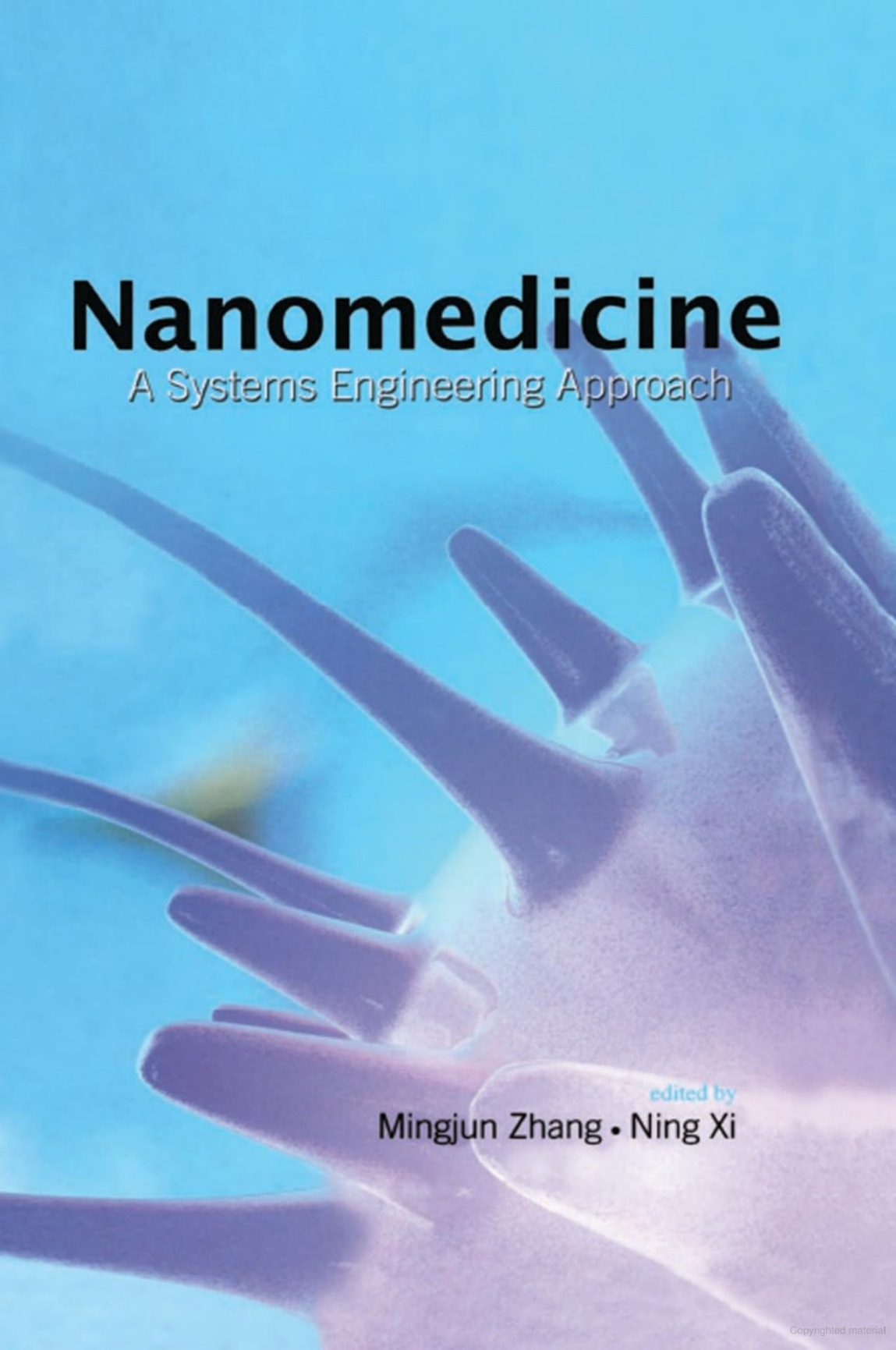
Nanomedicine

A Systems Engineering Approach



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NANOMEDICINE

A Systems Engineering Approach

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Nanomedicine: Dynamic Integration of Nanotechnology with Biomedical Science

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1.1 INTRODUCTION

The recent emergence of nanotechnology is setting high expectations in biological science and medicine, and many scientists now predict that nanotechnology will solve many key questions of biological systems that transpire at the nanoscale. Nanomedicine, broadly defined as the approach of science and engineering at the nanometer scale toward biomedical applications, has been drawing considerable attention in the area of nanotechnology. Given that the sizes of functional elements in biology are at the nanometer scale range, it is not surprising for nanomaterials to interact with biological systems at the molecular level. In addition, nanomaterials have novel electronic, optical, magnetic, and structural properties that cannot be obtained from either individual molecules or bulk materials. These unique features can be precisely tuned in order for scientists to explore biological phenomena in many ways. For instance, extensive studies have been done with chip-based or solution-based bio-assays, drug delivery, molecular imaging, disease diagnosis, and

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pharmaceutical screening.[1–4] In order to realize these applications, it is crucial to develop methods that investigate and control the binding properties of individual biomolecules at the fundamental nanometer level. This will require enormous time, effort, and interdisciplinary expertise of physical sciences associated with both biology and engineering. The overall goal of nanomedicine is to develop safer and more effective therapeutics as well as novel diagnostic tools. To date, nanotechnology has revolutionized biomedical science step by step not only by improving efficiency and accuracy of current diagnostic techniques, but also by extending scopes for the better understanding of diseases at the molecular level.[5–8] In this chapter, nanomaterials and their applications in biomedical research will be discussed.

1.2 DESIGNING NANOMATERIALS FOR BIOLOGY AND MEDICINE

One of the important technological aspects in nanomedicine lies in the ability to tune materials in a way that their spatial and temporal scales are compatible with biomolecules. That said, materials and devices fabricated at the nanometer scale can investigate and control the interactions between biomolecules and their counterparts at almost the single molecule level. This, in turn, indicates that nanomaterials and nanodevices can be fabricated to show high sensitivity, selectivity, and control properties, which usually cannot be achieved in bulk materials. The wide range of the scale of biointeractions is described in Fig. 1.

Given that one of the major goals of biology is to address the spatial-temporal interactions of biomolecules at the cellular or integrated systems level, the integration of nanotechnology in biomedicine would bring a breakthrough in current biomedical research efforts. In order to apply nanotechnology to biology and medicine, several conditions must be considered: (i) nanomaterials should be designed to interact with proteins and cells without perturbing their biological activities, (ii) nanomaterials should maintain their physical properties after the surface conjugation chemistry, and (iii) nanomaterials should be biocompatible and non-toxic.

In general, there are two approaches to build nanostructures or nanomaterials: "top-down" and "bottom-up" methods. Typically, the bottom-up approach utilizes self-assembly of one or

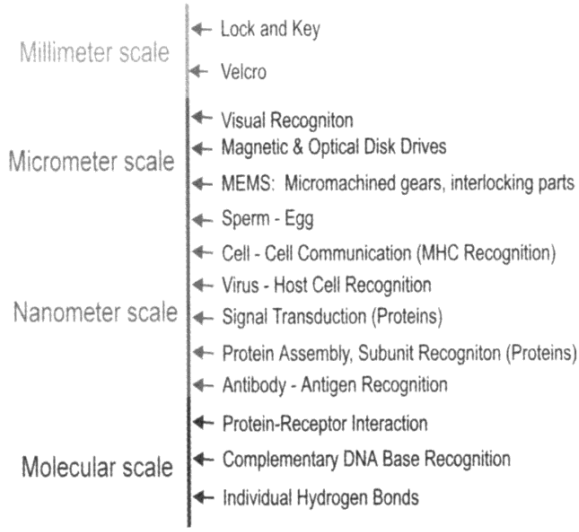

Figure 1. Scale of biomolecular interactions.

more defined molecular building blocks to create higher-ordered functional entities. For the bottom-up approach, the physical and chemical criterion, such as pH, concentration, temperature, and intrinsic properties of building blocks, must be fulfilled. On the other hand, the top-down approach usually involves processes such as lithography, etching, and lift-off techniques to fabricate micro- and nanoscopic structured materials from bulk materials. In many cases, nanomedicine strategies have been derived from what was originally a conventional biomedical application, with a certain degree of modification to address some scientific questions or technical limitations. As far as the applications of nanostructures are concerned, we will examine two examples, nanoparticles and nanoarrays/biochips, which are heavily used in biomedical applications.

1.2.1 Inorganic Nanoparticles

Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), peptides, and proteins are nanometer scale components that are the best examples of nanomaterials found in nature.[9,10] For example, DNA has a double-stranded helical structure with a diameter of 2 nm, RNA

has a single strand structure with a diameter of 1 nm, and most of protein sizes are less than 15 nm. Likewise, the sizes of functional elements in biology are at the nanoscale level, which inevitably generate significant interests at the intersection between nanotechnology and biological science. Even though much progress in the life sciences has been achieved over the last few decades, biological and physiological phenomena still remain beyond our understanding, because the interactions between elementary biomolecules and other higher components, such as viruses, bacteria, and cells, are complex and delicate. Moreover, the interactions of two biocomponents start from the single molecule level, where the recognition sites lie in a nanoscale domain. Thus, studies of these biological components require not only an ability to handle the biological properties, but also to develop highly advanced tools or techniques to analyze the biological systems.[11–14]

Bioconjugated nanomaterials have recently been used as cellular labeling agents to study the biological phenomena at the nanometer level. With significant advancements in synthetic and modification methodologies, nanomaterials can be tuned to desired sizes, shapes, compositions, and properties.[15] Inorganic nanoparticles are one of the most promising examples, since they can be synthesized easily in large quantities from various materials using relatively simple methods. Also, the dimensions of the nanoparticles can be tuned from one to a few hundred nanometers with monodispersed size distribution. Moreover, they can be made up of different metals, metal oxides, and semiconducting materials, whose compositions and sizes are listed in Table 1. Given many distinct properties, nanoparticles can be readily tailored with biomolecules via combined methodologies from bioorganic, bioinorganic, and surface chemistry.

Despite many significant advances in synthetic and surface modification methods, the fundamental development of bio-conjugation methods must first be achieved in order for the nanoparticles to be fully utilized. The bioconjugation strategies involve procedures for coupling biomolecules to nanomaterials, enabling the nanoparticles not only to be applied for clinical applications but also to ask and answer fundamental questions in cell biology. For the past few years, many methods have been developed for bio-labeled nanocomposites in various applications in cell biology: cell labeling, cell tracking,[19–22] and *in vivo* imaging.[23,24]

Table 1. Selection of available nanoparticle compositions, sizes, and shapes.

Particle Composition	Particle Size (nm)
Metals	
Au	2–250
Ag	1–80
Pt	1–20
Cu	1–50
Semiconductors	
CdX (X = S, Se, Te)	1–20
ZnX (X = S, Se, Te)	1–20
TiO_2	2–18
PbS	3–50
ZnO	1–30
GaAs, InP	1–15
Ge	6–30
Magnetic	
Fe_3O_4	6–40
Various polymer compositions	20 nm to 500 μm

1.2.2 Coupling of Nanoparticles with Biomolecules

Interdisciplinary knowledge from molecular biology, bioorganic chemistry, bioinorganic chemistry, and surface chemistry must be employed to functionalize nanostructures with biomolecules. Although nanostructures can be synthesized from various materials using several methods, the coupling and functionalization of nanostructures with biomolecules should be carried out in controlled manners such as a specific salt concentration or pH.[9] Three common methods of functionalizing nanoparticles with biomolecules are: (i) direct interaction between nanoparticles and biomolecules via electrostatic interactions or physical adsorptions, (ii) typical conjugation chemistry using organic linker molecules, and (iii) streptavidin-biotin affinity between functionalized nanoparticles.

Typically, solution phase synthesis of nanostructures is carried out in the presence of surfactants such as citrate, phosphates, and

alkanethiols. The surfactants not only interact with the atoms of nanostructures by either chemisorption or physisorption at the surface of nanostructures, but also stabilize nanostructures and prevent interparticle aggregation. Using the exchange reactions, surfactant molecules attached on the nanoparticles can be replaced by biomolecules, making direct biomolecule-nanoparticle covalent bonds. For example, gold nanoparticles can be modified with proteins consisting of cysteine residues or with thiol functionalized DNA molecules.

There are different types of coupling methods, where the electrostatic forces between proteins and citrate stabilized nanoparticles are used for the coupling. For the nanoparticles that are relatively unstable, the core-shell strategies can be applied to stabilize the nanoparticles.[25,26] For example, silver core-shell nanoparticles coated by thin layer of gold can be successfully functionalized with thiol-functionalized DNA.[25] Many semiconductor nanoparticles can also be linked with proteins or DNA by adding a hydrophobic silica shell.[16,27,28] Silica surfaces can be tailored with biomolecules, utilizing well known cross-linking methodologies, such as silanization chemisty and self-assembly monolayer (SAM) chemistry.[29–32]

1.2.3 Fabrication of Nanoarrays and Biochips

The search for novel ways to explore and understand biomolecular interactions has been sought in many ways, since interactions between biomolecules are fundamentally intriguing. For example, how proteins such as fibrinogen, fibronectin, and retronectin influence the adhesion of cells and control their morphology and physiology has been a central question of cell biology.[33,34] Several approaches have been examined over the past few years to comprehend these phenomena, and one of these approaches is the assembly of interfacial proteins constructed on micro- or nanoscale.[35] The biomolecular patterned surfaces are not only useful for probing biochemical interactions within whole cells but also crucial for biosensing. However, the realization of these applications is challenging, since the control of the interactions between proteins and surfaces with respect to a binding direction and biomolecular density is technically and biologically not easy to achieve.[36] Therefore, patterning techniques capable of high resolution — molecular to submicron scale — and compatible with biomolecules will be

required. Typically, current chip-based biodetection strategies pattern molecules with an analyte-capturing ability on the chip surfaces in the micro-/nano scale. This application of chip-based biosensing will allow scientists to detect various analytes at concentrations as low as picomolar in massively parallel ways. The aforementioned features are invaluable for the advancement in genomics and proteomics via generating DNA and protein arrays.[15] Yet, the key challenge lies in the fabrication of miniaturized surface structures in the form of nanoarrays that would allow for multi-magnitude orders of complex detection in the same chip and for improved detection sensitivity. There are many techniques available for patterning surfaces in terms of resolution and compatibility with soft materials (Table 2). Among different techniques, one primary distinction is whether the method uses a resist-based process or deposition of materials onto a surface directly. Although the indirect, resist-based patterning methods, such as photolithography, are multi-step processes that require specialized resists and etching protocols, they are currently by far the most widely used methods in industrial applications.

By contrast, direct-printing methods are typically useful for patterning soft materials such as small organic or biological molecules

Table 2. Features of selected lithography techniques.

Technique	Resolution Limit	Mode	Comments
Photolithography	Currently ~100 nm	Parallel only	Resist based, indirect
Electron beam lithography	5–10 nm	Serial only	Resist based, indirect
Indirect SPM methods	AFM 5–10 nm STM atomic to nm	Serial only	Resist based, indirect
Microcontact printing	~ 100 nm	Parallel only	Direct write or indirect
Ink-jet printing	6 μm	Serial	Direct write
Dip-pen nanolithography	5–10 nm	Parallel or serial	Direct write

with ease. Microcontact printing, developed by Whitesides and coworkers,[37–44] is a good example of a direct-printing method using elastomer stamps which can be "inked" with molecules and then used to transfer the inking molecules in a form of desired patterns to various substrates. This technique has been used to generate large area patterns of soft materials on surfaces with pattern resolutions approaching 100 nm. However, the technique is limited in its capability to generate multiple, chemically diverse, high-resolution patterns in alignment on a surface. Therefore, in terms of resolution to sub-100 nm, there is a high demand to develop methods of high-resolution lithographic techniques. Since the invention of scanning probe microscopes (SPMs), many scientists have realized that it might be possible to manipulate matter, atom-by-atom or molecule-by-molecule.[45,46] The early attempts to develop patterning methodologies from SPMs were able to demonstrate the high-resolution capabilities of these instruments.

The majority of SPM surface patterning methods have focused on either impressive but inherently slow scanning tunneling microscope (STM) based methods for moving individual atoms around on a surface in ultra high vacuum (UHV), or on indirect methods using atomic force microscope (AFM) and STM for stepwise etching of organic monolayers on a surface and backfilling with the molecule of interest.[37,47–50] However, resist-based approaches are inherently restricted to serial processes, and can only be used for a few molecule-substrate combinations. In 1999, Mirkin and his coworkers developed dip-pen nanolithography (DPN). DPN uses an AFM tip to transfer "ink" molecules onto a substrate through a water meniscus (Fig. 2).[3,51,52] DPN is simple, and its resolution is comparable to electron-beam lithography.

1.3 APPLICATION OF NANOMATERIALS IN BIOMEDICAL RESEARCH

Biomedical scientists have seen a great potential in the nanotechnology application. As a result, they have tried to incorporate the intrinsic properties of nanomaterials with conventional techniques in an attempt to improve detection methods and treatments for greater results.[53,54] Recently, many studies have reported that innovative nanotechnology has improved biomolecular detection sensitivity, diagnostic accuracy, and treatment efficiency.[55–59]

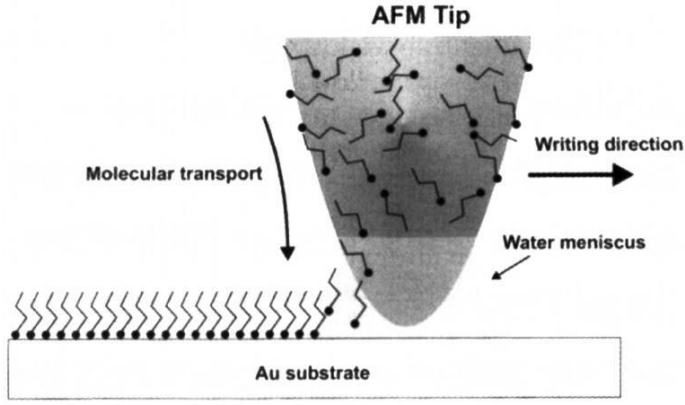

Figure 2. Schematic representation of Dip-Pen Nanolithography. "Ink" molecules coated on the AFM tip are transferred to the Au substrate through a water meniscus. Reprinted from Ref. 52 with permission from AAAS.

1.3.1 Molecular Imaging for Diagnosis and Detection

Optical imaging methods, such as fluorescence microscopy, differential interference contrast microscopy (DICM), and UV-Vis spectroscopy, are one of the most widely used methods for studying biological systems. These methods are simple and highly sensitive, yet tend to have high background noise that is mainly caused by cellular autofluorescence from labeled molecules.[60] Also the lack of quantitative data, requirement for the long observation time, and the loss of signals due to photobleaching in biological systems have initiated a need for new imaging agents. In order to complement conventional optical imaging methods, the approach using nanoprobes is one of the major research efforts in nanomedicine mainly due to their ability to recognize and characterize pre-symptomatic diseases.[61] Typically, the nanoprobes comprise of hybrid organic materials such as nanoliposomes and polymeric nanosystems, or inorganic nanoparticles such as quantum dots and magnetic nanomaterials. Depending on the composition and properties of nanomaterials, they can be further utilized *in vitro*, *ex vivo*, and *in vivo* imaging applications. Typical examples include vast arrays of functions such as cell or DNA labeling, molecular imaging, and angiogenesis as an imaging agent, particularly in tumor tissues.[15]

Quantum dots (QDs) are fluorescent semiconducting nanocrystals that can be used to overcome limitations associated with the

more commonly used organic fluorophores. QDs offer many advantages such as high quantum yields, high molar extinction coefficients, wide range of absorption spectra from UV to near IR, narrow emission spectra, resistance to photobleaching and chemical degradation, and long fluorescence lifetimes (>10 ns). Their unique photophysical properties allow time-gated detection for separating their signal from that of the background noise resulting from cell autofluorescence.[62–65] Quantum dots are typically 2–8 nm in diameter and their shapes and sizes can be customized by modifying the variables — temperature, duration, and ligand molecules — used in their synthesis. Thus by changing their size and composition, it is possible to precisely tune the absorption and emission spectra to increase or decrease the band gap energy. Due to their narrow emission spectra, they can be used effectively in multiplexing experiments where multiple biological units can be labeled simultaneously. One such study was demonstrated by Jain and coworkers.[66] The QDs were applied *in vivo* to spectrally distinguish multiple species within the tumor tissue.[66] More specifically, they demonstrated that QDs can be customized to concurrently image and differentiate the tumor vessels. The group also examined the accessibility to tumor cells depending on the size of QDs. Sizes and compositions of QDs have been extensively studied by many groups.[59,67–70] Wright and coworkers[71] studied QDs with CdSe core with ZnS shell (CdSe/ZnS) and found that they have potent brightness which is advantageous for optical imaging. The group further studied the core/shell QDs conjugated with an antibody against the respiratory syncytial virus (RSV), a virus which is responsible for causing infections in the lower respiratory tract. It was shown that the use of QDs reduced the detection time from over four days to one hour. In fact, the results were very valuable from a therapeutic point of view as the available antiviral agent against the RSV is effective only when administered in the initial stages of the infection. Moreover, QDs enable scientists to study live cells and to track down the mechanism of biological processes in a real-time manner due to their resistance to photobleaching over long periods of time. The ability to track cells *in vivo* without having to sacrifice animals signifies a great improvement over the current techniques. Figures 3 and 4 show two examples of QD imaging: one is the high sensitivity and multicolor capability of QD imaging in live animals and the other is the detection of cancer marker Her2 with QD-streptavidin.[21]

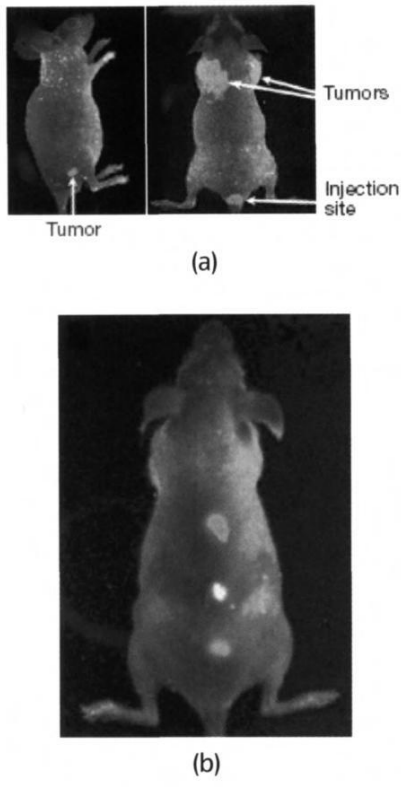

Figure 3. Imaging in live animals using quantum dots (QDs). (a) Molecular targeting and *in vivo* imaging using antibody-(QD) conjugate. (b) *In vivo* imaging of multicolored QD-encoded microbeads. Reprinted from Ref. 62 with permission from Nature Publishing Group. (See page 337 for color illustration.)

Cytotoxicity is a primary issue in QD applications,[72] because the release of Cd^{2+} and Se^{2-} ions from QDs could interfere with cell viability or function.[73,74] While the toxicity may not be critical at low concentrations optimized for labeling, it could be detrimental for the embryo development at higher concentrations. Yet, the problem can be solved by coating the QDs and making them biologically inert or by maintaining a safe concentration range. Several studies have reported that QDs can effectively accomplish their task without adversely affecting cellular processes.[75,76]

Other noble metal nanoparticles, such as Au, Ag, and Cu nanoparticles, are receiving as much attention as QDs, because they exhibit other unique and modifiable optical properties.[77] Typically,

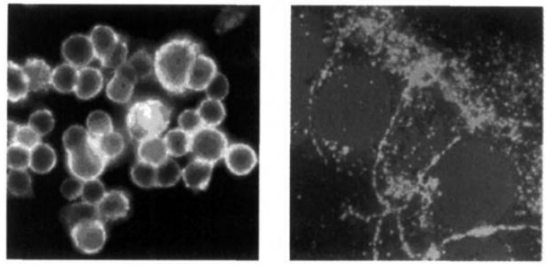

Figure 4. Detection of cancer marker Her2 *in vitro* with QD-streptavidin. (a) Her2 detected on surface of free cells using QD 560-streptavidin (yellow). (b) Her2 detected on a section of mouse mammary tumor tissue using QD 630-streptavidin (red). Reprinted from Ref. 21 with permission from Nature Publishing Group. (See page 337 for color illustration.)

when spherical nanoparticles are exposed to electromagnetic field at a certain frequency, the free electrons on a metal surface, known as plasmons, undergo coherent oscillation, resulting in a strong enhancement of absorption and scattering of electromagnetic radiation. In case of noble metal nanoparticles, the surface plasmon resonance (SPR) of a metal surface especially yields intense colors and unique optical properties.[78] These noble metals have a greater potential for applications than other materials, because the resonance for Au, Ag, and Cu lie at visible frequencies and they have a high stability.[77] Furthermore, Au nanoparticles are insusceptible to photobleaching and can be easily synthesized in a wide range of sizes (4–80 nm) for tunable size-dependent optical properties. They are biocompatible and devoid of cytotoxicity, which are big advantages over QDs where cytotoxicity can be a limiting factor. The use of biocompatible, nontoxic capping material is critical for medical applications of Au nanoparticles.[79] Another reason that makes gold nanoparticles more attractive for optical imaging in biology is the well-defined chemistry between biomolecules with a gold surface. By modifying the surface of Au nanoparticles with an amine or thiol moiety,[80] for instance, the nanoparticles can mount antibodies and specifically target tumor cells and biomolecules, such as folic acid[81,82] and transferrin,[83,84] for imaging and drug/gene delivery. This was well demonstrated in a study by Richards-Kortum and coworkers,[85] where the group used 12 nm Au nanoparticles conjugated with anti-EGFR (epithelial growth factor receptor) monoclonal antibodies

to image cervical epithelial cancer cell which exhibited an over expression of EGFR as compared to healthy cells. In their study, the conjugation was due to the electrostatic adsorption of the antibody molecules onto the citrate-capped and negatively charged Au nanoparticles.

The use of nanowires and nanotubes in the electrical detection method of analytes at extremely low concentration is one of the hot topics in nanomedicine. Their usage has two major advantages — high sensitivity and fast responses without tedious labeling steps. However, these nanostructures are not as readily functionalized as aforementioned quantum dots or nanoparticles. The unique advantages of these nanomaterials come from their one-dimensional morphological structures, and many researchers are trying to utilize them as a highly sensitive and selective signal transduction medium. For example, Lieber and coworkers[86] synthesized silicon nanowires with peptide nucleic acid (PNA) functionalization, and demonstrated how the synthetic material could detect DNA without labeling. A subsequent study modified silicon nanowires with biotin to detect picomolar concentrations of streptavidin and demonstrated high sensitivity to change in conductivity of the nanowires upon the biotin-streptavidin binding. Similar studies have also been carried out by Dai and coworkers,[87] where they focused on the application of carbon nanotubes as a material for the sensitive detection. The scheme (Fig. 5)[86] illustrates a basic structure of electrical detection of biomolecules with nanowire sensor.

Magnetic nanoparticles are emerging as novel contrast reagents for magnetic resonance imaging (MRI),[88–95] revolutionizing current diagnostic tools. Since their unique properties allow precise control of size and composition, magnetic nanocrystals offer great potential

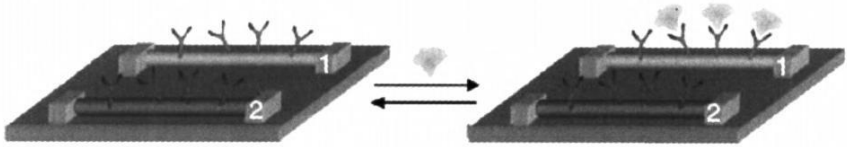

Figure 5. Schematic showing two nanowire devices, 1 and 2, within an array, where nanowires were modified with different (1, green; 2, red) antibody receptors. Reprinted from Ref. 86 with permission from Nature Publishing Group. (See page 338 for color illustration.)

for highly specific MRI for biological systems.[96–99] The nanocrystals tend to behave as a single magnetic domain in which all nuclear spins couple to create a single large magnetic domain. At certain temperatures and crystal sizes, these moments wander randomly (superparamagnetic), or become locked in one direction, making the material ferromagnetic.[88,94] Magnetic nanocrystals of differing compositions and sizes can be synthesized to generate ultra-sensitive molecular images. Cheon and coworkers[98] developed iron oxide magnetic nanoparticles which were doped with +2 cations such as Fe, Co, Ni, or Mn. It was observed that Mn-doped iron oxide nanoparticles were highly sensitive for detecting cancer cells. The nanoparticles even made it possible to image small tumors *in vivo*. In the same study, it was noted that 12 nm Mn-doped nanoparticles were bioconjugated with herceptin to have specificity towards the cancer cells. This approach is expected to improve the early diagnosis of diseases, which is critical for increasing survival rates.

1.3.2 Treatment of Diseases

The motivation to develop nanomedicine stemmed from the limitations of current treatments, such as surgery, radiation, or chemotherapy, which often damage healthy cells as well as tumor cells. To address the problem, novel therapeutics which could passively or actively target cancerous cells were developed. To date many nanomedicines are being administered to treat patients. For instance, nanomedicines such as liposomes (DaunoXome),[100–102] polymer coated liposomes (Doxil),[100,103,104] polymer-protein conjugates (Oncaspar),[105,106] antibodies (Herceptin),[107–109] and nanoparticles (Abraxane)[110] are already bringing clinical benefits to patients around the world. Most of the nanotherapeutics take two main paths to achieve their goals — passive targeting and active targeting.[111]

Rapid vascularization takes place within tumor tissues in order to supply nutrients for fast tumor growth. This inevitably causes the development of a defective, leaky architecture along with damaged lymphatic drainage. This leads to the enhanced permeation and retention (EPR) effect, which helps the injected nanoparticles to preferentially permeate and accumulate in the tumor tissue.[111,112] In order for the passive mechanism to be efficient, the size and surface properties of the nanoparticles should be well controlled. The nanoparticle size should be less than 100 nm, and the surface should

be hydrophilic to prevent the uptake by the macrophages of the reticuloendothelial system (RES), which would significantly improve the circulation half-life of the nanoparticles. This is achieved by coating their surfaces with hydrophilic polymers such as polyethylene glycol (PEG), poloxamers, poloxamines, and polysaccharides[113] since a hydrophilic nanoparticle surface avoids the adsorption of plasma proteins.

Another passive targeting method utilizes the unique environment around the tumor, such as cancer-specific enzymes, high or low pH of the tumor tissue, to release the drug/biomolecules within the tumor tissue.[114] Otherwise inactive nanoparticle-drug conjugate molecules can specifically be activated by the tumor-specific environment when they reach the tumor site. The release takes place within the tumor tissue, considerably increasing the drug efficiency. Yet another method to passively target the tumor is by using direct local delivery of anticancer agents into the tumors. This is an effective technique, but can be highly invasive and the access to certain tumors such as lung cancers may be impossible.[114]

Active targeting is usually achieved by conjugation of targeting moieties such as proteins, peptides, antibodies, and carbohydrates with nanoparticles. These ligands act as guided missiles which deliver the nanoparticles to the specific cancer tissue and cells. For example, when Doxil (liposomal doxorubicin) is attached to an antibody against a growth factor overexpressed in breast cancer (ErbB2), it shows faster and greater tumor regression compared to unmodified Doxil.[115] Similarly, several targeting ligands are available for nanocarriers to deliver drugs at specified locations.[116]

1.3.3 Nanocarriers for Drug Delivery

Nanocarriers, 2 to 100 nm in diameter, are designed to deliver multiple drugs and/or imaging agents.[117] They have high surface to volume ratios which allow high density functionalization of their surfaces with targeting moieties. Promising nanocarriers summarized in Fig. 6 include liposomes, polymeric micelles, dendrimers, carbon nanotubes, and inorganic nanoparticles.[111]

Polymers such as poly lactic acid (PLA) and poly lactic coglycolic acid are the most extensively studied and commonly used materials for nanoparticle-based delivery systems.[57,118–122] Additionally, natural polymers such as chitosan and collagen have

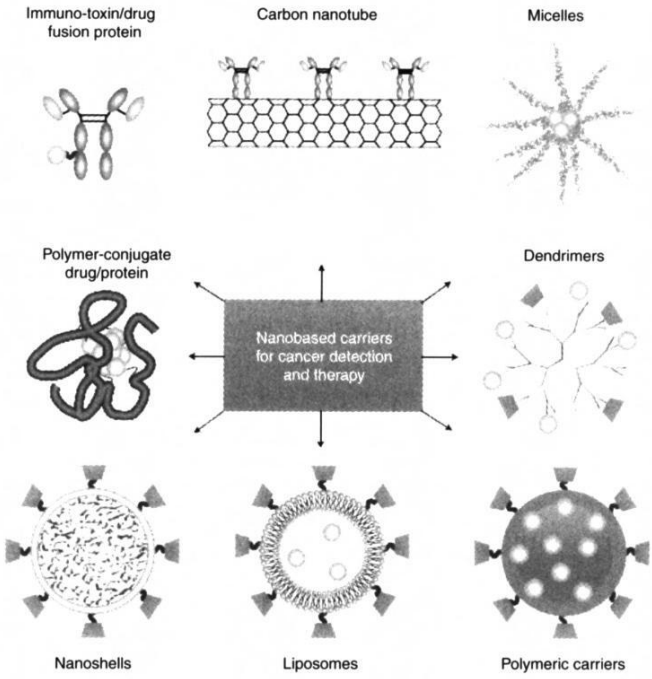

Figure 6. Examples of various drug delivery agents which typically include three components: a nanocarrier, a targeting moiety conjugated to the nanocarrier, and a therapeutic agent. Reprinted from Ref. 111 with permission from the Nature Publishing Group.

already been vigorously studied for encapsulation of drugs with their intrinsic biocompatibility.[123–126] The use of polymers has significant advantages because the drugs or biomolecules can be encapsulated easily without chemical modification processes, while maintaining a high cost efficiency and yield. Release of the encapsulated cargo of the polymeric nanoparticles takes place via diffusion through the polymer matrix, or swelling of the nanoparticles[127–132] due to changes in a local environment such as pH or specific enzyme. Efficiency can be further improved by attaching targeting moieties, and also by passivating the surfaces for longer circulation half-lives. For example, using PEG, Huang and coworkers formulated carbohydrate conjugated chitosan nanoparticles which effectively targeted the liver cancer cells (HepG2). In the study, the carbohydrate used for targeting was galactose, which specifically bound to the asialoglycoprotein (ASGP) receptors overexpressed in the HepG2 cells.[133]

Lipid-based carriers are attractive due to several properties such as biocompatibility, biodegradability, and ability to protect drugs from harsh environments. These properties came from the unique amphiphilic structure of liposomes, where the spherical amphiphiles consist of closed structures that contain one or more concentric lipid bilayers with an inner aqueous phase. Due to the amphiphilic nature, lipid-based carriers can be easily modified to entrap both hydrophobic and hydrophilic drugs.[134–136] However, Liposomes are rapidly cleared from circulation by the Kupffer cells in the liver. Thus, coating them with PEG could increase their circulation half-life.[137,138] Overall, liposomes have shown reduced toxicity and preferential accumulation in the tumor tissue by EPR effect.[127] They could also be actively targeted by attaching antibodies to their surface.

Dendrimers are well defined molecules which are built at a nanoscale with monodispersed systems with sizes ranging from 3 to 10 nm. They have unique molecular architectures and properties, both of which result in easy conjugation chemistry with targeting molecules, making them attractive for the development of nanomedicines.[127,139–142] Several studies have confirmed that conjugated dendrimers can be found concentrated in the tumor and liver tissue in contrast to non-targeted polymer folates. Increased therapeutic activity and marked reduction in toxicity was also observed.[143]

The synthesis of colloidal gold was first reported in 1857.[144] However, it was about 100 years later when scientists found out that the gold particles could bind proteins without altering their activity, paving a way for their application in biological systems. Several studies confirm that gold nanoparticles are easily taken up by cells.[83,145–147] The biocompatibility of gold nanoparticles can be further enhanced by PEG coating that prevents uptake by RES. For example, PEG-coated gold nanoparticle formulation, developed by CytImmune Sciences,[78] which carries the tumor necrosis factor (TNF), is currently under human trials. These gold nanoparticles showed preferential accumulation in MC-38 colon carcinoma tumors and no uptake by liver or spleen. It was further reported that this system is less toxic and more effective in reducing tumor burden than native TNF.[148] Gold nanoparticles are also very useful for delivering nucleic acids. As seen previously, their surface allows for easy functionalization with thiols which makes attachment of

oligonucleotides on the surface relatively easy. In a recent study by Mirkin and coworkers, it was reported that the cellular uptake of gold nanoparticles increased with the increase in density of the oligonucleotides on the particle surface.[149]

Magnetic nanoparticles are extremely promising as novel drug delivery agents.[150] They show several advantages which are not seen in most nanoparticle-based systems. They can resolve problems related to polydispersity and irregular branching, both of which are common in polymeric nanocarriers. Magnetic nanoparticles, for example, can act as contrasting agents for MRI application.[151] Also, drug loaded magnetic nanoparticles can be guided or held in place with applied external magnetic field using their superb magnetic susceptibility. The magnetic nanoparticles can also be heated to induce hyperthermia of the tissue upon exposure to the external magnetic field. The magnetic nanoparticles can be further functionalized with targeting ligands to improve their uptake efficiency, minimize their toxicity, and prevent them from aggregating.[152,153] This is typically done by coating them with hydrophilic to neutral compounds such a PEG, dextran, or HSA, which not only stabilizes the particles but also increases their half-life in blood.

With photothermal cancer therapy, which uses optical heating for tumor ablation, doctors can deal with tumors in a non-invasive manner.[154–159] It could be a method of choice for tumors which are inaccessible for surgery or radiotherapy, both of which kill healthy cells along with tumor cells. For this specific application, gold nanoparticles are most widely used, because the gold nanoparticles have ability to effectively convert strongly absorbed NIR light to localized heat, and hence are useful in selective photothermal therapy. Using active and passive targeting, these nanoparticles can be localized into the tumor tissue, increasing the effectiveness of the heat produced and at the same time minimizing the non-specificity of treatment. In a recent study, Li and coworkers developed gold nanocages (< 45 nm) which were specifically designed to strongly absorb in the NIR region for photothermal therapy.[160] These nanocages were conjugated to anti-HER2 monoclonal antibodies against the epidermal growth factor receptor (EGFR), overexpressed on the surface of breast cancer cells.[160,161] They concluded that the death of cancer cells increased linearly with increase in irradiation power density, thus making the immunogold nanocages effective photothermal therapeutics agents.

1.4 NANOSYSTEMATIC APPROACH FOR CELL BIOLOGY

Cells are single living units of organisms which first receive the input perturbation signals from disease and injury, and then return the output signals to their microenvironments. Conventional experimental studies on particular cellular responses are typically conducted on a large cell population, which inevitably produces data measured from inhomogeneous distribution of cellular responses. Unless cellular behaviors and processes are isolated from inhomogeneous signals at the level of single cell, it would be extremely difficult to elucidate the intricate cellular systems and analyze the complex dynamic signaling transductions. In order to better study and control the responses of cells towards outer stimuli, scientists need to characterize the full range of cell behaviors, such as self-renewal, differentiation, migration, and apoptosis, from the single cell level or even the single molecule level. In particular, understanding how a genotypic aspect affects a cell phenotype is a complicated process, which can barely be revealed by conventional biomedical approaches. In order to understand these processes, the two distinct approaches – bottom-up and top-down – can be applied in a combinatorial way.

1.4.1 Microfluidics & Micropatterns

Microfluidic devices offer a robust analytical approach, allowing rapid analysis of cell assays in a parallel way to investigate complex cell behaviors (Fig. 7).[162–165] Microfluidic devices have advantages over the macroscopic setting, such as reduced sample/reagent volume, high surface-to-volume ratios, an improved control of the physical/chemical microenvironments, and high throughput/automatic capabilities.[166,167] These characteristics would be beneficial for understanding new aspects of complex cell dynamics (e.g. stem cell differentiation and cancer cell apoptosis) and tissue engineering. Although there have been a few examples of microfluidic systems used to culture and assay stem cells,[166–169] the stem cell assays in microfluidic gradients have not been fully explored. Generation of microfluidic gradients have been demonstrated by Whiteside and coworkers,[170] and further studies have been done to study cell behaviors in gradients (Fig. 8).

The use of micro- or nanometer scale patterned surfaces is also one of the useful approaches to study individual cells at

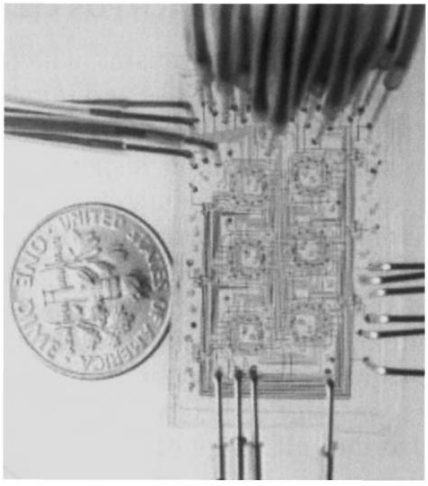

Figure 7. An optical micrograph of a microfluidic device. The coin is 18 mm in diameter. Reprinted from Ref. 171 with permission from AAAS.

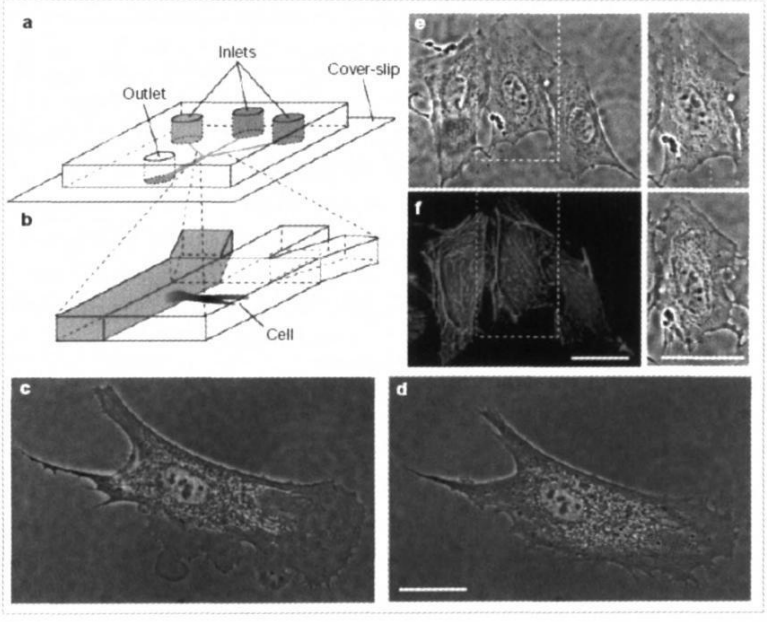

Figure 8. Microfluidics to study a single bovine endothelial cell using multiple laminar streams which deliver membrane permeable molecules to selected subcellular domains. Reprinted from Ref. 170 with permission from Nature Publishing Group.

the single cell level. Typically, the patterns are generated by microcontact printing, where a soft-lithographed material transfers molecules through conformal contact on substrates with planar or non-planar topographies to form SAMs. The key advantage of microcontact printing is that a variety of features can be generated simultaneously on a surface in a single step, thus making patterning easier and faster as compared to scanning probe techniques which require longer time and effort to generate patterns.

1.4.2 Nanopatterning for Stem Cell Research

Although stem cells hold great potential for the treatment of a number of devastating injuries and damages caused by degenerative diseases,[172,173] a better control of microenvironment that closely interacts with stem cells should be achieved before therapeutic applications can be fully realized. It is because stem cell fate is controlled by two prime factors; the intrinsic regulators, such as growth factors and signaling molecules, and the extracellular microenvironments, such as extracellular matrix (ECM). To date, there are few conventional methods available to study regulatory and extracellular microenvironmental cues that control stem cell fate at the single cellular level as well as in a combinatorial way. Stem cells normally reside within specific extracellular microenvironments,[174,175] known as "stem cell niches", comprising a complex mixture of soluble and insoluble ECM signals. The signals regulate stem cell behavior, such as self-renewal, migration, and differentiation.[176–178] For example, cell adhesion and ECM play important roles in early stem cell development. Cell adhesion process, which keeps the inner cell mass intact, is mediated by cadherins and integrins, which are further regulated post-translationally via protein kinase C and other signaling molecules (Fig. 9). The process determines cellular allocation and spatial organization of the inner cell mass (ICM) in the blastocyst.[179] Likewise, *in vivo* stem cells come in contact with various soluble and insoluble ECM components that affect their differentiation. *In vitro* studies also have shown how ECM components and growth factors regulate the differentiation of stem cells.[180] Several combinatorial high-throughput screening approaches on the function of soluble signal molecules on stem cell differentiations have been reported.[181]

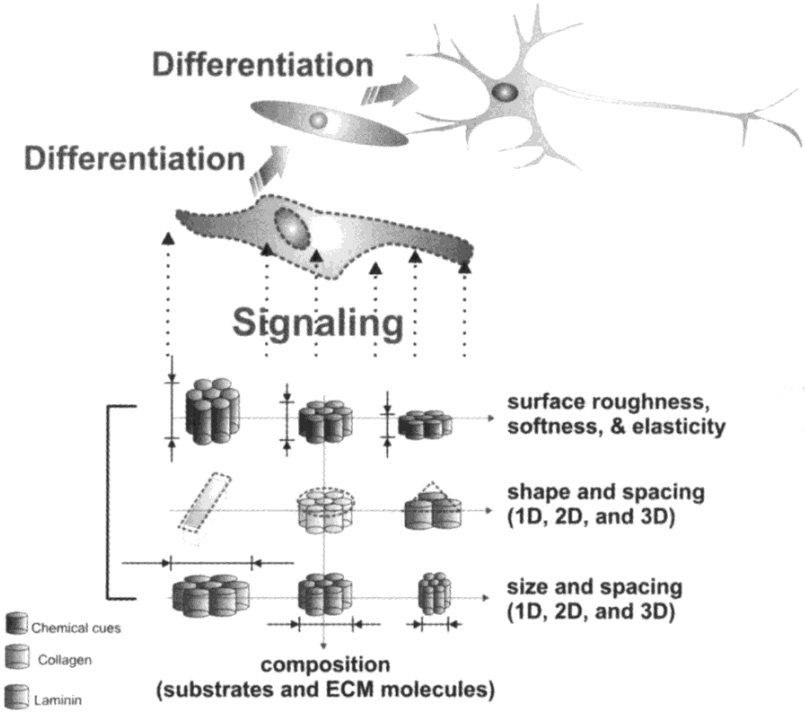

Figure 9. Application of micro-/nanoscale surface engineering in stem cells. Micro- and nanostructures that interact with stem cells at the molecular level can be utilized to control stem cell fate. (See page 338 for color illustration.)

However, similar approaches for screening the function of insoluble cues are limited due to the technical difficulty of identifying, isolating, and modulating individual stem cells from their surroundings. For instance, an extracellular matrix array format fabricated by conventional macro/micro patterning techniques has been used to probe cellular differentiation and migration.[182,183] Very recently, a combinatorial ECM microarray was used to investigate the role of the ECM components in the differentiation of mouse embryonic stem cells (mESCs) towards an early hepatic fate.[182] This technology required 1000 times less protein than a conventional method. There still is plenty of room for improvement in this approach in terms of pattern density, recognition sensitivity, and small sample requirement. In addition, relatively little is known about the subcellular interaction mechanisms between stem cells and ECM molecules and how such events eventually influence stem cell differentiation and

migration. Moreover, it is still unknown how insoluble or soluble extrinsic signaling molecules identify their molecular target proteins and receptors at the single molecule level. The application of nanotechnology in stem cell biology will help to address those challenges.

1.5 CONCLUSION

Nanomedicine is where traditional biomedical science meets nanotechnology. The synergetic effect offers new possibilities in diagnosis and treatment of many malicious human diseases. Moreover, the advancement in nanomedicine allows scientists in cell biology and physiology to investigate targeted bio-interactions at the fundamental molecular level. However, for the potential of nanomedicine to be fully realized, multimodal technologies for cell recognition and molecular imaging, and targeted delivery should be developed. This challenge requires an interdisciplinary approach from a high level of expertise in each field of science. In the near future nanomedicine may bring revolutionary breakthroughs in not only the entire field of medicine, but also in fundamental biology.

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