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Translational Medicine: Nanoscience and Nanotechnology to Improve Patient Care

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15.1
Introduction

Nanomedicine, also termed *nanotechnology-enabled medicine* [1], is the science and technology of diagnosing, treating, and preventing diseases and traumatic injuries, of relieving pain, and of preserving and improving health using molecular tools and molecular knowledge of the human body according to the European Science Foundation [2]. To clearly distinguish nanomedicine from established treatment forms, it can alternatively be defined as characterizing hard and soft tissues on the nanometer scale and tailoring nanostructured man-made materials for improving human health. The understanding of tissue organization down to its nanometer-size components and the development of interrelated tools to prevent, diagnose, and treat diseases are essential steps in current clinically applied science. The societal need for cost-effective improvements of patient health thereby motivates the research activities in nanomedicine and their scientific approaches.

The present chapter focuses on selected clinically relevant challenges that can realistically be overcome by nanomedicine approaches in the near future. The four most prevalent diseases in Europe are cancer, cardiovascular and neurodegenerative diseases, as well as disorders of the musculoskeletal system. Here, we will focus on selected solutions to cardiovascular diseases and musculoskeletal disorders. In these fields, therapeutic strategies are based upon quantitative understanding of the nanostructure and mechanical properties of human hard and soft tissues. Deeper understanding of diseases based on nanometer- and micrometer-scale mechanical characterization methods and, above all, structural imaging down to the molecular scale help in clarifying the roots of underlying pathological processes, allowing for elaborating preventive strategies and enabling the development of adaptive local drug delivery. For instance, one main focus is the design of mechanosensitive nanocarriers and nanocontainers to deliver active substances at target locations in predefined doses [3].

These efforts are converged on targeting applications in orthopedics and dentistry; oral and musculoskeletal biology are both largely driven by mechanical

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forces, and the prosthesis materials in both applications require matching the unique nanostructure and material properties of the host tissues [4].

Mechanical stresses are present not only in the musculoskeletal but also in the cardiovascular system [5]. Similar mechanical and structural approaches will target vascular ischemic disease and stroke with drugs released by mechanosensitive nanocontainers. To target cardiac insufficiency, containers sensitive to mechanical forces generated by the beating heart should become employed. This approach will allow for efficient, localized drug delivery that can increase the pump function. It is evident that the clinical realization of these envisioned applications, moving beyond very recent proof-of-concept studies [5, 6], will require careful design and manufacture of nanometer-sized containers and carriers.

Dealing with structural units ranging from 1 to 100 nm in size is a challenging feature of nanomedicine and nanotechnology (see Figure 15.1). Since, precisely at this size range, a variety of biological mechanisms are regulated, the design of man-made materials at these scales offers a huge potential to significantly improve patient management. While the understanding and manipulation of the human body at the nanometer scale will eventually revolutionize medicine, the challenges to realize the related innovations are considerable. Figure 15.1 illustrates the wide

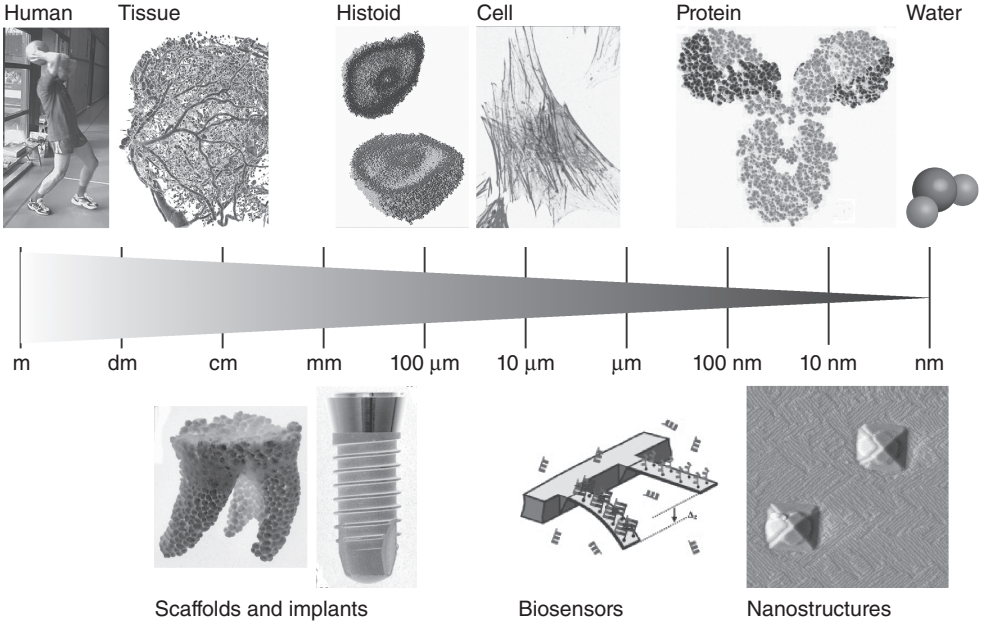

Figure 15.1 The logarithmic length scale for one direction in space classifies the life science disciplines: medicine from meter to submillimeter regime, cell biology on the micrometer scale, and biochemistry dealing with entities in the nanometer range. Below the scale, engineering and natural sciences are represented via man-made materials applied for diagnosis and therapy.

variety of disciplines involved and shows that all medical considerations start with the human body. Physicians consider and treat their patients in their entirety. For an increasing number of patients, medical doctors in general and radiologist in particular apply imaging techniques to diagnose or even assist treating a multitude of diseases. Current medical imaging is restricted to a spatial resolution down to a fraction of a millimeter, that is, far from the limits of imaging techniques applied not only in other life sciences such as cell biology and biochemistry but also in other natural sciences and engineering especially in materials science and solid state physics. Significant efforts have to be invested to bridge the gap between imaging techniques used in microtechnology and nanotechnology and those used in clinical practice. Frequently, these challenges in bridging nanoscience and medicine are simply considered as a matter of scale. For instance, the number of cells within the human body and of atoms within a single cell corresponds to the huge figure of 10^{13} to 10^{14}, which is at least three orders of magnitude larger than the number of stars in the Milky Way. Therefore, visualization of the entire human body cell-by-cell has not yet been achieved and the description of the body using individual atoms or molecules is currently impossible.

Nonetheless, models and other tools [7] can be successfully applied to describe macroscopic and microscopic material properties in terms of the arrangement of atoms in crystalline structures. These tools can yield useful insights into human tissue organization as well as diseases and healing processes. Such approaches, however, still require substantial developments in physics, chemistry, and engineering to effectively translate knowledge to solving challenges that clinicians are faced with in their daily practice. And in particular, it requires a convergence of all disciplines, focusing on one goal.

The research efforts in nanomedicine require a detailed understanding of the human body down to the molecular level using highly sophisticated methods for *post mortem, ex vivo,* and *in vivo* structural characterization. This knowledge will allow for not only the regeneration and repair of diseased tissue in a biomimetic, this means in a nature-analog manner, but also the fabrication of implants with mesoscopic surfaces to optimize the material–tissue interfaces.

Figure 15.2 shows how the Humboldt principle of coexistence of research and teaching forms the basis for nanomedicine's translation to the industry and the clinic. Essential to this approach is the idea of research-oriented teaching and the transfer of knowledge and technology developed in research. Students and teachers are joined in a rather flat hierarchy with the endeavor to critically examine traditional bodies of knowledge and to actively advance learning. Thesis projects on the distinct academic levels will become the seed for clinical research in hospitals (translation) and research and development (R&D) in MedTech and related industries with the final aim to create employment in clinics and high-tech companies. In this way, the clinically relevant knowledge and technology can be efficiently transferred to MedTech industry and the benefitting patients of the aging society.

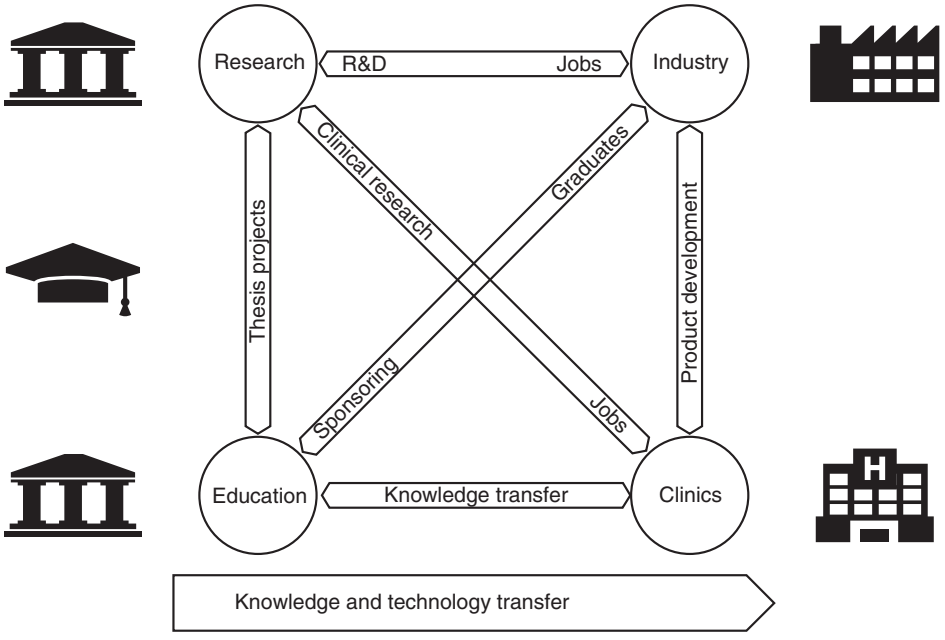

Figure 15.2 Translational research offers several pathways from the academic research via industry to clinics.

15.2 Nanoanatomy

Current medical imaging in clinical environment is restricted to the submillimeter range at its best. The next coherent step is the imaging of human tissues, that is, an entire organ or a reasonable part of the organ, down to the molecular scale *ex vivo.* Analog to the well-established macroanatomy and microanatomy, one considers this emerging field as *nanoanatomy.* These basic research activities in translational nanomedicine not only are limited to the characterization and understanding of human tissues in health and disease but also embrace its interrelation with body functions. They require a set of sophisticated tools that allow for probing the relevant parts of organs and tissues at the nanometer scale. Through cross-pollination from condensed matter physics, methods based on X-ray scattering have been developed to investigate human tissue function and disease progression [8–10]. Figure 15.3 illustrates a currently available experimental setup for spatially resolved X-ray scattering using synchrotron radiation. A highly intense, monochromatic X-ray beam is focused to a few micrometers in diameter, which perpendicularly impinges tissue slices about 100 μm thick. Although most of the X-ray photons pass the tissue and are absorbed on the beam stop, a significant fraction is scattered from the nanometer-size features of the tissue slice and counted on the highly efficient detection system. Moving the tissue in x- and y-directions and acquiring the related scattering patterns, micrometer resolution

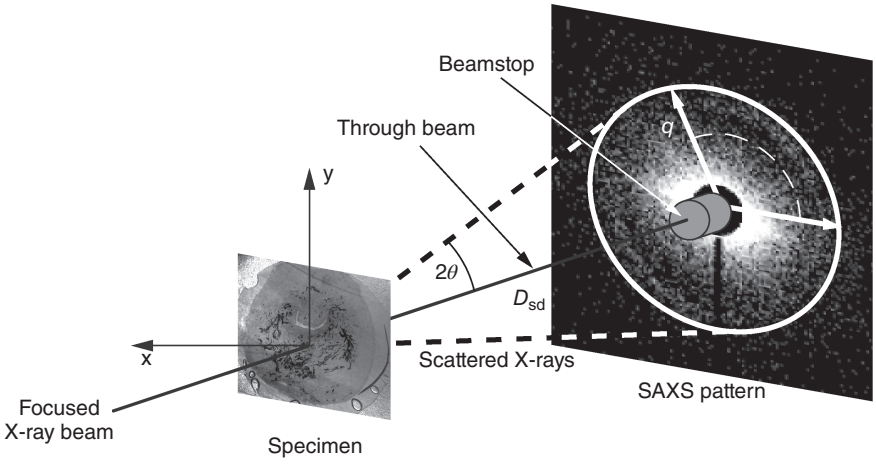

Figure 15.3 X-ray scattering using monochromatized synchrotron radiation can be performed in a spatially resolved manner combining micrometer resolution in real space and averaged nanometer information from reciprocal space.

in the real space is achieved and an enormous amount of data in reciprocal space covering the entire range from atomic distances to several 100 nm are obtained. Comparing these X-ray scattering data with histological characterizations, the abundance and the preferential orientation of numerous nanometer-size components including collagen fibers and elongated hydoxyapatite crystallites can be extracted. Such an experimental setup has been used to quantitatively characterize the nanometer-size components of hard and soft tissue slices from bone/cartilage, urethra, human teeth, and brain (see, for example, Ref. [11]). Figure 15.4 shows an example of the nanostructure orientation of nanostructures within the sheep urethra [12]. The left image is a virtual cut through synchrotron radiation-based micro-computed tomography data of the sheep urethra, which displays the orientation of characteristic micrometer-sized anatomical features. One can clearly differentiate between the lumen of the urethra and the well-organized surrounded tissues. The epithelium and the lamina propria form the 1 to 2 mm-thick tunica mucosa. Caverns in the lamina appearing in black are characteristic for connective tissue in mammalians. As in histological slices, an interface separates clearly between the tunica mucosa and the tunica muscularis (muscular tissue) with a highly oriented microstructure. The color image on the right exhibits the scattering signal related to the presence of oriented nanostructures in the range between 7 and 11 nm. The main orientation of these nanostructures is given according to the color wheel. Overall, both cross-sectional modalities demonstrate the high similarity between the microanatomic and nanoanatomic features of the urethra.

Similar to the extension of two-dimensional (2D) radiography to three-dimensional (3D) hard X-ray computed tomography, tomographic imaging can be developed on the basis of these scattering approaches. For example, the spatial distribution of bio-membranes formed by myelin was recently revealed in the

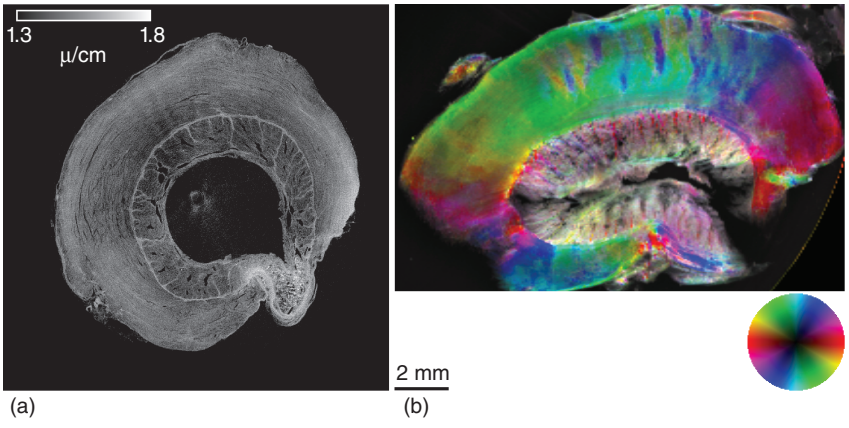

Figure 15.4 Virtual cut through a sheep urethra, as seen in the synchrotron radiation-based micro computed tomography (a). Main orientation of the scattering signal related to nanostructures with sizes between 7 and 11 nm according to the color wheel (b).

rat brain by applying small-angle X-ray scattering (SAXS)-tomography [13] at the Swiss Light Source, Paul Scherrer Institute that currently hosts one of the world's premiere beamlines for spatially resolved X-ray scattering [14]. Other X-ray-based techniques at the Paul Scherrer Institute, above all the high-resolution microscopy technique and ptychographic coherent diffractive imaging (P-CDI), have recently been expanded from two to three dimensions and applied to bony tissues [15].

Next to taking a snapshot of the current state of tissues, assessing tissue dynamics constitutes an integral part of understanding human nanoanatomy. Interfacing microfluidics with state-of-the-art microscopy and SAXS is a strong emerging tool for investigating self-assembly processes of biomaterials and cell motility *in vitro* [16–20]. This microfluidics-based approach allows for deeper insights into the formation of tissue networks of different complexity [21, 22] and understanding of the self-organization, invasion, disruption, and healing of human membranes [23–25].

Data acquisition through probing tissues at the mesoscopic scales is a complex step, but only the first of several ones that will lead to the understanding of human nanoanatomy. The acquired imaging data are of tremendous size, often coming from multiple modalities with dissimilar spatial and density resolutions. The data thus require registration (i.e., alignment and merging) to unleash their full potential. Various image registration approaches have been proposed in the last two decades [26] and future developments may focus more on the handling of large data sets [27]. Past research efforts mainly concentrated on the task of co-registering thousands of images [28], but the registration of highly resolved images in three dimensions requires more efficient algorithms with respect to data size. Current image registration techniques can be separated into two main approaches, the landmark-based [29] and intensity-based methods [30]. While

the landmark-based approaches seem to be attractive for registering large data sets due to their inherent dimensionality reduction ability, matching the landmarks between images of different modalities can be challenging. Nevertheless, first steps in the direction of multimodal landmark matching have been made recently [31]. In intensity-based registration, in contrast, mutual information is used as a well-established similarity measure for multimodal registration [32]. Future intensity-based approaches may also be able to handle large data sets efficiently. Their development is already in progress for image data registration [33, 34] and for their application to mesoscopic scale three-dimensional imaging problems [35, 36].

15.3 Nanorepair

Human tissues are generally organized in three dimensions as known for the inner organs and the musculoskeletal system, but the membranes of the inner ear, for example, are two-dimensional arrangements of cells. The hierarchical organization of microstructures and nanostructures is characteristic for both the 2D and 3D human tissues. Therefore, the repair of human tissues should not only be restricted to 3D bony tissues but also include membranes commonly present in the human body. The increasing number of prenatal diagnostics, for example, is resulting in an increasing number of procedures penetrating the fetal membrane. Although its healing capacity is well appreciated, the risk for premature rupture is high [37]. Therefore, strategies have to be developed to suitably repair the membrane after amniocentesis. Such research initiatives might be termed *nanorepair*.

One approach to form biomimetic materials is given in a bottom-up fashion. Biomimetic materials have been shown to control cell migration and tissue regeneration [38]. The controlled hierarchical organization using layer-by-layer and printing approaches has allowed for the *in vitro* formation of tissue-like structures [39]. It is obvious that by taking such approaches to the next level, the border between implant material and tissue becomes more and more diffuse. The implant becomes basically a replacement tissue. Indeed, using growth factor immobilization and release methods [40, 41], cyclic loading protocols [42], techniques to mimicking fetal membranes tissue formation, injury, and healing *in vitro* have been developed. Additionally, gluing materials have been tested for their properties to seal fetal membranes [37, 43].

15.4 Nanoorthopedics

Nanoanatomy will help to identify nature-analog implant surfaces. In principle, nature-analog implant surfaces have been available for decades. For example,

titanium bone implants exhibit a surface with a distinct roughness on the micrometer and nanometer scales generated via sandblasting and/or etching procedures to achieve improved osteointegration [44]. Nevertheless, the effects of the microstructured and nanostructured surfaces are only partly understood [7]. Therefore, the roughness of load-bearing implants on these length scales has to be further optimized to avoid, for example, inflammatory reactions and oral diseases such as peri-implantitis. Several underlying mechanisms were revealed, but the optimal roughness for the numerous man-made implants has not yet been found and further extended studies will be initiated. Such research activities for load-bearing implants might be summarized as *nanoorthopedics.* The understanding of nanoanatomy is necessary for intelligent and targeted manipulation of artificial materials at the nanometer scale to interface with human tissue. The importance of the interaction between tissues and engineered materials has been known ever since the first implants were investigated. A clinically successful, functionally stable tissue transition from host to implant requires orderly tissue ingrowth that is influenced by the implant surface chemistry and surface topography [45–48]. The development of next generation biomaterials will feature nanometer and micrometer scale surface structures designed to elicit specific host responses to control tissue ingrowth to an implant and avoid tissue scarring [49]. Orthopedic medicine is on the frontline of such developments, posing important clinical challenges [50–52]. Nanotechnology to tailor surfaces requires state-of-the-art techniques that allow processing of various materials, starting from ones that can be applied for exploratory *in vitro* studies to those finally used as constituents of medical implants for patients. Submicron scale patterns have been applied to surfaces and embedded in materials by using various techniques [53–55]. Advanced polymer constructs with mesoscopic surface patterns have been fabricated using up-scalable techniques including injection molding and roll embossing [56, 57], allowing for the application of secondary nanostructuring by use of hybrid molds [58–60]. Other methods include the grafting of polymer brushes onto chemically inert polymers such as fluoro-polymers and poly-olefins based on selective plasma activation [61]. Electron beam lithography exposures can be used to produce polymer brush structures with high spatial resolution and complexity [62–64].

Despite the smart application of specific nanostructures or the production of biomimetic materials, only testing of the respective tissue's response can guarantee the viability of an implant. The host–implant response is often characterized via phenotypic cell behavior by assessment of gene and protein expression [65–70]. Murine, rat, equine, and human fibroblast model systems are used to elucidate cell and matrix interplay in healing and implant integration [71–74]. Quantitative microscopy [73, 75, 76] has been applied to explore and understand the function of tissue structures [71–74] and cell–matrix interactions [69, 70, 77–79]. Nanostructured and microstructured sensors were employed to explore inflammatory reactions [7, 80] and contractile cell forces that have been relevant to myofibroblast behavior [81].

15.5 Nanovesicles

A wide variety of medical applications might benefit from nanometer-size components for targeted drug delivery. Thereby, liposomes offer a rather natural path to transport and release pharmaceuticals at desired locations. A recent prominent example is the shear-sensitive release of vasodilators [5, 6]. Such approaches can be classified as *nanovesicles.*

Given that the human body is based on nanostructures, it is intuitive that the tools to intervene in the body's function in a highly targeted fashion should also be of that scale. Building such small structures with classical engineering tools such as lithography is expensive and cumbersome. However, nature can guide us toward better approaches: self-assembly of amphiphiles into soft matter nanosystems, a fundamental principle of nature [82].

Arguably, nature's most important amphiphiles are phospholipids. They contain both a hydrophilic or polar head and hydrophobic or water-insoluble tails on the same molecule. In water, this arrangement leads to a hydrophobic effect and aggregation of the molecules into ordered structures. This association of phospholipids into superstructures minimizes the contact between bulk water and the hydrophobic parts of the molecules [83]. Depending mainly on the geometry and concentration of the phospholipids, various structures can be formed, the most attractive being self-closed and water-filled spherical particles composed of lipid bilayers [84]. They are termed *liposomes* or *phospholipid vesicles.* Drug delivery systems made from liposomes have shown a high potential as therapeutics in the past decades in selected fields such as cancer and antifungal therapy, where the vesicle can be loaded with drugs such as amphotericin B (AmBisome) or doxorubicin (Caelyx) [85]. This technology is currently in use in the clinic [86]. The formulations show high tolerability and can be designed to have a prolonged circulation in the blood stream. Liposomal formulation can be lyophilized and have a shelf life of up to 2 years.

The fundamental research on liposomes has, however, for a long time, diverged from medical goals. Instead, fundamental soft matter physics has taken the upper hand in the study of, for example, supported bilayers [87] and nanocontainer morphology [88]. Recent years have seen a trend in soft matter physics to study facetted vesicles which, for example, have a capsid-like icosahedral morphology [89]. The studies showed how liposome shapes are governed by the membrane bending energy and its in-plane elasticity [90]. The conclusion is that rigid bilayers show a multi-curvature, facetted morphology with in-built membrane defects at the vertices that will have an impact on membrane permeability [91]. Although the membrane bilayers consist of tightly packed phospholipids, the application of an external physical trigger such as changes in osmolarity, pH, or shear stress can induce a transient increase in the passive trans-bilayer membrane transport of vesicle payload. This phenomenon promises attractive applications in the field of nanomedicine.

Artificial 1,3-diamidophospholipids that have been synthesized [92] self-assemble into faceted, lentil-shaped vesicles [5]. Indeed, as predicted by theory, liposomes formulated from these molecules were found to maintain their payload in the resting state; but upon the application of shear stress, triggered by simple shaking, the entrapped molecules were released from the vesicle. This fact stands in contrast to liposomes derived from natural sources that either release their cargo both spontaneously and when shaken or do not release their payload at either conditions. Therefore, the vesicles formulated from the artificial 1,3-diamidophospholipid Pad–PC–Pad represent an unprecedented category of nanocontainers [92]. This fact has led to a convergence of soft matter physics with phospholipid chemistry and has shown fresh perspectives in medical applications for liposomes that react to the mechanical forces that occur in the human body. Y. Barenholz [93] pointed out that "because non-spherical liposomes release their contents only at elevated shear stress, drugs can be targeted and released only in regions with rheological changes (such as inside a clogged artery) without the need for any recognition molecules or a remote trigger." Using lentil-shaped nanocontainers, a preferential release for clinically relevant stenosed artery models was demonstrated *in vitro* [5, 94]. In a constricted artery, wall shear stresses were found to be one order of magnitude higher than in a healthy artery. This change in shear stress may be used as a purely mechanical trigger for targeted drug delivery. Therapies for diseases in which mechanical effects are involved can thus be envisioned. This includes drug delivery to address heart insufficiency (beating of the heart acts as the mechanical trigger), to open occluded arteries sufficiently to achieve a sufficient blood flow in the case of heart attack or stroke (local hemodynamic changes are the trigger), and intra-articular applications to medicate osteoarthritis in load-bearing joints such as the temporomandibular joint.

Nanoparticle-based drug delivery systems are tools with the potential to improve efficacy and reduce systemic toxicity of a variety of drugs [95, 96]. In order to trace the *in vivo* distribution and functionality of those delivery systems, they can be enhanced with imaging functionalities such as for positron emission tomography (PET) [91]. In general, imaging-based drug development has shown promise for speeding up drug evaluation by supplementing or replacing preclinical and clinical pharmacokinetic and pharmacodynamic evaluations [91, 97, 98]. With radiolabeled drug compounds, the additional functionality allows for noninvasive imaging of bio-distribution and pharmacokinetics. This approach has shown the potential to monitor drug activity during preclinical and clinical drug development. Additionally, it might facilitate an early decision to select promising investigational compounds from compounds that seem likely to fail [99]. Furthermore, addition of the imaging functionality allows trace amounts of a drug to be tracked, leading to the development of the principle called *microdosing*. PET-based microdosing involves the administration of only microgram amounts of the respective drug. Thus, the potential toxicological risks to human subjects are limited. As a consequence, microdosing is anticipated to permit smarter candidate selection by taking investigational compounds into humans earlier. Microdosing might allow safer human studies and reduce the use of animals

in preclinical toxicology [100]. Clinical microdose studies are anticipated to be feasible with a variety of radiolabeled nanovesicles. These microdose studies have the potential to shorten time lines and cut costs along the critical path of clinical translation [101].

15.6 Nanodentistry

As aforementioned, human hard and soft tissues that generally exhibit preferential alignment of microstructures and nanostructures according to the loading directions form a sound basis of biomimetic implants. Although current dental fillings, for example, show superior mechanical stability, they have an average life span of only one or two decades, while the human crown can remain stable for many decades. Building bioinspired dental fillings with oriented and elongated nanocomposites might significantly improve the mechanical properties of artificial crown tissues [102]. Furthermore, remineralization of quality-reduced hard tissues, for example, of caries lesions, might reduce the volumes to be treated in a conventional manner. We classify the related topics as *nanodentistry* [103, 104].

Having the tools to characterize human nanoanatomy, to produce biomimetic materials, and to test their interaction with tissues opens the door to clinical applications. One of them is dentistry, where innovative implants have found widespread use [103, 105, 106]. The enamel that forms the outer surface of teeth has a complex anisotropic nanostructure as a result of biomineralization during formation and subsequent mechanical loading [107]. Such structural anisotropy is also observed for the underlying dentin. The anisotropic nature of human crown tissues became available for use in bioinspired dental fillings [102, 104]. The Clinical Editor of the journal *Nanomedicine: Nanotechnology, Biology, and Medicine* pointed out at the end of the article abstract [108] that synchrotron radiation-based X-ray scattering enabled a groundbreaking study of the caries pathology. It has been demonstrated that while bacterial processes dissolve ceramic components in enamel and dentin, the dentinal collagen network remains unaffected, enabling the development of future caries treatments that remineralize the dentin [108].

Inside teeth, mineral loss usually accompanies root canal infections, globally one of the most frequent dental disease indications. When developing improved root canal treatments, reliable binding of canal sealants to dentin and remineralization of root dentin for biomechanical re-constitution of the affected tooth would be immensely valuable [109]. Likewise, in bones, development of treatments for complex-shaped defects demand for “bone glues” where mineral release has been found to be a key advantage for bonding hard tissue interfaces. Re-establishing bone density after trauma or osteoporosis demands significant mass and release of minerals. For highly mineralized tissues, the highly active, inorganic biominerals and bioactive glass nanoparticles are among the most promising solutions [110]. Production methods were developed to

access nano-biomaterials at the ton scale, hence enabling their use in global clinical product development. A number of preclinical tests on nano-bioglass and amorphous (glassy) nano-calcium phosphates have proven the methods' capability to prepare materials of adequate purity and under quality protocols amenable to biomedical manufacturing.

15.7 Interactions of Disciplines in Nanomedicine

The five areas of nanomedicine selected above are closely related (see Figure 15.5), since equivalent materials and the same methods for characterization are applied, as recently reflected in a large national research proposal for Switzerland. The list of such emerging medical fields, which are based on nanoscience and nanotechnology, can be continued. The final goal is always the translation from basic research to clinical applications and its benefit for patients.

The translation will succeed gradually and the developments are expected to appear in clinics step by step, as illustrated in Figure 15.6. Imaging methodologies

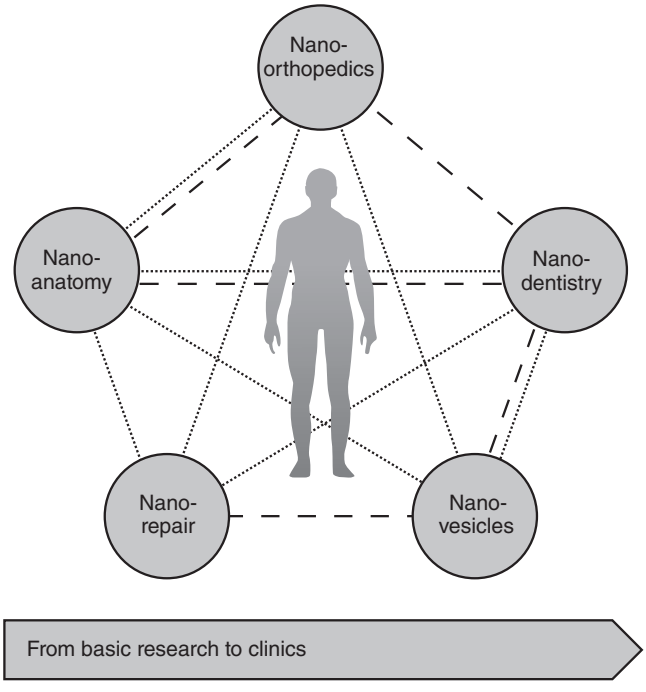

Figure 15.5 Selected research fields in nanomedicine with high translational impact: certain fields are clearly related to basic research whereas others are already in clinical use. Method- and material-related interactions are represented as dotted and dashed lines, respectively.

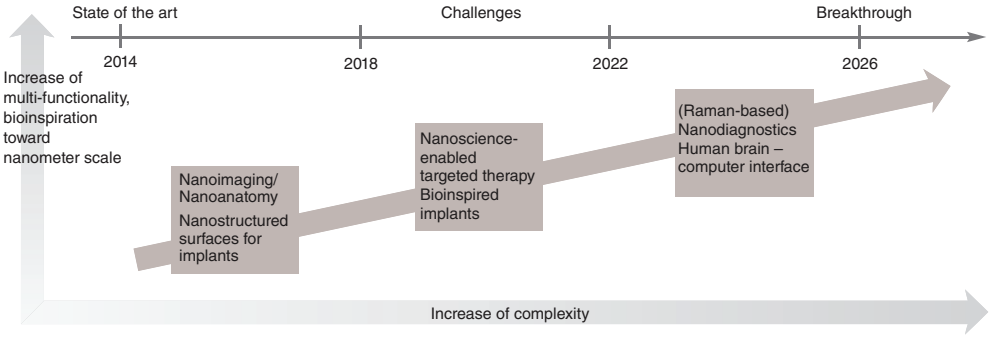

Figure 15.6 From current research activities toward the major breakthroughs one expects more than a decade, a time period, which is characterized by increasing complexity.

being currently only available for nanoscience may become applicable for clinical diagnostics in more than a decade. Insights from nanoanatomy and technical capabilities may enable the design of bioinspired implants and might lead to functional interfaces, such as a human brain–computer interface. In general, the achievements are expected to become more and more complex when the mimicking of nature will approach the nanometer scale. The successful completion of these complex tasks, however, is anticipated to generate improvements in patient care in a wide variety of clinical disciplines.

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