Chapter 1
Engineering in Translational Medicine: An Introduction

Weibo Cai

1.1 Man Text

Translational medicine has attracted tremendous attention over the last decade. Personalized medicine is the future for twenty-first century healthcare, where translational research is the indispensable bridge between basic science discoveries and clinical patient management. In the broadest sense, translational research is a continuum that spans the majority of preclinical and clinical research. In a more strict sense, translational research is referring to research that can be immediately translated into clinical investigation, as well as those that can be translated into the clinic within a decade. In many cases, the biggest hurdle for clinical translation is not the lack of adequate technology development for clinical applications; instead it is the regulatory requirement that causes a "valley of death" for translational research. Given the recent crisis in global economy and significant budget cut in virtually all agencies that fund research over the last decade, which may not change dramatically in the near future, translational research is facing unprecedented challenges since it is quite costly to translate new discoveries (e.g., agents and devices) into clinical investigation [1, 2].

Nonetheless, scientists and researchers remain highly enthusiastic and devoted to translational research. To provide a forum to disseminate research findings in cutting-edge translational research, many major publishers have launched peer-reviewed journals that are focused on translational research/medicine. The words "research" and "medicine" are often used interchangeably, and there is no clear-cut boundary regarding where "research" stops and "medicine" begins, although the latter is certainly more clinically oriented than the former. Some representative

W. Cai (✉)
Departments of Radiology and Medical Physics, University of Wisconsin, Madison, WI, USA

University of Wisconsin Carbone Cancer Center, Madison, WI, USA
e-mail: wcai@uwhealth.org

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journals that are focused on this topic include *Science Translational Medicine* (Science), *American Journal of Translational Research* (e-Century Publishing Corporation), *Translational Research* (Elsevier Science Inc.), and *Journal of Translational Medicine* (Biomed Central Ltd.).

The field of engineering has witnessed spectacular advancement over the last several decades, which has affected the everyday life of billions of people worldwide in all areas ranging from energy to medicine. However, currently, a comprehensive reference book that focuses on "Engineering" and "Translational Medicine" does not exist. To fill this gap and meet the urgent need, I have worked with an international ensemble of leading experts in the field to organize this book entitled "Engineering in Translational Medicine", which covers most major topics of the field in the 34 subsequent chapters of this book. I am deeply grateful to the total of $\sim$80 contributors of this book for their tremendous effort in writing these exceptional chapters on a diverse array of topics that are related to "Engineering in Translational Medicine", which we all firmly believe will be an invaluable resource for scientists/students/clinicians both new to this topic and currently working in this area for many years to come.

Encompassing a broad spectrum of state-of-the-art engineering research in translational medicine, the book is categorized into 5 parts: "Cell and Tissue Engineering" which includes 6 chapters, "Genetic and Protein Engineering" which has 10 chapters, "Nanoengineering" which also contains 10 chapters, "Biomedical Instrumentation" which is composed of 4 chapters, and "Theranostics and Other Novel Approaches" with 4 chapters (Fig. 1.1).

Part I of this book, which includes Chaps. 2–7, is focused on cell and tissue engineering. Regenerative medicine holds tremendous potential in the treatment of a wide variety of human diseases; for many of those, it may be the only solution [3]. In Chap. 2, Dr. Ray and co-workers discussed about these "therapeutic wonders" (i.e., stem cells) and reviewed the ongoing investigations on stem cell biology, as well as their clinical applicability which can bring revolutions to future clinical care. Engineering of stem cells, non-invasive imaging of stem cells, as well as clinical applications of stems cells in a number of human diseases were all covered in this chapter. In Chap. 3, Dr. Cooper and co-workers focused on the treatment of cancer with engineered T cells. Since tumor microenvironment is often immune suppressive, which protects cancer cells from recognition and elimination by effector cells, engineered T cells can be used for effective cancer cell killing. This chapter provided a comprehensive summary of adoptive cell therapy with genetically modified T cells to redirect specificity to tumor cell antigens using a number of approaches, to improve T-cell effector function, as well as to control these T cells with suicide genes to ensure safety.

Much recent research effort has been devoted to the development of various scaffold systems that can carry cells, using a broad array of biomaterials. In Chap. 4, Dr. Wang and his co-worker gave a thorough review on engineering biomaterials for the delivery of anchorage-dependent and non-anchorage-dependent therapeutic cells. Some cells (e.g., muscle cells and neurons) are dependent on anchorage to the extracellular matrix. Therefore, they require extensive cell adhesion to a substrate

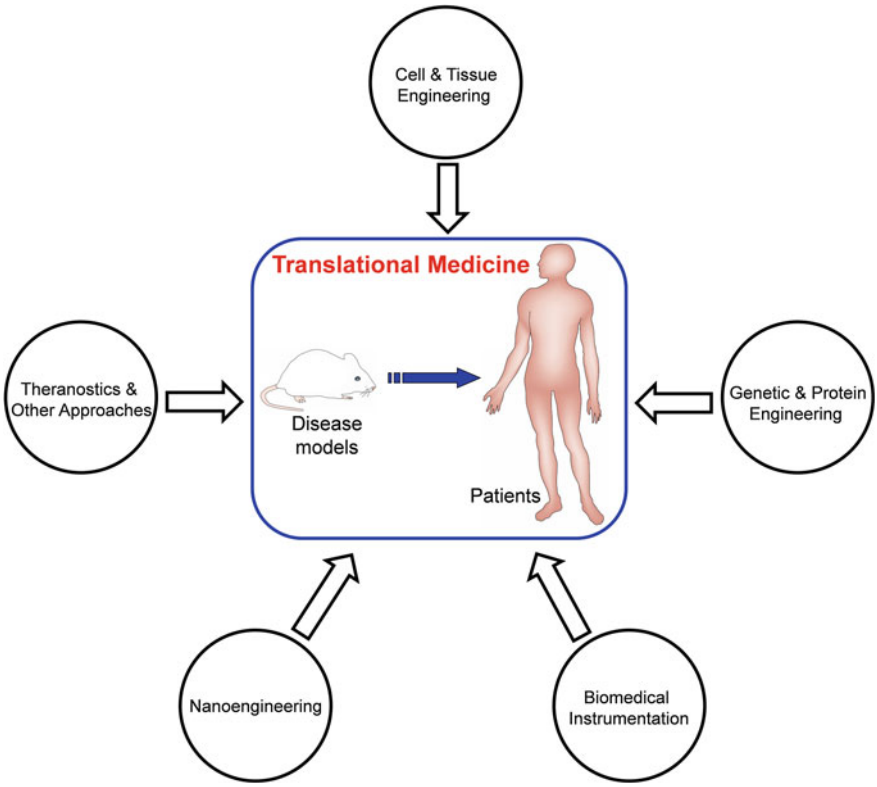

Fig. 1.1 An outline of this book, which is organized into 5 parts

to survive and elicit biological function upon transplantation. On the other hand, non-anchorage-dependent cells (e.g., chondrocytes and hepatocytes) do not have such requirement, which often exhibit a rounded morphology in their native environment. Clearly, different cell delivery structures are needed for these cells to achieve maximal therapeutic effect, which were illustrated in detail in this chapter.

The next three chapters in Part I of this book are focused on tissue repair. Peripheral nerve injuries can lead to variable levels of functional loss, depending on the extent of injury. In Chap. 5, Dr. Orbay and I had a detailed survey into the components of tissue-engineered nerve grafts, as well as reviewed the relevant clinical studies. A tissue-engineered nerve is typically composed of a biodegradable scaffold, a neurogenic cell line, and growth factors. Therefore, how to improve the cell–scaffold and scaffold–tissue interactions is a key challenge for peripheral nerve repair. Perhaps even more important than nerve repair, the establishment of blood vessel networks is a matter of life and death for tissues and organisms. In Chap. 6, Dr. Murphy and co-workers discussed about the structure of blood vessels and key signaling molecules, which play significant role in vasculogenesis, angiogenesis, and maturation of nascent blood vessels. A description of

promising approaches specific to tissue-engineered blood vessels, various tissue engineering approaches to identify the appropriate sources of endothelial cells, a brief introduction to some clinical results, as well as the regulatory challenges were all included in this chapter. Cartilage tissue is prone to significant dysfunction after damage, which also lacks the innate ability to effectively repair itself. Chapter 7, written by Dr. Zhang and his co-worker, was focused on engineering gene-activated matrices for the repair of articular cartilage defect. Although growth factors were shown to promote cartilage repair, the structure of cartilage tissue can hinder direct addition of growth factors into the traditional system, due to the difficulty in controlling the amount of growth factors added and the short biological half-lives. Therefore, gene-activated matrix systems have been developed for the repair of articular cartilage damage, which can secret large quantity of growth factors continuously and serve as a promising therapeutic approach for repairing cartilage damage.

The ten following chapters in Part II of this book cover a broad array of areas related to genetic and protein engineering. Many luciferases have been isolated and investigated for broad biomedical applications, ranging from cell-based studies to in vivo applications in small animal models [4, 5]. In Chap. 8, Dr. Walls and Dr. Loening provided an excellent and comprehensive review of engineered luciferases. They discussed in detail about how protein engineering can be used to improve their stability, light output, emission wavelength, chemical sensitivity, and substrate specificity among others for better in vitro/in vivo performances and broader biomedical applications. After significant improvement and optimization of the naturally occurring luciferases (e.g., firefly luciferase and Renilla luciferase, as discussed in Chap. 8) and other enzymes (e.g., herpes simplex virus type-1 thymidine kinase), they can be employed for a wide variety of biomedical applications, two of which are detailed in the two following chapters.

In Chap. 9, Dr. Massoud and Dr. Paulmurugan focused on the use of engineered split reporter systems for in vivo molecular imaging of protein–protein interactions, which can pave the way for functional proteomics in living animals and provide a tool for whole-body evaluation of new pharmaceuticals that can modulate protein–protein interactions. The main strategies currently available for imaging protein–protein interactions in living subjects using molecularly engineered and rationally designed split reporter gene, as well as the broad (potential) uses of these strategies, were illustrated in exquisite detail. A few of the major strategies adopted for split reporter gene-based imaging include intein-mediated reconstitution and protein fragment complementation (which can be achieved via a variety of different mechanisms), which can enable the interrogation of many protein–protein interactions through in vitro assays and/or non-invasive imaging. Besides split reporter gene techniques, bioluminescence resonance energy transfer (BRET)-based sensors are rapidly expanding, which can also be adopted for investigation of protein–protein interactions, protein dimerization, signal transduction, etc. [6, 7]. In Chap. 10, Dr. De and co-workers presented an in-depth overview on the engineering requirements of BRET components such as donor, acceptor, substrate chemistry, and instrumentation. Both genetically engineered

and synthetic BRET systems were described, which have witnessed significant advancement over the last decade and can be used in a variety of systems including both in vitro assays and in vivo applications.

Besides luciferases and other enzymes, a variety of other engineered proteins can be used for biomedical applications, which are the topics of the subsequent 4 chapters. Antibodies are a major constituent of the human immune system and have become an indispensable class of therapeutics in cancer and many other diseases [8, 9]. In Chap. 11, Dr. Fischer summarized the topic of antibody engineering in translational medicine, which offered an overview of current strategies to tailor antibodies for biomedical applications. After a brief introduction, this chapter covers many related topics which include approaches to reduce immunogenicity of therapeutic antibodies, optimize pharmacokinetics, and enhance therapeutic efficacy (e.g., with Fc engineering to improve effector function, generation of antibody–drug conjugates, and radioimmunoconjugates). In addition, engineered antibody fragments and bispecific antibodies were also discussed, which can be advantageous than intact antibodies for many applications due to the much shorter circulation half-life and other desirable properties (e.g., the capability to bind to two different antigens simultaneously). Together with the conventional antibodies, many of which have been approved for clinical use over the last two decades, these novel engineered antibody-based imaging agents and therapeutics will have a major impact on future diagnosis and treatment of many diseases, including but not limited to cancer.

Affibodies are an emerging class of small (7 kDa) protein scaffold-based affinity ligands, which have attracted much attention recently and are the focus of Chap. 12, written by Dr. Cheng and co-workers. Because of the small size (58 amino acid residues), affibodies can be readily produced via both peptide synthesis and recombinant expression in Escherichia coli. General structures and engineering strategies used to optimize affibody molecules for imaging and therapy applications are described in this chapter. As evidenced by the constantly emerging literature reports on this class of exciting proteins, affibodies have been extensively investigated for imaging applications with different techniques (e.g., upon labeling with various radionuclides for positron emission tomography or single-photon emission computed tomography, fluorescent dyes or quantum dots for optical imaging, and magnetic nanoparticles for magnetic resonance imaging), and most of the current research effort is focused on cancer research. In addition, affibodies have been explored for therapeutic applications as well (e.g., upon labeling with ^{177}Lu or ^{90}Y, or used as targeting ligands to redirect cytotoxic T lymphocytes and natural killer cells). With recent clinical studies indicating safety and efficacy of affibody-based imaging agents, affibodies hold promising potential in future clinical patient management.

In Chap. 13, Dr. Hackel provided a comprehensive summary of various protein scaffolds that can be used for molecular imaging and therapy applications. Since effective targeting of imaging/therapeutic agents requires specific binding with high-affinity, facile and robust conjugation of effectors (e.g., drugs, genes, radioisotopes, and therapeutic proteins) without compromising the binding/biological

activity, and efficient in vivo delivery, protein scaffolds can serve as promising platforms for these applications. Aside from affibodies that were covered in Chap. 12, other validated scaffolds include the fibronectin domain, knottin, designed ankyrin repeat protein, anticalin, etc. With clinical trials in therapy or imaging already ongoing with many of these scaffolds, the future is bright for newly developed agents that are based on these protein scaffolds.

In Chap. 14, Dr. Cochran and Miss Liu focused on the topic of engineering multivalent and multispecific protein therapeutics. Since many proteins utilize the principles of multivalency and multispecificity to ensure optimal biological function in nature [10], their mechanisms of action have inspired the development of next-generation protein therapeutics with improved efficacy and safety profiles. The main advantages conferred by multivalency and multispecificity include increased target-binding affinity through avidity effects and selectivity for a diseased versus normal state, which can lead to better therapeutic control over an intended biological response and in the meantime offer reduced side effects. Both the basic biophysical principles underlying multivalency/multispecificity and how they influence protein design parameters were illustrated in detail in this chapter, which also included several examples regarding how these principles can be utilized to develop next-generation protein therapeutics.

After seven chapters covering many aspects of protein engineering, as described above, the next three chapters focus on DNA/RNA. Aptamers are single-stranded DNA or RNA oligonucleotides that can be selected for specific binding to a wide range of targets, primarily through the systematic evolution of ligands by exponential enrichment (SELEX) technology [11]. Chapter 15 is the first of two chapters focusing on engineering aptamers for biomedical applications, written by Dr. Li and Dr. Cao. Because of their many desirable properties such as small size, ease of synthesis, and versatile chemistry, aptamers have attracted considerable attention in many disciplines of biomedicine. After a brief introduction and description of the different strategies for selecting optimal aptamers for biomedical applications, this chapter provided a detailed overview of engineering aptamers for use in biosensors. A number of approaches were covered, which include engineering aptamers to improve the bioavailability, to generate detectable signals (e.g., through incorporation of functional nucleic acids or molecular reporters) and to achieve signal amplification for high-sensitivity/selectivity biosensing. In Chap. 16, Dr. Cerchia and co-workers provided a comprehensive summary on the development of multifunctional aptamer-based bioconjugates for targeted delivery of therapeutics and imaging agents to diseased cells and tissues. Many approaches have been utilized to conjugate aptamers with a broad array of agents, which include siRNA/miRNA, drugs, synthetic polymers, nanoparticles (e.g., synthetic nanoparticles, liposomes, gold nanoparticles, quantum dots, magnetic nanoparticles, and silica-coated nanoparticles), and radioisotopes (e.g., ^{99m}Tc for single-photon emission computed tomography imaging). Lastly, various strategies used to enhance the resistance of aptamers to nuclease degradation were also described in this chapter.

One intriguing approach for the treatment of cancer is to use vaccines. Dozens of DNA vaccines have entered clinical trials for a variety of malignancies, with demonstrated efficacy in eliciting immune responses and potential clinical responses [12, 13]. Recently, a DNA vaccine was approved for the treatment of canine melanoma, which represents a landmark achievement in this area [14]. In Chap. 17, Dr. McNeel and Dr. Olson presented an excellent overview of engineering DNA vaccines for cancer therapy. Generally speaking, a DNA vaccine for cancer is a bacterial DNA plasmid that encodes the cDNA of a tumor antigen, which can elicit humoral and/or cellular immunity against tumor cells expressing the encoded antigen when injected into recipients. A distinct advantage of DNA vaccines over other methods of antigen delivery is that DNA vaccines can be easily constructed, purified, and delivered to recipients. In this chapter, the engineering efforts to enhance the immune and anti-cancer efficacy of DNA vaccines were illustrated in extensive detail, focusing on specific modifications that can be made to the DNA backbone to enhance the expression, processing, and presentation of the encoded antigen, in addition to improve the inherent immunogenicity of the vaccine itself.

The next ten chapters, part III of this book, are related to the exciting topic of nanoengineering. Nanotechnology, an interdisciplinary research field involving physics, chemistry, engineering, biology, and medicine among others, holds tremendous potential for early detection, accurate diagnosis, and personalized treatment of diseases [15–17]. With the sizes several orders of magnitude smaller than human cells, nanoscale agents/devices can offer unprecedented interactions with biomolecules both on the surface of and inside cells, which can revolutionize disease diagnosis and treatment. In light of this, there has been numerous nanotechnology centers established worldwide over the last two decades [18–20]. One major area of applications for nanotechnology is biomedicine, and it is expected that nanotechnology will mature into a clinically useful field in the near future, which has already made significant impact in many aspects to date.

In Chap. 18, Dr. Seto and Dr. Conroy gave an excellent overview of the multifunctional nanoscale delivery systems for nucleic acids. Although nanoscale systems are attractive platforms for in vivo delivery of nucleic acids, their potential for improving human health has yet to be fully realized in the clinic. Engineering efforts is critical for modifying and optimizing synthetic and viral delivery systems to include drugs, imaging agents, and targeting moieties, which can simultaneously minimize toxicity and increase delivery efficiency/specificity. This chapter focuses on the recent advances in RNA/DNA delivery with an emphasis on the progress toward human therapies, the challenges that have been encountered, and the engineering approaches that have been employed. The delivery systems used (e.g., viral, non-viral, and directed delivery) and the therapeutic components incorporated (e.g., DNA, RNA, and nucleic acid analogs) were all described in exquisite detail. Lastly, three major challenges were identified, namely the development of optimal systems for preclinical testing, integration of clinically useful imaging approaches, and rapid translation of directed delivery technologies, which should all be adequately addressed before broad future clinical applications.

In Chap. 19, Dr. Chang and co-workers centered on engineering nanomaterials for biosensing and therapeutic applications, which provided a timely overview on the synthetic methodology, surface engineering, physiological itinerary, and theranostic applications for various inorganic and organic nanostructures. After an introduction and a brief review of the applications and preparative methods of inorganic nanomaterials, the effects of surface modification with polymers and morphology on the physiological itinerary and toxicity of these nanomaterials were discussed, which can offer critical insights for future design of targeted imaging/therapeutic agents. Subsequently, in this chapter, several examples of promising nanotechnologies and theranostic applications were given, such as biosensors, magnetic hyperthermia, and photodynamic therapy for diagnosis and treatment of cancer. To take full advantage of these innovative ideas and demonstrated proof of principles of nanomaterials for biomedical applications, it is crucial to emphasize and expand efforts on translational research and development in the near future.

The following five chapters in part III of this book are related to several classes of nanomaterials that have been extensively investigated over the last decade, all of which hold tremendous potential for clinical applications: fluorescent nanoparticles (Chap. 20), magnetic nanoparticles (Chap. 21), upconversion nanoparticles (Chap. 22), mesoporous silica nanoparticles (Chap. 23), and carbon nanomaterials (Chap. 24). Fluorescent nanoparticles have been extensively studied in preclinical research for cancer imaging and/or theranostic applications [21, 22]. In Chap. 20, Dr. Chen and Dr. Silvestre offered in-depth discussions about the design of fluorescent nanoparticles, which has to take into consideration not only the parameters that are related to enhanced fluorescence (e.g., absorption coefficient, quantum yield, stability, and excitation/emission wavelengths), but also characteristics that can lead to optimal blood circulation half-life, tumor specificity, efficient delivery, biocompatibility, and low toxicity. Three major classes of fluorescent nanoparticles were discussed: quantum dots and fluorescent dye-loaded inorganic and organic nanoparticles (e.g., those that are based on calcium phosphate, silica, lipid, or polymer). The characteristics, advantages, limitations, and the engineering strategies employed to enhance their in vivo use were discussed for each class of nanoparticles with ample preclinical examples. Significant emphasis was also devoted to clinical translation, which has remained a major challenge for most nanoparticles, and a few examples as well as the hurdles that exist were illustrated and discussed.

Magnetic nanoparticles are also popular candidates for many biomedical applications because of their low toxicity, biocompatibility, and unique magnetic properties [23]. In Chap. 21, Dr. Xu and co-workers gave a general overview of their applications in diverse areas such as separation of biological samples, in vitro diagnostics, in vivo imaging, drug/gene delivery, treatment of iron deficiency, cancer therapy with hyperthermia, anti-bacterial agents, tissue engineering, and regenerative medicine. Although magnetic nanoparticles hold great promise for numerous biomedical applications, as listed above, many challenges still exist and should be addressed before newly developed magnetic nanoparticle-based agents can be translated into clinical investigation.

Upconversion nanoparticles are a relatively new class of materials with intriguing properties for biomedical applications, such as sharp emission lines, long signal lifetime, large anti-Stokes shift, superb photostability, and no blinking/bleaching [24]. In addition, doping of these intriguing nanoparticles with rationally selected ions (e.g., Gd^{3+}, Er^{3+}/Yb^{3+}, Tm^{3+}/Yb^{3+}, or combination of these ions) can lead to many exciting new features. In Chap. 22, Dr. Shi and co-workers summarized the recent advances in engineering upconversion nanoparticles for biological imaging and therapy, focusing on size/morphology, optimization of properties for in vivo multimodality imaging (e.g., to achieve near-infrared emission or confer sensitivity for magnetic resonance imaging), as well as the integration of therapeutic entities for cancer therapy.

Another class of promising nanoparticles is mesoporous silica nanoparticles, which possess many attractive properties such as excellent biocompatibility, large surface area, high pore volume, and uniform/tunable pore size [25, 26]. In Chap. 23, Dr. Chen, Dr. Hong, and I reviewed the progress to date and potential future directions of engineering these nanoparticles for biological imaging and/or therapy in vivo. After a brief introduction, efforts in engineering the morphology and surface of these nanoparticles were summarized, followed by a systematic review of in vivo imaging with functionalized mesoporous silica nanoparticles, which include the use of optical techniques, positron emission tomography, magnetic resonance imaging, as well as various combinations of multiple techniques. Subsequently, the use of (hollow) mesoporous silica nanoparticles for cancer therapy was discussed, which include loading of these nanoparticles with chemotherapeutic drugs, genes, or photodynamic therapy agents. Furthermore, mesoporous silica nanoparticles have also been investigated for image-guided ultrasound therapy and combination therapy applications, which were also covered in this chapter.

The 2010 Nobel Prize in Physics was awarded to Andre Geim and Konstantin Novoselov "for groundbreaking experiments regarding the two-dimensional material graphene," which is a class of carbon nanomaterials that have attracted tremendous attention over the last decade [27–29]. In Chap. 24, Dr. Nayak and I focused on the recent studies pertaining to engineering and development of carbon nanomaterials and their composite for applications as synthetic scaffolds in tissue engineering and regenerative medicine. Because of the unique intrinsic physical and chemical properties, carbon nanotubes and graphene have been engineered via different methods to develop suitable two-dimensional and three-dimensional scaffolds, which were reported to sustain growth, proliferation, and adhesion of many types of stem cells [29]. Whereas some of these scaffolds were found to accelerate the osteogenic, neurogenic, and adipogenic differentiation of certain stem cells in the presence of specific medium, many other reports supported spontaneous differentiation of stem cells into specific adult tissues even in normal medium. Several underlying mechanisms such as nanotopography, preconcentration of growth factors, and electrostatic/chemical interactions have been proposed for such behavior of stem cells growing on different carbon nanomaterial-based scaffolds. Since most of the literature reports to date are based on in vitro studies,

more in vivo studies with relevant toxicity and biocompatibility data need to be in place for their future applications as implantable biomaterials for tissue engineering and regenerative medicine.

Some of the major goals of optimizing drug/gene delivery systems include increasing cell specificity, incorporating organelle targeting, and improving overall delivery efficiency [30]. Peptides/proteins can potentially meet the requirement for reaching these goals because of several desirable properties, such as the ability to condense DNA into compact particles for transport, to disrupt endosomal membrane, to escape proteasomal degradation, to traffic various therapeutic molecules to targeted intracellular compartments, and to minimize cytotoxicity/immunogenicity. In Chap. 25, Dr. Numata and co-workers gave a comprehensive overview of engineering peptide-based carriers for drug and gene delivery. Since silk is well known for its biodegradability and biocompatibility, silk proteins derived from spiders and insects can be utilized for various biomedical applications such as drug/gene delivery, which is the focus of this chapter. The ability of silk to self-assemble, in combination with many other inherent features, makes it a unique and versatile delivery platform for small molecules, large proteins, as well as DNA/RNA. Not limited to drug/gene delivery, it may also be useful for tissue engineering, imaging, and regenerative medicine applications.

To achieve effective therapy and minimize toxic effects to the normal tissue, the drug/gene delivery systems need to target the disease sites (e.g., tumor) effectively. An alternative approach to enhance the therapeutic efficacy while minimizing side effects is to use activatable agents, which can be designed to release biologically active agents (e.g., drugs and genes) in response to internal or external stimuli upon systemic administration. In Chap. 26, Dr. Law and his co-worker presented a comprehensive summary of activatable agents and provided specific examples to illustrate their mechanisms and potential applications for imaging and therapy of various diseases (e.g., cancer, diabetes, and pulmonary embolism). Complementary to nanotechnology, such activatable agents with various built-in sophisticated mechanisms have been engineered, which can dramatically facilitate broad future use of nanotechnology for clinical applications. The activation approaches described in this chapter include pH (e.g., with the use of acid-sensitive linkers or microgels, disassembly of micelles under certain pH range, and decomposition/desorption of inorganic materials), enzyme (e.g., proteases, esterases, myeloperoxidase, and many other enzymes), heat, light, and magnetic field. Although most of these agents are still in the preclinical development stage, for many of which only in vitro data are available, continued research effort in this exciting area will ultimately lead to more sophisticated activatable systems that are biocompatible and possess favorable pharmacokinetic properties as prospective candidates for future clinical trials.

Molecular beacons are useful in a number of biomedical applications, since they can provide fluorescence readout upon biomolecular recognition [31, 32]. With the use of photosensitizers, photodynamic molecular beacons hold potential as new tools for not only disease diagnosis but also therapy. In Chap. 27, Dr. Lovell and his co-worker discussed about the opportunities for new photodynamic molecular

beacon designs. After an introduction to the classic molecular beacons and photodynamic molecular beacons, they offered important insights into the design considerations for next-generation molecular beacons, including the optimization of fluorophores and quenchers, rational design of loops and stems, as well as several other aspects. Enormous opportunities lie ahead in converting some of the already existing molecular beacons into new photodynamic molecular beacons, where seamless conversion of an imaging probe to a therapeutic agent can be achieved by replacing the fluorophores with light-activatable photosensitizers.

The design/optimization of new agents should proceed in parallel with the development of new instrumentation that can offer sensitive detection of various imaging agents described above, as well as providing high-resolution real-time images in preclinical animal models and cancer patients. Part IV of this book, composed of the next four chapters, is focused on biomedical instrumentation which has witnessed tremendous recent advances in terms of engineering. Three of the chapters (Chaps. 28–30) are related to the development of new imaging systems for various contrast agents, whereas the last chapter (Chap. 31) is focused on preclinical radiotherapy systems.

With the use of contrast agents that contain positron emitters, positron emission tomography (PET) is widely used in the clinic for disease diagnosis and treatment monitoring, particularly in oncology [33, 34]. In Chap. 28, Dr. Levin and Dr. Vandenbroucke provide the readers with an overview of novel techniques in the engineering of next-generation PET detectors. After a brief introduction to PET and current state-of-the-art commercially available clinical systems, the characteristics of current and novel scintillating materials used for detection were discussed in detail. Subsequently, improvements in spatial resolution through depth-of-interaction measurements and novel optical photon extraction methods were illustrated, followed by a discussion on various photodetectors which include photomultiplier tubes (widely used in current clinical PET scanners) and silicon-based solid-state photodetectors (e.g., avalanche photodiodes and silicon photomultipliers), as well as semiconductor detectors that do not require the use of photodetectors. Since accurate time-of-flight information can significantly improve image signal-to-noise ratio, how to improve time resolution and the consequences for time-of-flight imaging was also covered in this chapter. With the recent commercial availability of clinical PET/MR scanners, the compatibility of various detector materials with magnetic resonance imaging was also reviewed. Lastly, improvements in image reconstruction techniques were briefly discussed. The authors believe that many of these techniques currently being developed will make their way into the clinic, which will significantly improve patient management.

Photoacoustic imaging, which converts short light pulses into ultrasound waves for detection, can generate three-dimensional maps of tissues with high spatial resolution and good signal penetration depth [35]. As a relatively new technique, photoacoustic imaging can overcome many limitations of conventional optical imaging. In Chap. 29, Dr. de la Zerda gave a thorough review on the development of imaging systems and molecular contrast agents for photoacoustic imaging. After an introduction and brief review of the physical basis for photoacoustic imaging,

different photoacoustic scanner implementations were discussed (e.g., photoacoustic tomography and photoacoustic microscopy), along with their biomedical applications. Since the basic mechanism that gives rise to a photoacoustic signal is light absorption, many endogenous molecules can be used for photoacoustic imaging, such as hemoglobin and melanin. To fully realize the potential of photoacoustic imaging, exogenous contrast agents (both molecularly targeted and non-targeted) have also been developed, which were comprehensively covered in detail in this chapter.

Aside from scanners that can be used for non-invasive imaging in humans or preclinical animal models, miniature imaging instruments are also needed for endoscopy applications. Many such instruments have been developed, which exhibit millimeter dimensions for in vivo imaging with performance approaching that of conventional microscopy [36]. In Chap. 30, Dr. Wang and Dr. Qiu presented representative miniature imaging technologies that are currently under active development, including scanning fiber endoscopy, optical coherence tomography endomicroscopy, photoacoustic endomicroscopy, confocal endomicroscopy, dual-axes confocal endomicroscopy, and multi-photon endomicroscopy. Significant advances have been made in endomicroscopy technology such as optical designs/fibers, light sources, and miniature scanners, which can allow for improved resolution, greater signal penetration in tissue, as well as multi-spectral imaging. With the significant reduction in size, these systems can enable minimally invasive visualization of pathology in hollow organs to guide biopsy, identify surgical margins, and localize diseases. Major engineering challenges in this field include the need for large displacements, high scan speed, linear motion, and mechanical stability in a miniature instrument. To ultimately achieve fast two- and three-dimensional beam scanning, novel methods for cross-sectional imaging with deep tissue penetration, wide-area surveillance, and high-resolution microscopy were also illustrated in this chapter.

Chapter 31, written by Dr. Graves and Dr. Bazalova, was focused on engineering small animal conformal radiotherapy systems which are important tools for translational research in radiation oncology. Since it is critical that the effects of radiotherapy on preclinical animal models are studied under the same conditions as how clinical radiotherapy is delivered, the engineering aspects of small animal radiotherapy systems were thoroughly discussed in this chapter, which include the sources of radiation, beam collimation, and imaging techniques. Quality assurance (e.g., mechanics, dose output, beam targeting, and imaging) of small animal conformal radiotherapy systems was also illustrated, which is an extremely important aspect in radiotherapy. Many preclinical radiotherapy systems have been developed at various institutions, which were also reviewed and compared. Lastly, various applications of such small animal conformal radiotherapy systems were briefly described. The authors are convinced that the models derived from small animal studies will facilitate the development of future personalized medicine, which can help physicians to decide on the best treatment protocol for individual cancer patients.

Part V of this book, which includes the remaining four chapters, is related to theranostics and other novel approaches. The area of theranostics has been an extremely vibrant field over the last decade, which can integrate diagnosis and therapy [37, 38]. Since the field has evolved so rapidly, the journal *Theranostics* was launched in 2011, which has already gained widespread recognition with an impressive 2012 impact factor of 7.806. In Chap. 32, Dr. Lapotko and Dr. Lukianova-Hleb gave a comprehensive review regarding cancer theranostics with plasmonic nanobubbles. After an introduction and description of the limitations of various other approaches, the physical properties of plasmonic nanobubbles were described (e.g., generation, detection, tunability, and spectral properties). Next, generation and detection of plasmonic nanobubbles in cells and tissues were reviewed in exquisite detail. In addition, theranostic applications of plasmonic nanobubbles were also discussed with concrete and specific examples, both in vitro and in vivo. Lastly, the translational potential/challenges of these plasmonic nanobubbles were mentioned, which are mainly associated with the limited penetration of light and gold nanoparticles into heterogeneous tumors, as well as possible phototoxicity. As a new paradigm of nanomedicine, plasmonic nanobubble-based theranostics was expected to achieve cell-level efficacy and safety, as well as high speed through the novel intracellular diagnostic and therapeutic mechanisms for potential future clinical applications.

Microfluidics, the study and use of fluid flow at small volumes (e.g., microliter or less), has been an exciting area of research over the last decade [39, 40]. In Chap. 33, Dr. Lang and co-workers focused on cell-based microfluidic assays in translational medicine, which hold great potential in many engineering and medical arenas such as point-of-care diagnostic tests. Two major areas where microfluidic cell-based assays have been used for clinical applications are chemotaxis (i.e., gradient-dependent cell migration) and the isolation/analysis of rare cells (e.g., circulating tumor cells), which were both reviewed in this chapter.

Chapter 34 is focused on engineering of photo-manipulatable hydrogels for translational medicine, which was written by Dr. Zhang and her co-worker. Since spatial and temporal resoluted control of their property and function can be realized through light irradiation, photo-manipulatable biomaterials are of high significance in translational medicine. Hydrogels, an important class of biomaterials that are based on natural or synthetic polymers [41, 42], have been engineered to have photo-reactive chemical moieties for post-gelation photo-manipulation. In this chapter, the chemistry involved in the engineering of photo-manipulatable hydrogels was summarized, followed by representative examples of photo-manipulatable hydrogels (e.g., those that are photo-polymerizable, photo-degradable, or photo-patternable, as well as smart supramolecular hydrogels with sensitive photo-response). In addition, the applications of these photo-manipulatable biomaterials in regenerative medicine and tissue engineering were illustrated using recent examples. With the continued discovery of new bio-orthogonal reactions, it is certainly possible to engineer photo-manipulatable and multi-functional biomaterials for translational medicine in the near future.

In the last chapter of this book, written by Dr. Cai and his co-worker, a new class of peptidomimetics termed "AApeptides" was comprehensively reviewed. Peptidomimetics, designed to mimic the structure and function of bioactive peptides, have found numerous applications in the biomedical arena, and novel classes of peptidomimetics have continued to emerge [43, 44]. Dr. Cai's research group recently developed AApeptides, which have unnatural backbones and hence are much more resistant to protease degradation than the naturally occurring peptides. Furthermore, they have limitless potential for derivatization with straightforward synthesis. In this chapter, the chemical development of AApeptides was illustrated with ample examples of AApeptides as potential therapeutics such as anti-microbial agents and anti-cancer agents.

Together, there are about 80 contributors to this book. Since they are all international leaders in the topic of each chapter, I am convinced that this book will be a comprehensive and authoritative reference book in the area of engineering and/in translational medicine. Several more chapters on other important topics were planned in the initial book proposal. However, due to various unforeseen circumstances, many authors were not able to write the book chapters that they initially committed to contribute. Since such situations were only discovered with very short notices, there was not sufficient time to find other experts in the field to write on these topics. A list of topics that would have been covered in this book include the following: engineering induced pluripotent stem cells for biomedical applications, carbohydrate-engineered cells for regenerative medicine, engineering tissues with spatial and temporal control of microenvironmental cues, engineering HaloTag for biomedical applications, engineering FRET sensors for biomedical applications, engineering nanoneedle systems for transdermal vaccine delivery, engineering combinatorial drug delivery systems, engineering fluorescence optical devices for non-invasive and intraoperative molecular imaging in patients, engineering small animal optical imaging systems, engineering tumor ablation systems, and a few others. Given the extremely competitive funding situation across the globe with grant funding rate at all-time low, I completely understand their time constraint and priorities. If there will be a second edition of this book in the future, I hope I will be able to include these exciting topics and other newly emerged areas.

I am deeply indebted to the scientists who have contributed to this book and will be forever grateful for their enthusiasm, support, tremendous effort, and scientific insight. Thank you very much for reading this introductory book chapter, and now, I present you the rest of the book which I am sure you will be as intrigued by each chapter as I was during the editing process.

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