15.4
anti-infectives	25.6	29.3	33.5	68.0	15.2
anti-inflammatories	20.8	24.7	28.3	57.7	15.3
cardiovascular	12.0	13.7	15.7	28.8	12.9
others	5.5	5.5	6.2	14.5	18.5
total nanopharmaceuticals	159.7	182.9	209.4	412.1	14.5
all nanodiagnostics	25.3	31.3	38.9	115.9	24.4
total nanomedicine market	185.0	214.2	248.3	528.0	16.3

profile that requires substantial due diligence for initiation and conduct of these steps. A recent comprehensive analysis of data for new drug development shows that the chance that a new compound entering Phase 1 will succeed in reaching regulatory approval is <12% in the United States.[168] This high risk, along with the substantial cost of drug development (estimated to be around $2.6 billion),[169] has created a unique opportunity for nanotechnology-enabled drugs to make a significant impact in enhancing existing therapeutics as well as developing novel classes for unprecedented targets/drugs. This prospect is quite evident with the rapid growth of the nanomedicine market, as shown in Table 1.[170]

Early stage and *in vitro* development of nanobased research has been significant, but assuring the safety and efficacy of these solutions requires a transition into clinical testing as the next step. Notably, critical obstacles that occur in translational research[171] are generally applicable to nanomedicine. However, due to the nature of the underlying technology and lack of regulatory clarity, concerns about issues such as funding, manufacturing, and safety are more pronounced in nanorelated products. Next, we briefly comment on several relevant technical and nontechnical aspects related to the successful translation of nanotechnologies: funding, manufacturing aspects, and regulatory matters.

Funding. Substantial resources are available for funding and support of various nanobased early research and discovery efforts. However, transition from preclinical to clinical research often requires a sizable investment that is not commonly supported by conventional government and nonprofit organizations. This situation creates a substantial gap in funding needed for the translation of early stage, discovery research into late-stage clinical trials (often referred to as the "valley of death"). Many federal funding sources (such as the National Institutes of Health, NIH) have recognized this challenge and have shifted a portion of their extramural funding toward support of translational efforts. Additionally, as activities within private investment pick up, with additional flow of new capital, some venture capital groups (as well as their corporate counterparts) have increased their investment in early stage nanomedicine opportunities, which is a welcome sign within the field.

Recent increases in public investment and the success of biotech and nanobased initial public offerings have expanded the range of options for financing translational research for nanotechnology-enabled therapeutics and diagnostics.

Manufacturing Challenges. A number of promising nanobased platforms can play significant roles in the development of small molecules and biologics. These platforms, commonly developed in academic settings, are capable of producing batch sizes in quantities sufficient for small investigational *in vitro* or animal studies. However, moving into clinical trials critically requires production based on good manufacturing practices, which, in turn, requires substantial initial investment and validation. Additionally, as a compound moves to later stages of development and postapproval introduction to the market, its scale-up at a commercially competitive cost becomes a major challenge to be addressed.

Scaling up production from the lab to clinical testing is a complex process that requires optimization steps of multiple parameters. At times, significant changes in unit operations are required. For example, scaling up a production system of a nanomedicine candidate might require changing mixing strategies from stirring in vessels to agitating using a static mixer in the lines. These variations in nanomedicines and formulations through scale up of the process production could change the original formulation and alter the effectiveness of the drug.

Researchers should adopt several important steps during the small-scale formulation preparation to facilitate scaling up. These steps include monitoring the entire nanomedicine preparation process (*e.g.*, pH, temperature, and ionic strength, with detectors inside the preparation vessels), collecting and analyzing relevant data, defining critical key parameters during process development, keeping consistency in the nanomedicine constituents, and selecting scalable equipment for the production of the nanomedicine. Microfluidic mixers are an emerging technology for high-quality synthesis of drug-loaded NPs, since they enable a rapid screening of different formulations with a high encapsulation rate.[91,92]

Regulatory Issues. The possibility that nanobased products may differ substantially from other drug development technologies has been an issue of debate for quite some time. The general position of regulatory agencies has been that existing regulations and tools are sufficient to address the safety and efficacy of nanobased products. However, due to perceived public safety concerns, a series of activities have been initiated for the development and introduction of regulatory guidance in the United States and European Union.[172–174]

The absence of clear regulatory guidance and associated processes has led to a broad range of diligence in safety and risk assessment, which could lead to significant overrun of cost and inconsistency in product development. The current regulatory processes can sufficiently address clinical trials for nanobased products; however, an efficient and harmonized clarification (and perhaps updated guidance) is needed to support preclinical and clinical development for nanobased products in order to

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expedite safe and efficacious therapeutics that will meet existing and future medical needs. Of note is that several researchers and start-up companies have received quick (less than 6 months) regulatory approval for their nano structured medical devices (mostly in the orthopedic space), when implementing nanoscale surface features and not introducing new chemistries to predicate devices to improve tissue growth, inhibit infection, and decrease inflammation.[116] Such approaches have provided for the immediate commercialization of nanostructured medical devices.

Despite all these translational challenges, the pace of nanomedicine research and product development has picked up significantly across all therapeutic areas. This acceleration is especially the case for cancer,[175] where the number of clinical trials has increased 10-fold in the past 10 years.[176] Additionally, several nanobased platforms for reformulation and development of new molecular entities are leading the efforts for introducing improvements in existing drugs as well as introducing novel therapeutics to address unmet medical needs.

CONCLUSIONS AND PROSPECTS

The currently available micro- and nanotechnologies are reshaping the way we prevent, diagnose, and treat disease. Nevertheless, the highest potential for breakthrough solutions to our current medical challenges involves the synergistic integration of micro- and nanotechnologies.

The highest potential for breakthrough solutions to our current medical challenges involves the synergistic integration of micro- and nanotechnologies.

Microfluidics is reaching maturity as a well-established technology, and it will continue to be an enabling platform for new medical applications. Many other microtechnologies have reached the market, facilitating diagnosis and continuous monitoring of vital signs and metabolites, such as glucose or even viral levels. Over-the-counter pregnancy and paternity assays are now routinely found in convenience stores. Conversely, the need for development of POC applications for cost-effective diagnosis of infectious diseases (*e.g.*, Ebola, influenza, and malaria) continues to grow, particularly in underdeveloped and remote regions. Conventional technologies are frequently ineffective at addressing the prevalent global health threats, so the need for portable and cost-effective POC diagnostic (and eventually therapeutic) technologies is another important factor driving the use of micro- and nanotechnologies. The integration of simple but ingenious ideas can take us far into the design of POC diagnostic devices. Micro- and nanocomponents are already available for use: nanosensors, antibodies, antibody fragments and aptomers, NPs (rods, posts, and discs), quantum dots, *etc.* One concern is the relatively high cost of full-length antibodies, the most expensive reagent in POC immunoassays. The use of antibody fragments[17] or aptamers[177,178] has been proposed as a strategy to lower the cost of POC applications. The increasing availability of highly sensitive fast-response sensors and biosensors is further contributing to the development of highly effective diagnostic devices and online monitoring applications.

Many more microtechnologies, including those based on continuous flow applications,[148,179] will reach research and development laboratories in the next few years. Microelectroporators and other lab-on-chip applications are rapidly being adopted in laboratories around the world. Biotech start-ups and pharmaceutical and biopharmaceutical companies are expected to be important end-users of lab-on-chip applications. Organ-on-chip platforms, now exclusively experimental, will see future adoption in the pharmaceutical industry for early drug development and drug screening applications. In the meantime, more examples of multiorgan-on-chip integration will become available in the scientific literature.

Nanotechnology is rapidly evolving from promise to reality for many diagnostic and treatment scenarios. The need for the delivery of pharmaceutical molecules to local target cells drives the search for smaller, yet stable, drug release vehicles. As the scales become increasingly miniaturized, the surface-to-volume ratios increase. Nanoparticle-based therapeutic applications promise higher specificity, selectivity, and efficacy (at lower doses) than are achievable with regular therapies. For the clinician and the patient, this advance translates into higher efficacy at lower doses, with fewer secondary effects. For instance, NPs appear to be an excellent fit for the release of many drugs and nucleic acids;[180,181] higher penetration into tissues and cells can be achieved with NPs.

Nanotechnology is rapidly evolving from promise to reality for many diagnostic and treatment scenarios.

Nevertheless, many issues remain to be resolved before nanoproducts can see extensive clinical use. Safety concerns and a strict regulatory framework are among the important hurdles to be faced before nanomedicines reach the patient. Other aspects, such as the availability of funding for the translation of nanotechnologies into clinical applications or the requirement of more scalable manufacturing platforms for NPs, need to be addressed before the full potential of nanomedicine will be reached.

Arguably, cancer nanomedicine constitutes the most advanced therapeutic front of application of nanotechnology in medicine. However, NPs have proven to have other relevant and important applications, including exciting opportunities as gene- and drug-delivery systems for cardiac tissue repair.[182−184] The design of antibacterial surfaces based on the use of NPs has opened a new avenue for combating infection without the need for antibiotics—an increasingly limited resource due to the remarkable ability of bacteria to develop resistance. Nanofabrication using nucleic acids[185−187] or selective and precise "cutting and/or pasting" of the genetic code using clustered regularly interspaced short palindromic repeat technologies are expected to open the door to unprecedented diagnostic and therapeutic nanotechnology applications.[188] Nanoscale strategies have also used membrane curvature to their advantage in attacking persistent bacterial cells.[189]

Our ability to turn to nature for inspiration, or bioinspiration, has provided us with additional strategies for solving medical problems through the integration of elements from the micro- and nanoscale. Nature has evolved a remarkable spectrum of examples where micro- and nanoprocesses enable remarkable biological events to occur at a relatively low energy cost. Nature inspires us to design and to build materials with combined micro- and nanostructures that confer novel physical, and even chemical, properties to surfaces and devices (*i.e.*, anti-

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bacterial activity, hydrophilic character, or superhydrophobic behavior). Nature can teach us how to achieve underwater adhesion in dynamic settings, how to move fluids through complex channels, and how to achieve long circulation times in the bloodstream.

Biomimicry consists of copying nature, usually in every detail; however, solving problems by simply mimicking nature is generally futile. Bioinspiration, by contrast, can be used to overcome seemingly insurmountable challenges. A basic idea in nature can be tailored or even improved upon for one's own purposes. Consider that every living thing around us has overcome insurmountable challenges through evolution, presenting us with an unlimited supply of ideas and solutions to complex problems. To get to a practical solution, one needs to impose design criteria that consider the full translational spectrum, including large-scale manufacturing. We must understand the problem and go beyond just looking at nature; it requires a highly iterative process and multidisciplinary team.

The integration of micro- and nanotechnologies will continue to revolutionize the diagnosis and treatment of infectious and chronic diseases and will provide us with novel platforms to study diseases at the tissue, cellular, and even molecular levels.

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ABBREVIATIONS

NPs, nanoparticles; POC, point of care; CTC, circulating tumor cells; HIV, human immunodeficiency virus; EpCAM, epithelial cell adhesion molecule; HbCTC-Chip, herringbone CTC-Chip; CTC-iChip, inertial focusing CTC-Chip; RT-LAMP, reverse transcription loop-mediated isothermal amplification; ELISA, enzyme-linked immunosorbent assay; 3D, three-dimensional; ExM, expansion microscopy; CoPhMoRe, corona phase molecular recognition; SWCNT, single-walled carbon nanotubes; RITC-PEG-RITC, rhodamine isothiocyanate-difunctionalized poly(ethylene glycol); BA-PhO-Dex, boronic acid substituted phenoxy-dextran; NO, nitric oxide; DCs, dendritic cells; NC, nanocarrier; FDA, Food and Drug Administration; MoA, mode of action; EPR, enhanced permeability and retention; CSC, cancer stem cells; PET/CT, positron emission tomography-computed tomography; MRI, magnetic resonance imaging; siRNA, small interfering RNA; LbL, layer-by-layer; HA, hydroxyapatite; MSCs, mesenchymal stem cells; rGO, reduced graphene oxide; GELMA, gelatin methacryloyl; PEI, polyethylenimine; BMMSCs, bone marrow mesenchymal stem cells; VEGF, vascular endothelial growth factor; DEX, dexamethasone; EPD, electrophoretic deposition

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