Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2012, Article ID 789841, 14 pages
doi:10.1155/2012/789841

Review Article

Microfluidic Platforms for Evaluation of Nanobiomaterials: A Review

Venkataraman Giridharan,[1] YeoHeung Yun,[1] Peter Hajdu,[2] Laura Conforti,[2] Boyce Collins,[1] Yongseok Jang,[1] and Jagannathan Sankar[1]

[1] *Engineering Research Center, North Carolina A&T State University, Greensboro, NC 27411, USA*
[2] *College of Medicine, University of Cincinnati, Cincinnati, OH 45267, USA*

Correspondence should be addressed to Jagannathan Sankar, sankar@ncat.edu

Received 18 February 2012; Accepted 19 April 2012

Academic Editor: Haifeng Chen

Biomaterials, especially those based on nanomaterials, have emerged as critical tools in biomedical applications. The applications encompass a wide range such as implantable devices, tissue regeneration, drug delivery, diagnostic systems, and molecular printing. The type of materials used also covers a wide range: metals (permanent and degradable), polymers (permanent and degradable), carbon nanotubes, and lipid nanoparticles. This paper explores the use of microfluidic platforms as a high-throughput research tool for the evaluation of nanobiomaterials. Typical screening of such materials involves cell/tissue cultures to determine attributes such as cell adhesion, proliferation, differentiation, as well as biocompatibility. In addition to this, other areas such as drug delivery and toxicity can also be evaluated via microfluidics. Traditional approach for screening of such materials is very time-consuming, and a lot of animals should be sacrificed since it involves one material and a single composition or concentration for a single test. The microfluidics approach has the advantage of using multiple types of drugs and their concentration gradients to simultaneously study the effect on the nanobiomaterial and its interaction with cell/tissue. In addition to this, microfluidics provides a unique environment to study the effect of cell-to-extracellular interaction and cell-to-cell communication in the presence of the nanobiomaterials.

1. Introduction

The field of microfluidics has seen great advances in the past decade. Two important application areas of microfluidics have been the use of it as a platform for medical diagnostics and biosensing. Microfluidic platforms like lab-on-a-chip have the advantage of using very small quantities of reagents, typically in the nanoliter to microliter range. This feature is very valuable in medical diagnoses like cell-based assays, drug screening, and screening for diseases [1–9]. Microfluidic devices are especially useful for cell-based assays because of the comparable scale of microfluidic channels and cells. In addition to this, the scale of the devices allows for things like growth factors to accumulate and form a stable environment for cell culture. Microfluidics also presents the potential to influence stem cell research, particularly for high-throughput analysis of signals that affect stem cells [4]. In light of this potential, one of the leading areas for microfluidics research will be its use as a platform for nanobiomaterials-based cell cultures [1–6, 10–20]. Such a high-throughput method will be a key factor in the future in enabling more realistic predictions of nanobiomaterial behavior in relation to cell toxicity, cell proliferation, and differentiation. This predictive capability is very important for preclinical methods since this has a direct impact on improving the attrition rates of candidate materials during clinical testing [21, 22].

This paper reviews the use of microfluidics as a high-throughput analysis platform for evaluation of nanobiomaterials. First, we provide a brief overview of microfluidic devices and their advantages over traditional cell culture technology. The following section discusses nanobiomaterials and some of their biomedical applications. Section 3 discusses the advances in microfluidic technology and how it is being applied as a high-throughput method in areas such

as material screening, toxicity testing, and drug discovery. Finally, Section 4 reviews research performed in evaluating nanobiomaterials using microfluidics. We conclude the paper with suggestions for next steps in specific areas.

2. Overview of Nanobiomaterials and Their Applications

Nanomaterials can provide the cells with the desired matrices that mimic the native environment of the cells. Usage of these materials includes hip replacements, fracture plates, bioresorbable sutures, tissue engineering scaffolds, and drug delivery devices [24–35]. Nanomaterials which mimic the matrix composition of the body are important regulator of stem cell differentiation towards specific cell lineages. These materials provide sites for cell adhesion and initiation of matrix-generated signal transduction pathways.

A nanobiomaterial can be defined as a biomaterial substrate composed of nanometer scale components. One example of a naturally occurring nanobiomaterial is inorganic bone matrix which is composed of hydroxyapatite crystals. Significant property changes can occur at the nanoscale, especially related to surface energy and reactivity. There are more atoms at the surface of nanostructured biomaterials which results in a marked increase in surface area to volume ratio when compared to micron scale biomaterials. Correlations of surface properties with stability, toxicity, and biodistributions are essential for *in vivo* applications [36]. This is very important considering that, for example, cell adhesion, proliferation, and migration during tissue repair are dependent on protein adsorption on the surface of implanted biomaterials.

The application potential of nanobiomaterials varies widely from tissue engineering to biosensing and diagnostics to drug delivery and disease therapy. Examples of some such applications are (a) nanohydroxyapatite for orthopedic implants and drug carriers for bone diseases, (b) carbon nanotubes and nanofibers as novel drug delivery devices, (c) gold nanoparticles for cancer diagnostics, and (d) quantum dots as biological sensors [37–43]. The use of carbon nanotubes and various types of nanoparticles in medicine is very prevalent in research and hence described in separate sections here.

2.1. Carbon Nanotubes in Medicine. Carbon nanomaterials are being used to develop the next generation of biomaterials for applications in therapeutics and regenerative medicine. Carbon nanomaterials, mainly in the form of nanotubes and graphene, have become the focus of intensive research because of their unique physical and chemical properties such as their hollow structure, their high surface area-to-volume ratio, electrical conductance, thermal conductivity, mechanical stiffness, and the possibilities of functionalizing them to change their intrinsic properties. Functionalization can increase their solubility and biocompatibility under physiological conditions. The nanomaterials can be further conjugated with specific biomolecules such as polymers, peptides, proteins, nucleic acids, and other therapeutic agents, which can target specific types of cells, tissue, and organs.

Carbon nanomaterials demonstrate several significant features that have promise for use in nervous system repair. Carbon nanomaterials have the type of nanosurface features that have been demonstrated to encourage nervous tissue regeneration, including the physical shape (a linear geometry), the nanoscale surface topology, and the high aspect ratio of nanomolecules or larger structures made from carbon nanotubes, like carbon nanotube thread. These closely resemble the microenvironment that nerve fibers migrate along during embryonic development and regeneration. Carbon nanomaterials also offer high mechanical strength to support process outgrowth and flexibility to avoid further damage of soft surrounding tissues during movement. Two applications of carbon nanomaterials of especial interest to neurobiologists are discussed here, their use in scaffolds to repair damaged nervous tissues, and their use as biocompatible electrodes for recording from or stimulating nervous tissues.

2.2. Nanoparticles in Medicine. Naturally occurring nanoparticles (NPs; such as LDL, HDL, and VDL), which are endogenously produced in developed organism, are utilized in transportation of hydrophobic molecules such as cholesterol and triglycerides into various parts of the body via the circulation. In the past twenty years, several groups started to develop various nanoparticles that can be an appropriate tool in diagnostics as well as cell/tissue-specific therapy. At present, nanoparticles are applied in several field of medicine: cancer treatment, high-resolution imaging, siRNA-based gene therapy and so forth. In this section, we give a brief summary on the up-to-date knowledge on NPs used in medicine.

At the dawn of the application of nanometer-sized particles, liposomes, which encapsulated highly hydrophobic anticancer agents (doxorubicin, daunorubicin), were used to deliver these compounds via circulation to the tumor cells. Due to passive distribution of these lipid vesicles in the body, toxic side-effect might occur causing arrhythmia, which could lead to heart failure. To overcome this problem, active targeting of lipid vehicles has been extensively studied and advanced: antibodies or their fragments, oligopeptides, nucleic acids (aptamers), small molecules, or others (vitamins or carbohydrates) are conjugated to the surface of nanoparticles [45].

In the presence of malignant tumors, hypocholesterolemia may arise due to the enhanced LDL uptake of cancerous cells. Thus, LDL vesicles, as naturally targeted lipoprotein vesicles, have been studied in diagnostics and treatment of cancer for several years: (1) LDL particles loaded/conjugated with photosensitizers are applied in photodynamic therapy; (2) ^{99m}Tc, ^{131}I, ^{125}I, or ^{68}Ga/^{111}. In labeled LDLs were used in *in vivo* tumor detection with gamma camera or PET (positron emission tomography) in animal models, respectively; (3) with the help of Gd or iron-loaded LDL vesicles could adequately image the boundary of the tumor with MRI [46–52]. It was also demonstrated that HDL

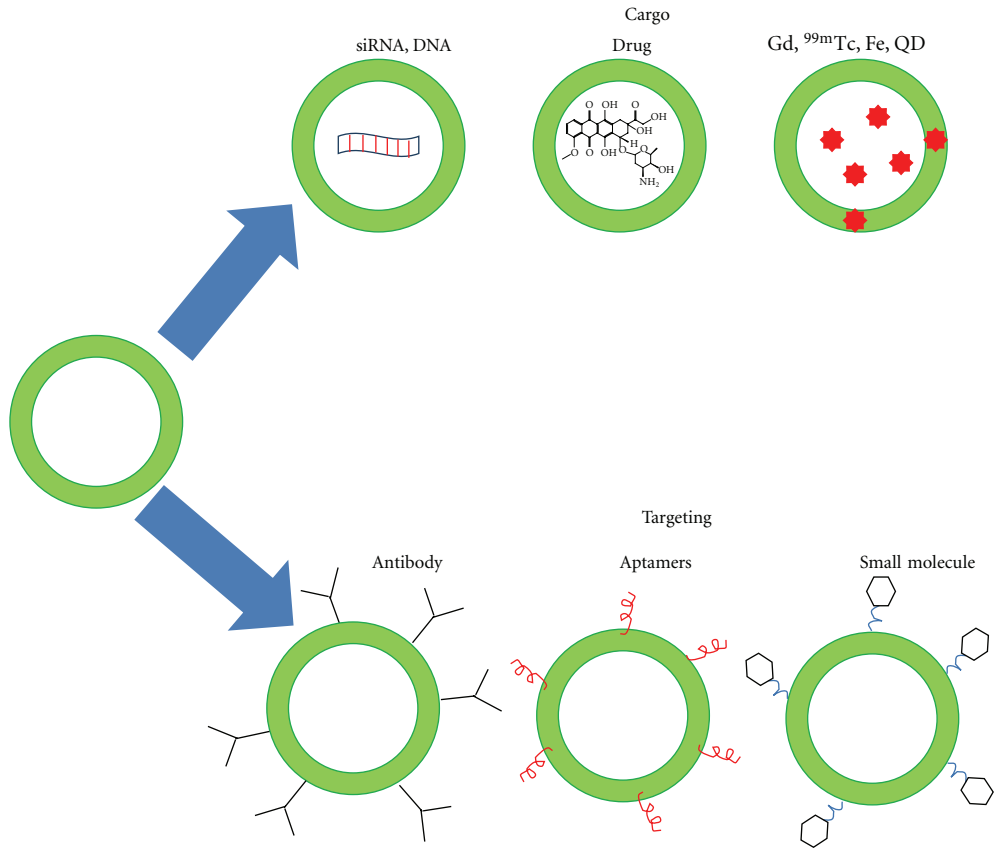


Figure 1: Classification of lipid-based nanovesicles mainly used in medicine.

cholesterol level was lower in certain malignancies, which can be also attributed to increased cholesterol consumption of cancer cells. Since several cancer cell lines express scavenger receptor class B type I (SR-BI), which is responsible for HDL uptake, anticancer agent loaded HDL or HDL mimicking, targeted lipid-protein vesicles can be used to deliver their cargo the tumor cells [53–55]. Furthermore, nanovesicles can also be applied to boost the immune system: injections of anticancer liposome vaccine resulted in hindered E.G7-OVE tumor progression in mice, and, in a clinical study, the survival time of patients with nonsmall cell lung cancer was longer compared to control group [56–58].

Atherosclerosis is the chief risk factor in the onset of cardiovascular diseases, which is a leading cause of death in the adult population and characterized by a complex etiology. In a recent paper, Lewis et al. reviewed several novel, nanometer-sized particle approaches on the therapy and detection of atherosclerotic plaques in model system or in human (Figure 1) [59].

2.3. TiO_2 Photocatalyst in Medicine. Biomedical research applications of titania (TiO_2) have primarily focused on harnessing its potential as a photocatalyst, specifically its ability to perform oxidative or reductive chemistry under illumination of UV light [61, 62]. Recent advances in doping, nanoparticle assembly, and dye conjugation have extended this photochemistry into the visible [63, 64]. Applications in biomedical field are becoming more routine due in part to the use of titania as an implant material and a desire to modify the surface of the titanium and make the implant "smarter." For example, photolysis of TiO_2 and nanotube TiO_2 with X-ray radiation was performed to demonstrate a possible drug release methodology [65]. In another study, precursors to hydroxyapatite formation were embedded in TiO_2 nanotubes and shown to influence bone formation in adult pig model [66]. Titania particles have also been used in disinfection applications and the killing of cancer cells [67, 68]. The ability to vary TiO_2 properties, morphology, and surface functionalities as a result of years of materials research allows for a wide range of possible medical benefits.

3. Microfluidics Devices for High-Throughput Analysis of Nanobiomaterials

There are numerous parameters of nanobiomaterials that can affect cellular behavior. The complexity of interacting parameters is one of the main motivations for the high-throughput screening of nanobiomaterials.

3.1. Introduction to Microfluidics. Microfluidic devices that enable high-throughput analysis have typically been produced using photoassisted and soft lithography techniques.

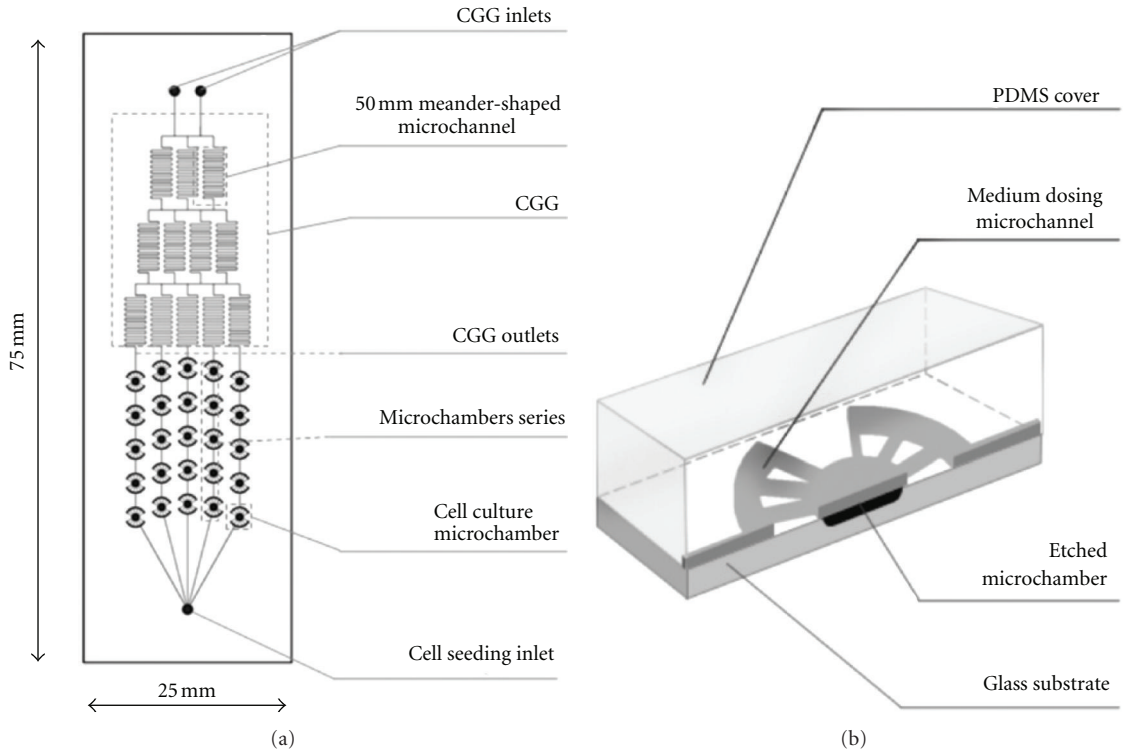


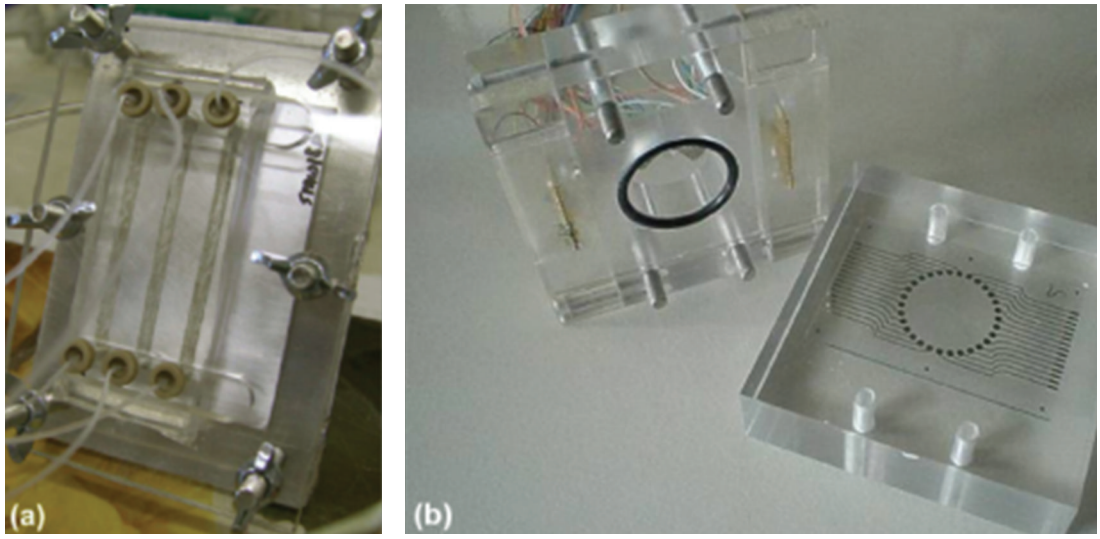
Figure 2: (a) The geometry of a microfluidic cell culture array for high-throughput analysis, (b) cross-section of cell culture microchamber [23] (reprinted from [23]).

An increasing demand for fully automated and quantitative cell culture technology has resulted in the development of microfluidic chip-based arrays. Compared to traditional culture tools, microfluidic platforms provide much greater control over cell microenvironment and rapid optimization of media composition using relatively small numbers of cells. Because a group of cells can more easily maintain a local environment within microchannels than in traditional culture flasks, cells grow significantly slower in microchannels than cells in traditional culture flasks [23, 70]. Figure 2 shows a typical microfluidic cell culture array for high-throughput analysis. The bonded PDMS cell culture microchambers provide a stable and uniform microenvironment for the cells [23].

3.2. Microfluidic Assays for Implant Material Screening. Recent advances in microfluidic techniques have increased the potential of high-throughput biochemical assays on individual mammalian cells. A microfluidic assay for bacterial chemotaxis was developed, in which a gradient of chemoeffectors was established inside a microchannel via diffusion between parallel streams of liquid in laminar flow [19, 71]. Bacterial adhesion often occurs in implant surgeries. Biomaterial-related infection starts with the adhesion of infectious bacteria, which is considered as one of the main causes of failure in implant surgery. Bacterial adhesion to surfaces is usually present in aqueous flows since such flows can promote the transport of microorganisms to surfaces. Considering that nanomaterial surfaces can be extremely reactive, the problem of bacterial adhesion becomes even more important. A microfluidic flow system was utilized to investigate the behaviors of biological cells under various flow conditions. This system offers precise kinetic control of the cellular microenvironment [20]. Microfluidics can also be applied for high-throughput and combinatorial electrochemistry where numerous channels operating in parallel can provide for synthesis of large arrays of materials and subsequently characterized for corrosion properties [44]. Figure 3 shows examples of such systems.

Microfluidic biochips offer the advantage of being able to include a whole new set of technologies that can preserve cellular function *in vitro* over a long period of time. This in turn would allow the proposal of relevant and alternative models to reduce the animal experimentation and their costs. They offer the possibility of dynamic cultures and kinetic studies on microengineered tissues simulating the cellular organizations that are found *in vivo*. This would minimize animal use as well as offer assays more relevant than traditional techniques [73].

3.3. Microfluidics Screening in Tissue Engineering. Applications of microfluidic systems based on cell and tissue culture are now emerging, as platforms for high-throughput screening, drug discovery, and toxicity testing. A new generation of microfluidics-based approaches are designed for specific tissue and organ applications, incorporating microvascular



(a) (b)

Figure 3: (a) Microfluidic assembly for high-throughput corrosion experiments, (b) multielectrode electrochemical testing cell [44] (reprinted [44]).

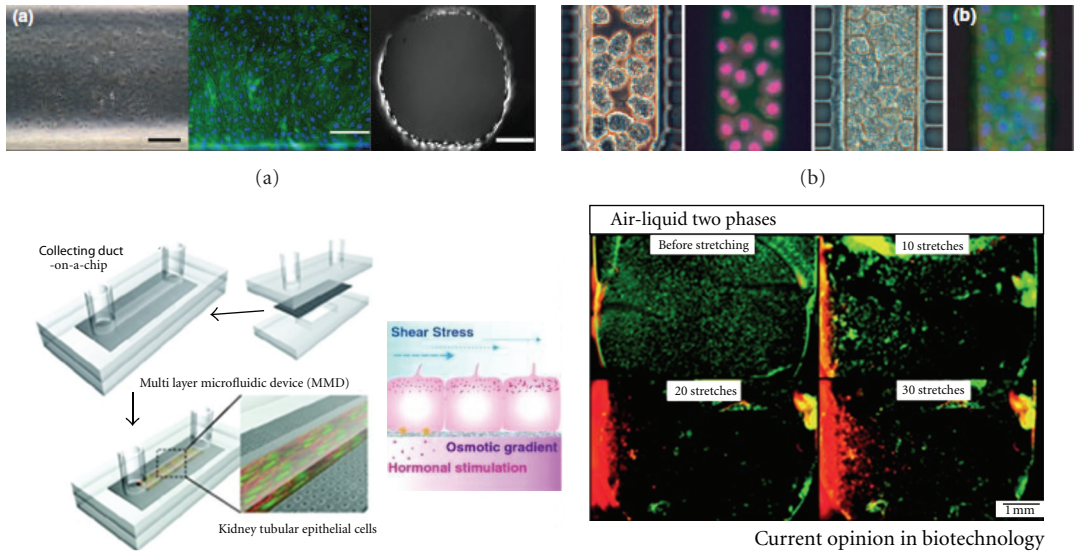


Figure 4: Organ-specific tissue-engineered microfluidic devices: (a) vasculature, (b) liver sinusoid, (c) renal tubule, and (d) alveolar fluid-liquid interface [16] (reprinted from [16]).

networks, structures for transport and filtration, and a three-dimensional microenvironment suitable for supporting phenotypic cell behavior, tissue function, and implantation, and host integration [16]. Figure 4 shows a range of such devices.

Microvascular networks are key sites for many of cell-cell and particle-cell interactions during physiological and/or pathological processes. Advances in targeted drug delivery to the microvasculature often involve encapsulating drugs in delivery vehicles ranging from microparticles to nanoparticles. Development of *in vitro* microfluidic devices to mimic these microcirculatory processes has been a critical step forward in our understanding of the inflammatory process, developing of nanoparticulate drug carriers, and developing realistic *in vitro* models of the microvasculature and its surrounding tissue [17].

3.4. Microfluidics with Microarrays for High-Throughput Multiplexing. A microarray consists of a support onto which hundreds to thousands of different molecular reporter probes are attached or immobilized at fixed locations in

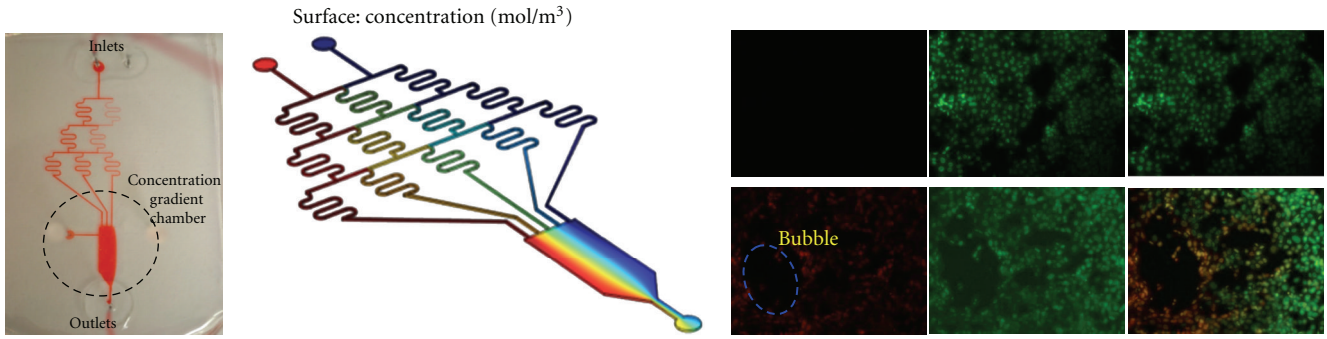


Figure 5: (a) Layout of microfluidic gradient mixer used to generate a linear concentration gradient. (b) Linear concentration gradient obtained from a three-dimensional numerical simulation using COMSOL. (c) Osteoblast cell live/dead assay using the microfluidic network shown above.

either a two-dimensional or three-dimensional format. The multiplexing capabilities of microarray-based assays are produced by spatially encoding the array, in which each location on the array is used as a reporter of a specific analyte. For conventional microarrays, shortcomings include (a) the analyte binding process on supports requires long incubation times to produce the optimal signal due to slow diffusional kinetics and (b) consumption of large amounts of precious sample material for interrogating the array due to the large area occupied by high-density arrays. The use of microfluidic platforms can directly address these issues as well as offer the potential for parallel processing of multiple samples [5].

Chemotaxis is the directional migration of cells in response to chemical gradients of molecules called chemoattractants. The process is crucial in numerous biological processes. Most of the traditional methods (e.g., different types of chambers or puffer pipettes) are nonideal in that the generated gradients are created in macroscopic environments, are nonlinear, and change with time in an uncontrolled manner. By making use of microfluidic gradient generators, chemotaxis studies can be carried out with precise spatial and temporal control of the chemical environment around cells [6, 76]. Figure 5 shows an osteoblast cell live/dead assay using a microfluidic gradient generator. Such a setup can be used for screening of cell interaction with nanomaterials in the presence of growth factors.

4. Evaluation of Nanobiomaterials Using Microfluidics

4.1. Combinatorial Screening. The application of combinatorial approaches to the discovery of new nanomaterials provides exciting opportunities to produce materials designed to give optimal performance for specific applications. This process has been facilitated by the development of the automated fabrication and analysis of polymer microarrays, produced largely by contact or ink-jet printing. Studies have focused on the discovery of materials that support cell attachment for particular cell types. In addition to applications of microarrays in screening for desirable material properties, the size of the sample set provides enormous potential to be able to elucidate key underlying principles that govern biological-material interactions [2, 77–79]. As mentioned previously in Section 2, the use of microfluidics in conjunction with microarrays enables a high-throughput mode for screening of nanomaterials and their interactions with the cell environment.

Cell-based assays are currently considered central to toxicity testing and biomaterials testing [6, 16, 81–86]. Despite the frequent lack of correlation between *in vivo* models and *in vivo* observations, cell models still seek validation as a useful screening bridge between materials quality analysis and *in vivo* deployment. Advances in high-throughput methods using microfluidics allow for toxicity and efficacy screening of multiple nanomaterials at multiple concentrations with multiple cell lines, simultaneously. By assaying numerous material types/functionalizations and material concentrations on numerous cell types, all in parallel, complex interactions between materials and cells may be ascertained through data analysis [87]. Nanomaterials for biomedical applications exhibit extremely high specific surface areas exposed to physiological environments. Due to the extremely reactive nature of nanomaterials surfaces, extensive characterization and correlation of nanophase surface properties with their stability, toxicity, and distributions are essential for *in vivo* applications [36, 60].

One such study used surface chemistry, nanostructures, and microfluidics to create a set of tools applicable for problems ranging from molecular to cellular analysis. Microfluidics allows the precise manipulation of fluids down to the nanoliter scale, and this is important since most biochemical processes take place in the aqueous phase. Surface gradients with arbitrary shapes from virtually any type of biomolecules can be formed by transferring the gradients in solution to a surface by adsorption as shown in Figure 6 below. One example presented in this study is that of axon specification during differentiation of neuritis into axons and dendrites. Microfluidic gradient generators similar to the one shown in Figure 6 were used to generate gradients of laminin in solution and transferred to the substrate by physical adsorption. The study indicates that

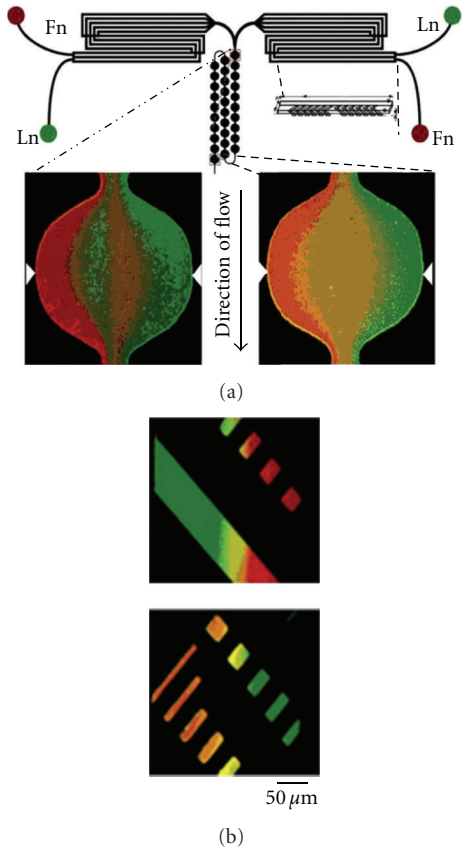


Figure 6: (a) Generation of complex gradients in solution by combining two microfluidic generators. (b) Transferring the gradient in solution into a gradient on surfaces through adsorption [60] (reprinted from [60]).

axon specification orients in the direction of increasing laminin surface density [60].

4.2. Microfluidic Evaluation in Tissue Engineering. A microfluidic 3D tissue model was used to evaluate the efficacy of PLGA micropatterns for promoting osteogenic development by osteoblasts and preventing biofilm formation. This study demonstrated the tremendous efficiency of the tissue model approach by significantly reducing the number of samples and experiments required to assess the *in vitro* efficacy of the micropatterns by conventional biofilm and cell culture experiments. This approach provided an ability to directly monitor how the 3D tissue development was positively influenced by the presence of biphasic calcium phosphate nanoparticles in the micropattern [15, 89]. Another study looked at biofilm-related infection of orthopedic implants in physiologically relevant microenvironments using a multichannel microfluidic device. It was used to observe in real time the development of osteoblasts into three-dimensional (3D) tissue-like structures how this development was influenced by phenotypes of a specific bacteria [90]. The microfluidics approach has also been used to build a three-dimensional heterogeneous multilayer tissue-like structure inside microchannels. Patterning of biological structures can be achieved not only on the surface but also over the thickness of the construct. The tissue formed from different types of cells and biopolymer components can be engineered to model layered *in vivo* living systems. This approach provides a novel solution to fabricate hybrid biopolymers and hierarchical tissue structures for tissue engineering and basic cell biology [91]. One of the keys to tissue engineering and cell therapy is the ability to identify materials that support cell adhesion, proliferation, and differentiation.

One such study utilizes microfluidics for nanoliter-scale synthesis of materials and simultaneous characterization of their interaction with embryonic stem cells using this high-throughput approach. This study simultaneously characterizes over 1700 embryonic stem cell material interactions. The identification of materials that selectively support the growth of specific cell types could be useful for the creation of complex tissue-engineered constructs. The proof-of-concept study was carried out with hES cells, and it identified polymers that allow for varying levels of hES cell attachment and spreading, cell-type specific growth, and growth factor-specific proliferation [92]. There are other studies that discuss the characterization of biomaterial interaction with stem cells using microfluidic approaches [3, 13, 93]. These approaches can be adopted for characterization of nanobiomaterials as well.

Another study describes a highly parallel cell-based microfluidic device where biomolecules are transported via mobile substrates, like micro/nano beads or cells. Such dynamic microarrays present several advantages over static microarrays like the ability to mix and match the beads or the cells to cater for the type of screening to be performed and introduce them into the microarray on demand. The beads or cells can be replaced, thus resulting in a reusable format that greatly reduces the cost of operation. The reaction on beads tends to be faster compared to conventional planar surfaces, as micro/nano beads have increased surface area to volume ratio and hence higher binding capacity. Combining such a device with automation will allow for high-throughput screening in an environment that closely mimics cell-cell interactions found in animals [69]. Figure 7 shows the potential contribution of MEMS and nanobiotechnology to society.

4.3. Nanobiomaterials Evaluation for Diagnostics and Sensors. A number of studies have focused on microfluidic and nanofluidic devices as diagnostic tools for cancer and other infectious diseases [29, 72, 94–97]. Inorganic nanoparticles, semiconductor quantum dots (QDs), carbon nanotubes, polymeric nanoparticles, as well as cantilevers and nanochips, all have the potential to be useful in the design of sensitive pathogen diagnostics [98]. The fabrication of nanodevices as probes is complex, most likely the assembly of building blocks including nanoparticles, nanowires, nanotubes, and substrates. Typical examples of nanodevices are nanofluidic arrays and protein nanobiochips. One of the most promising uses of nanofluidic devices is isolation and analysis of individual biomolecules, such as DNA, which could lead to new detection schemes for cancer. Devices

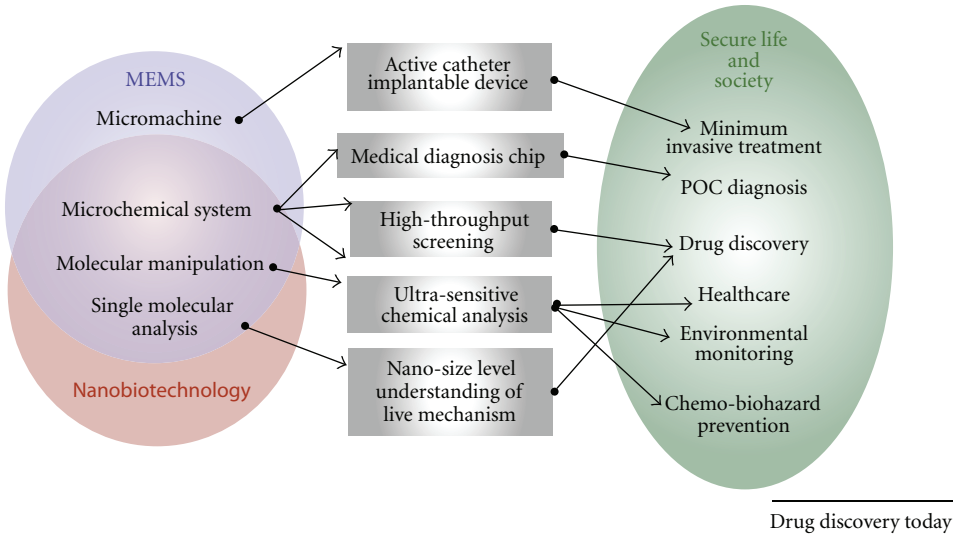


Figure 7: Contribution of MEMS and nanobiotechnology to the quality of life for society in the future through medical and pharmacological applications [69] (reprinted from [69]).

based on nanowires are emerging as a powerful and general platform for ultrasensitive, direct electrical detection of biological and chemical species [95]. Nanowire sensors may be formed into dense circuits which can be constructed within microfluidics environments, creating very dense sensor libraries. These enable measurements of many different genes and proteins from very small tissue samples or even single cells [97]. Nano/microfluidic diagnostic technologies are potentially applicable to global health applications, since they are disposable, inexpensive, portable, and easy-to-use for detection of infectious diseases. Nano/microfluidic technologies have been successfully integrated with current POC devices for on-chip diagnosis and monitoring of infectious diseases at resource-limited settings [72, 96]. Figure 8 shows a microfluidic NMR biosensor combined with magnetic nanoparticles.

Another study used microfluidics as an *in vitro* assessment of the cytotoxicity potential of quantum dots. A multicompartmental device was integrated with a syringe pump to establish a flow exposure system. This study enabled the exposure of cell cultures to variable concentrations of QDs simultaneously. The controlled flow conditions mimicked *in vivo* physiological conditions very closely. The results were compared to those from static exposure conditions. Both static and flow conditions are illustrated in Figure 9 below. The static exposure of cells to QDs resulted in a higher percentage of cell death and an increased number of detached and deformed cells. This study demonstrated the efficient utilization of microfluidic technology in nanotoxicity research [74].

Another QD study involved tracking the mechanism of nerve growth factor (NGF) signal propagation from the axon terminal to cell body. Axonal transport of NGF signals is critical to survival, differentiation, and maintenance of peripheral sympathetic and sensory neurons. One set of hypotheses for retrograde axonal transport of NGF states that NGF and its signaling proteins are transported in complex vesicles such as multivesicular bodies, lysosomes, or macropinosomes. To study this, dorsal root ganglion (DRG) neurons were cultured in a microfluidic chamber (shown in Figure 10 below) and treated with quantum dot-labeled NGF (QD-NGF). The conclusion from this study was that small vesicles (50–150 nm) are responsible for most retrograde axonal transport of QD-NGF in DRG axons [75].

In a third study involving QDs, an integrated microfluidic device capable of screening an anticancer drug has been presented by analyzing apoptotic cells using biofunctionalized QDs. The cell immobilizing structures and gradient-generating channels were integrated within the device. The technique utilizes Annexin V conjugated quantum dots as apoptosis detection probes as shown in Figure 11. This technique can bridge the gap between the quantum dots based *in vitro* cell imaging, and the analysis of individual apoptotic cell in a microfluidic system allows an easy operating protocol to screen some clinically available anticancer drugs [80].

Liposome nanoparticles have been evaluated for formulation composition and stability using a microfluidic biochip. Changes in size and surface chemistry of these nanoparticles are important since they can significantly alter *in vivo* distributions of these nanoparticles which affect therapeutic outcomes. The biochip was embedded with dielectric microsensors which enabled quantitative measurements of formulation using unique electrical properties of liposomes [99]. In another study, microfluidics has been used as a platform for monitoring lipid vesicle membrane permeability to tetracyclines. This approach allows for use of artificial nanovesicles to study the influence on permeability. Liposomes are immobilized onto the glass surface in a stripe pattern via an avidin-biotin bond. The biggest advantage provided by microfluidics in this case is the ability to reliably resemble an *in vivo* environment. The fluid flow provides

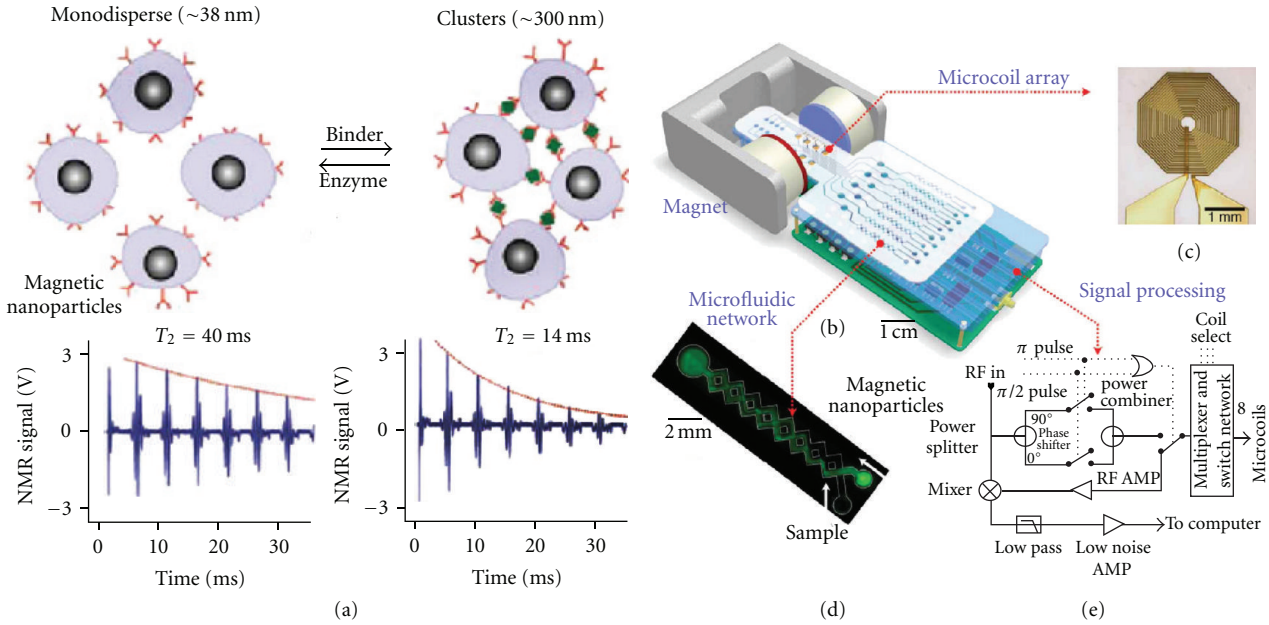


Figure 8: Microfluidic NMR biosensor combined with magnetic nanoparticles for potential applications of TB testing in resource-limited settings [72] (reprinted from [72]).

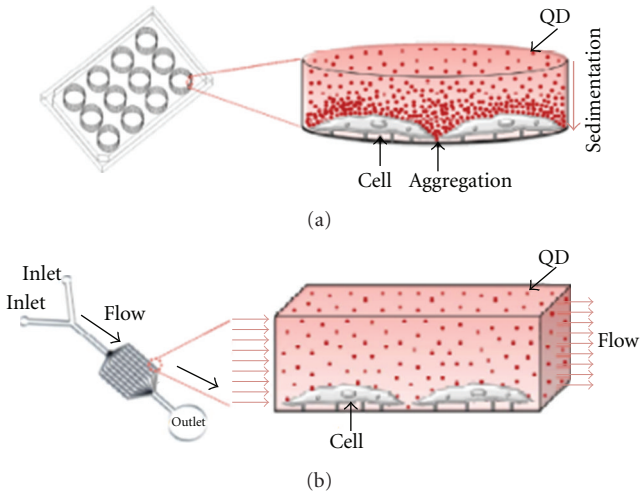


Figure 9: Schematic illustration of (a) static exposure resulting in sedimentation of quantum dots and (b) flow exposure resulting in homogeneous distribution of quantum dots [74] (reprinted from [74]).

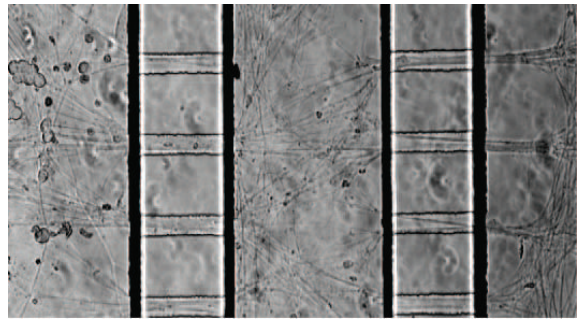


Figure 10: A representative image of DRG neurons cultured inside a microfluidic device. Axons were able to extend across two columns of microgrooves into the distal axon chamber [75] (reprinted from [75]).

a constant concentration profile and thereby resembles the drug transport via blood in the human body. Additionally, many different drug concentrations and pH conditions can be investigated in parallel with this approach. It allows the measurements of slow and fast kinetics with a good temporal resolution, requires only short measuring times, consumes very small volumes of drug solution and vesicle suspension, and allows sensitive detection at low concentrations using TIRF microscopy [88]. Figure 12 shows the setup used in this study.

Synthesis of nanoscale lipid vesicles using microfluidic channels has been studied where the device geometry and flow rate have been used to influence the vesicle size. This method enables a reliable control over vesicle size and homogeneity when compared to bulk liquid synthesis techniques [100].

5. Conclusions and Future Work

In summary, the use of microfluidics as a platform for biomedical research and applications is very widespread. The specific use of microfluidic devices in conjunction with nanobiomaterials is becoming an established method for fast-throughput analysis in biomedical engineering. The usage covers cell-based assays for toxicity and biocompatibility screening of materials, tissue engineering and cell therapy,

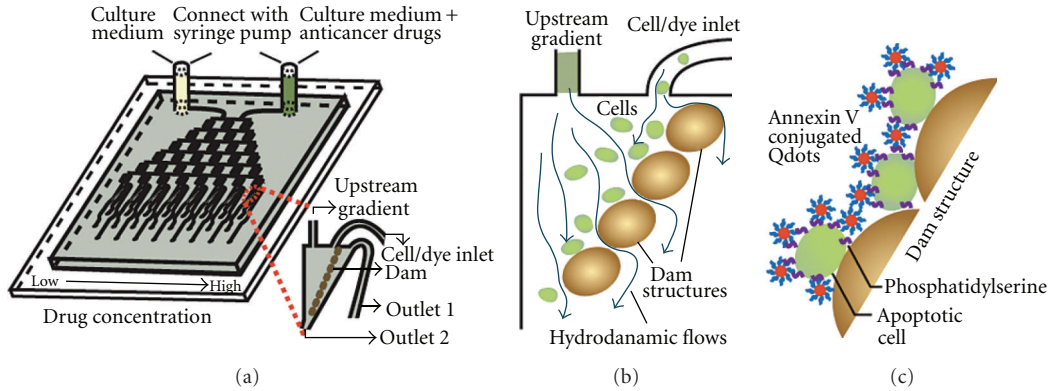


FIGURE 11: Schematic drawing of (a) microfluidic gradient generator, (b) cell trapping on sand-bag structures, and (c) detection of apoptotic cells immobilized on dam structures using Annexin V conjugated QDs [80] (reprinted from [80]).

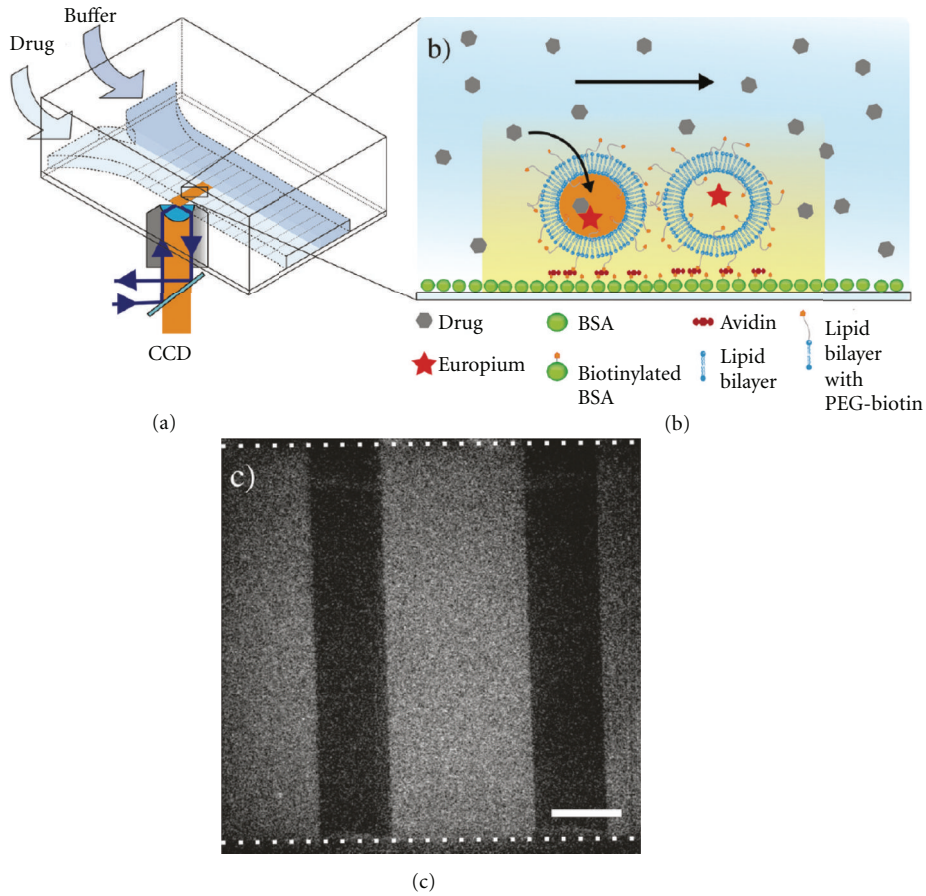


FIGURE 12: A microfluidic chip, mounted on a TIRF microscope, is used to supply buffer and tetracyclines through a microchannel to vesicles immobilized on the bottom glass slide. (b) Scheme of the detection assay. (c) Micrograph of the stripe pattern. Dotted lines indicate the channel walls [88] (reprinted from [88]).

nerve regeneration, diagnostics and sensing for infectious diseases and cancer biomarkers, and drug discovery. The nanomaterials used in the microfluidic environments present a challenge to characterization due to their unique properties and reactive nature, and this should be noted while performing future research. These microfluidic platforms have great potential for integration into lab-on-a-chip type of devices and providing point-of-care solutions.

Future work in this area by the authors will focus on using microfluidics as an assessment tool for nanobiomaterials in emerging research on degradable metallic biomaterials. The target applications for such materials include

craniofacial and orthopedic implants, nerve regeneration, bone and bone-ligament fixation, and airway stents. One of the objectives of the research will be to use computational tools to complement experimental efforts in microfluidics. For example, computational models can be used to predict distribution of species such as growth factors in cell culture chambers as a result of concentration gradient generation in microfluidic networks. Additionally, these models can be used to simulate various microenvironments in cell cultures which in turn mimic *in vivo* conditions. Finally, microfluidic platforms can be used to assess the effect of growth factors on nanobiomaterial-cell interactions, and the high-throughput capability can result in simultaneous assessment of multiple concentrations in conjunction with various biomaterial compositions.

Acknowledgments

This work was sponsored by the NSF ERC for Revolutionizing Metallic Biomaterials, http://erc.ncat.edu/. This project was supported in part by NIH Grant (1R21AR060966), Korea Grant (Project no. 00042172-1), and ONR Grant (N00014-11-1-0315).

References

[1] A. Astashkina, B. Mann, and D. W. Grainger, "A critical evaluation of *in vitro* cell culture models for high-throughput drug screening and toxicity," *Pharmacology & Therapeutics*, vol. 134, no. 1, pp. 82–106, 2012.

[2] A. L. Hook, D. G. Anderson, R. Langer, P. Williams, M. C. Davies, and M. R. Alexander, "High throughput methods applied in biomaterial development and discovery," *Biomaterials*, vol. 31, no. 2, pp. 187–198, 2010.

[3] T. G. Fernandes, M. M. Diogo, D. S. Clark, J. S. Dordick, and J. M. S. Cabral, "High-throughput cellular microarray platforms: applications in drug discovery, toxicology and stem cell research," *Trends in Biotechnology*, vol. 27, no. 6, pp. 342–349, 2009.

[4] M. Yliperttula, B. G. Chung, A. Navaladi, A. Manbachi, and A. Urtti, "High-throughput screening of cell responses to biomaterials," *European Journal of Pharmaceutical Sciences*, vol. 35, no. 3, pp. 151–160, 2008.

[5] C. Situma, M. Hashimoto, and S. A. Soper, "Merging microfluidics with microarray-based bioassays," *Biomolecular Engineering*, vol. 23, no. 5, pp. 213–231, 2006.

[6] J. Pihl, J. Sinclair, M. Karlsson, and O. Orwar, "Microfluidics for cell-based assays," *Materials Today*, vol. 8, no. 12, pp. 46–51, 2005.

[7] C. Rivet, H. Lee, A. Hirsch, S. Hamilton, and H. Lu, "Microfluidics for medical diagnostics and biosensors," *Chemical Engineering Science*, vol. 66, no. 7, pp. 1490–1507, 2011.

[8] S. le Gac and A. van den Berg, "Single cells as experimentation units in lab-on-a-chip devices," *Trends in Biotechnology*, vol. 28, no. 2, pp. 55–62, 2010.

[9] W. Siyan, Y. Feng, Z. Lichuan et al., "Application of microfluidic gradient chip in the analysis of lung cancer chemotherapy resistance," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 49, no. 3, pp. 806–810, 2009.

[10] P. G. Gross, E. P. Kartalov, A. Scherer, and L. P. Weiner, "Applications of microfluidics for neuronal studies," *Journal of the Neurological Sciences*, vol. 252, no. 2, pp. 135–143, 2007.

[11] S. Kim, H. J. Kim, and N. L. Jeon, "Biological applications of microfluidic gradient devices," *Integrative Biology*, vol. 2, no. 11-12, pp. 584–603, 2010.

[12] J. M. Klostranec, Q. Xiang, G. A. Farcas et al., "Convergence of quantum dot barcodes with microfluidics and signal processing for multiplexed high-throughput infectious disease diagnostics," *Nano Letters*, vol. 7, no. 9, pp. 2812–2818, 2007.

[13] A. Ranga and M. P. Lutolf, "High-throughput approaches for the analysis of extrinsic regulators of stem cell fate," *Current Opinion in Cell Biology*, vol. 24, pp. 1–9, 2012.

[14] Kshitiz, D. H. Kim, D. J. Beebe, and A. Levchenko, "Micro- and nanoengineering for stem cell biology: the promise with a caution," *Trends in Biotechnology*, vol. 29, no. 8, pp. 399–408, 2011.

[15] J.-H. Lee, Y. Gu, H. Wang, and W. Y. Lee, "Microfluidic 3D bone tissue model for high-throughput evaluation of wound-healing and infection-preventing biomaterials," *Biomaterials*, vol. 33, no. 4, pp. 999–1006, 2012.

[16] N. K. Inamdar and J. T. Borenstein, "Microfluidic cell culture models for tissue engineering," *Current Opinion in Biotechnology*, vol. 22, pp. 681–689, 2011.

[17] B. Prabhakarpandian, M. C. Shen, K. Pant, and M. F. Kiani, "Microfluidic devices for modeling cell-cell and particle-cell interactions in the microvasculature," *Microvascular Research*, vol. 82, pp. 210–220, 2011.

[18] A. Schober, C. Augspurger, U. Fernekorn et al., "Microfluidics and biosensors as tools for NanoBioSystems research with applications in the 'Life Science'," *Materials Science and Engineering B*, vol. 169, no. 1–3, pp. 174–181, 2010.

[19] C. Yi, C. W. Li, S. Ji, and M. Yang, "Microfluidics technology for manipulation and analysis of biological cells," *Analytica Chimica Acta*, vol. 560, no. 1-2, pp. 1–23, 2006.

[20] Y. Liu, J.-C. Wang, L. Ren et al., "Microfluidics-based assay on the effects of microenvironmental geometry and aqueous flow on bacterial adhesion behaviors," *Journal of Pharmaceutical Analysis*, vol. 1, no. 3, pp. 175–183, 2011.

[21] A. L. van de Ven, J. H. Sakamoto, B. Godin et al., "Enabling individualized therapy through nanotechnology," *Pharmacological Research*, vol. 62, no. 2, pp. 57–89, 2010.

[22] J. S. Murday, R. W. Siegel, J. Stein, and J. F. Wright, "Translational nanomedicine: status assessment and opportunities," *Nanomedicine*, vol. 5, no. 3, pp. 251–273, 2009.

[23] K. Ziolkowska, E. Jedrych, R. Kwapiszewski, J. Lopacinska, M. Skolimowski, and M. Chudy, "PDMS/glass microfluidic cell culture system for cytotoxicity tests and cells passage," *Sensors and Actuators B*, vol. 145, no. 1, pp. 533–542, 2010.

[24] S. Kobel and M. P. Lutolf, "Biomaterials meet microfluidics: building the next generation of artificial niches," *Current Opinion in Biotechnology*, vol. 22, pp. 690–697, 2011.

[25] A. Muralimohan, Y. J. Eun, B. Bhattacharyya, and D. B. Weibel, "Dissecting microbiological systems using materials science," *Trends in Microbiology*, vol. 17, no. 3, pp. 100–108, 2009.

[26] Y. Wang, J. D. Byrne, M. E. Napiera, and J. M. DeSimonea, "Engineering nanomedicines using stimuli-responsive biomaterials," *Advanced Drug Delivery Reviews*. In press.

[27] A. Seidi, M. Ramalingam, I. Elloumi-Hannachi, S. Ostrovidov, and A. Khademhosseini, "Gradient biomaterials for soft-to-hard interface tissue engineering," *Acta Biomaterialia*, vol. 7, no. 4, pp. 1441–1451, 2011.

[28] A. Kunze, M. Giugliano, A. Valero, and P. Renaud, "Micropatterning neural cell cultures in 3D with a multi-layered scaffold," *Biomaterials*, vol. 32, no. 8, pp. 2088–2098, 2011.

[29] J. V. Jokerst, A. Raamanathan, N. Christodoulides et al., "Nano-bio-chips for high performance multiplexed protein detection: determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels," *Biosensors and Bioelectronics*, vol. 24, no. 12, pp. 3622–3629, 2009.

[30] D. F. Williams, "On the nature of biomaterials," *Biomaterials*, vol. 30, no. 30, pp. 5897–5909, 2009.

[31] D. Chow, M. L. Nunalee, D. W. Lim, A. J. Simnick, and A. Chilkoti, "Peptide-based biopolymers in biomedicine and biotechnology," *Materials Science and Engineering R*, vol. 62, no. 4, pp. 125–155, 2008.

[32] A. A. Agrawal, B. J. Nehilla, K. V. Reisig et al., "Porous nanocrystalline silicon membranes as highly permeable and molecularly thin substrates for cell culture," *Biomaterials*, vol. 31, no. 20, pp. 5408–5417, 2010.

[33] H. Chen, C. Jiang, C. Yu, S. Zhang, B. Liu, and J. Kong, "Protein chips and nanomaterials for application in tumor marker immunoassays," *Biosensors and Bioelectronics*, vol. 24, no. 12, pp. 3399–3411, 2009.

[34] M. E. Furth, A. Atala, and M. E. V. Dyke, "Smart biomaterials design for tissue engineering and regenerative medicine," *Biomaterials*, vol. 28, no. 34, pp. 5068–5073, 2007.

[35] R. Langer and D. A. Tirrell, "Designing materials for biology and medicine," *Nature*, vol. 428, no. 6982, pp. 487–492, 2004.

[36] D. W. Grainger and D. G. Castner, "Nanobiomaterials and nanoanalysis: opportunities for improving the science to benefit biomedical technologies," *Advanced Materials*, vol. 20, no. 5, pp. 867–877, 2008.

[37] H. Liu and T. J. Webster, "Nanomedicine for implants: a review of studies and necessary experimental tools," *Biomaterials*, vol. 28, no. 2, pp. 354–369, 2007.

[38] E. Engel, A. Michiardi, M. Navarro, D. Lacroix, and J. A. Planell, "Nanotechnology in regenerative medicine: the materials side," *Trends in Biotechnology*, vol. 26, no. 1, pp. 39–47, 2008.

[39] R. P. Singh, "Prospects of nanobiomaterials for biosensing," *International Journal of Electrochemistry*, vol. 2011, pp. 1–30, 2011.

[40] L. Yildirimer, N. T. K. Thanh, M. Loizidou, and A. M. Seifalian, "Toxicology and clinical potential of nanoparticles," *Nano Today*, vol. 6, no. 6, pp. 583–607, 2011.

[41] F. Gelain, "Novel opportunities and challenges offered by nanobiomaterials in tissue engineering," *International Journal of Nanomedicine*, vol. 3, no. 4, pp. 415–424, 2008.

[42] W. Hu and C. M. Li, "Nanomaterial-based advanced immunoassays," *Wiley Interdisciplinary Reviews*, vol. 3, no. 2, pp. 119–133, 2011.

[43] B. G. Chung, L. Kang, and A. Khademhosseini, "Micro- and nanoscale technologies for tissue engineering and drug discovery applications," *Expert Opinion on Drug Discovery*, vol. 2, no. 12, pp. 1653–1668, 2007.

[44] T. H. Muster, A. Trinchi, T. A. Markley et al., "A review of high throughput and combinatorial electrochemistry," *Electrochimica Acta*, vol. 56, no. 27, pp. 9679–9699, 2011.

[45] M. K. Yu, J. Park, and S. Jon, "Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy," *Theranostics*, vol. 2, no. 1, pp. 43–44, 2012.

[46] I. R. Corbin, H. Li, J. Chen et al., "Low-density lipoprotein nanoparticles as magnetic resonance imaging contrast agents," *Neoplasia*, vol. 8, no. 6, pp. 488–498, 2006.

[47] R. A. Firestone, "Low-density lipoprotein as a vehicle for targeting antitumor compounds to cancer cells," *Bioconjugate Chemistry*, vol. 5, no. 2, pp. 105–113, 1994.

[48] D. Kessel, "Porphyrin-lipoprotein association as a factor in porphyrin localization," *Cancer Letters*, vol. 33, no. 2, pp. 183–188, 1986.

[49] S. M. Moerlein, A. Daugherty, B. E. Sobel, and M. J. Welch, "Metabolic imaging with gallium-68- and indium-111-labeled low-density lipoprotein," *Journal of Nuclear Medicine*, vol. 32, no. 2, pp. 300–307, 1991.

[50] E. Ponty, G. Favre, R. Benaniba et al., "Biodistribution study of 99mTc-labeled LDL in B16-melanoma-bearing mice. Visualization of a preferential uptake by the tumor," *International Journal of Cancer*, vol. 54, no. 3, pp. 411–417, 1993.

[51] P. C. N. Rensen, R. L. A. de Vrueh, J. Kuiper, M. K. Bijsterbosch, E. A. L. Biessen, and T. J. C. van Berkel, "Recombinant lipoproteins: lipoprotein-like lipid particles for drug targeting," *Advanced Drug Delivery Reviews*, vol. 47, no. 2-3, pp. 251–276, 2001.

[52] G. Zheng, H. Li, M. Zhang, S. Lund-Katz, B. Chance, and J. D. Glickson, "Low-density lipoprotein reconstituted by pyropheophorbide cholesteryl oleate as target-specific photosensitizer," *Bioconjugate Chemistry*, vol. 13, no. 3, pp. 392–396, 2002.

[53] S. Acton, A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Kriegert, "Identification of scavenger receptor SR-BI as a high density lipoprotein receptor," *Science*, vol. 271, no. 5248, pp. 518–520, 1996.

[54] Z. Zhang, J. Chen, L. Ding et al., "HDL-mimicking peptide-lipid nanoparticles with improved tumor targeting," *Small*, vol. 6, no. 3, pp. 430–437, 2010.

[55] W. J. McConathy, M. P. Nair, S. Paranjape, L. Mooberry, and A. G. Lacko, "Evaluation of synthetic/reconstituted high-density lipoproteins as delivery vehicles for paclitaxel," *Anti-Cancer Drugs*, vol. 19, no. 2, pp. 183–188, 2008.

[56] C. Butts, N. Murray, A. Maksymiuk et al., "Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer," *Journal of Clinical Oncology*, vol. 23, no. 27, pp. 6674–6681, 2005.

[57] N. Kojima, L. Biao, T. Nakayama, M. Ishii, Y. Ikehara, and K. Tsujimura, "Oligomannose-coated liposomes as a therapeutic antigen-delivery and an adjuvant vehicle for induction of *in vivo* tumor immunity," *Journal of Controlled Release*, vol. 129, no. 1, pp. 26–32, 2008.

[58] J. Neidhart, K. O. Allen, D. L. Barlow et al., "Immunization of colorectal cancer patients with recombinant baculovirus-derived *KSA* (Ep-CAM) formulated with monophosphoryl lipid a in liposomal emulsion, with and without granulocyte-macrophage colony-stimulating factor," *Vaccine*, vol. 22, no. 5-6, pp. 773–780, 2004.

[59] D. R. Lewis, K. Kamisoglu, A. W. York, and P. V. Moghe, "Polymer-based therapeutics: nanoassemblies and nanoparticles for management of atherosclerosis," *Wiley Interdisciplinary Reviews*, vol. 3, no. 4, pp. 400–420, 2011.

[60] Y. Sun, Y. Liu, W. Qu, and X. Jiang, "Combining nanosurface chemistry and microfluidics for molecular analysis and cell biology," *Analytica Chimica Acta*, vol. 650, no. 1, pp. 98–105, 2009.

[61] A. Fujishima and K. Honda, "Electrochemical photolysis of water at a semiconductor electrode," *Nature*, vol. 238, no. 5358, pp. 37–38, 1972.

[62] A. Fujishima and X. Zhang, "Titanium dioxide photocatalysis: present situation and future approaches," *Comptes Rendus Chimie*, vol. 9, no. 5-6, pp. 750–760, 2006.

[63] A. Zaleska, "Doped-TiO_2: a review," *Recent Patents on Engineering*, vol. 2, no. 3, pp. 157–164, 2008.

[64] S. G. Kumar and L. G. Devi, "Review of modified TiO_2 photocatalyst under UV/Visible light: selected results and related mechanisms on interfacial charge carrier dynamics," *The Journal of Physical Chemistry A*, vol. 115, pp. 13211–13241, 2011.

[65] F. Schmidt-Stein, R. Hahn, J. F. Gnichwitz et al., "X-ray induced photocatalysis on TiO_2 and TiO_2 nanotubes: degradation of organics and drug release," *Electrochemistry Communications*, vol. 11, no. 11, pp. 2077–2080, 2009.

[66] C. V. Wilmowsky, S. Bauer, R. Lutz et al., "*In Vivo* evaluation of anodic TiO_2 nanotubes; an experimental study in the pig," *Journal of Biomedical Materials Research*, vol. 89, no. 1, pp. 165–171, 2009.

[67] D. M. Blake, P. C. Maness, Z. Huang, E. J. Wolfrum, J. Huang, and W. A. Jacoby, "Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells," *Separation and Purification Methods*, vol. 28, no. 1, pp. 1–50, 1999.

[68] Y. Kubota, T. Shuin, C. Kawasaki et al., "Photokilling of T-24 human bladder cancer cells with titanium dioxide," *British Journal of Cancer*, vol. 70, no. 6, pp. 1107–1111, 1994.

[69] D. Collard, S. Takeuchi, and H. Fujita, "MEMS technology for nanobio research," *Drug Discovery Today*, vol. 13, no. 21-22, pp. 989–996, 2008.

[70] M. Huang, S. Fan, W. Xing, and C. Liu, "Microfluidic cell culture system studies and computational fluid dynamics," *Mathematical and Computer Modelling*, vol. 52, no. 11-12, pp. 2036–2042, 2010.

[71] J. P. Shelby, J. White, K. Ganesan, P. K. Rathod, and D. T. Chiu, "A microfluidic model for single-cell capillary obstruction by Plasmodium falciparum-infected erythrocytes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 25, pp. 14618–14622, 2003.

[72] W. G. Lee, Y. G. Kim, B. G. Chung, U. Demirci, and A. Khademhosseini, "Nano/Microfluidics for diagnosis of infectious diseases in developing countries," *Advanced Drug Delivery Reviews*, vol. 62, no. 4-5, pp. 449–457, 2010.

[73] R. Baudoin, A. Corlu, L. Griscom, C. Legallais, and E. Leclerc, "Trends in the development of microfluidic cell biochips for *in vitro* hepatotoxicity," *Toxicology in Vitro*, vol. 21, no. 4, pp. 535–544, 2007.

[74] S. K. Mahto, T. H. Yoon, and S. W. Rhee, "A new perspective on *in vitro* assessment method for evaluating quantum dot toxicity by using microfluidics technology," *Biomicrofluidics*, vol. 4, no. 3, pp. 1–8, 2010.

[75] B. Cui, C. Wu, L. Chen et al., "One at a time, live tracking of NGF axonal transport using quantum dots," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 34, pp. 13666–13671, 2007.

[76] C. Beta and E. Bodenschatz, "Microfluidic tools for quantitative studies of eukaryotic chemotaxis," *European Journal of Cell Biology*, vol. 90, no. 10, pp. 811–816, 2011.

[77] M. Taylor, A. J. Urquhart, D. G. Anderson et al., "A methodology for investigating protein adhesion and adsorption to microarrayed combinatorial polymers," *Macromolecular Rapid Communications*, vol. 29, no. 15, pp. 1298–1302, 2008.

[78] C. G. Simon Jr., N. Eidelman, S. B. Kennedy, A. Sehgal, C. A. Khatri, and N. R. Washburn, "Combinatorial screening of cell proliferation on poly(L-lactic acid)/poly(D,L-lactic acid) blends," *Biomaterials*, vol. 26, no. 34, pp. 6906–6915, 2005.

[79] S. Heungsoo, "Fabrication methods of an engineered microenvironment for analysis of cell-biomaterial interactions," *Biomaterials*, vol. 28, no. 2, pp. 126–133, 2007.

[80] L. Zhao, P. Cheng, J. Li et al., "Analysis of nonadherent apoptotic cells by a quantum dots probe in a microfluidic device for drug screening," *Analytical Chemistry*, vol. 81, no. 16, pp. 7075–7080, 2009.

[81] S. M. Ong, C. Zhang, Y. C. Toh et al., "A gel-free 3D microfluidic cell culture system," *Biomaterials*, vol. 29, no. 22, pp. 3237–3244, 2008.

[82] K. Anselme, P. Davidson, A. M. Popa, M. Giazzon, M. Liley, and L. Ploux, "The interaction of cells and bacteria with surfaces structured at the nanometre scale," *Acta Biomaterialia*, vol. 6, no. 10, pp. 3824–3846, 2010.

[83] W. H. Huang, F. Ai, Z. L. Wang, and J. K. Cheng, "Recent advances in single-cell analysis using capillary electrophoresis and microfluidic devices," *Journal of Chromatography B*, vol. 866, no. 1-2, pp. 104–122, 2008.

[84] D. Falconnet, G. Csucs, H. Michelle Grandin, and M. Textor, "Surface engineering approaches to micropattern surfaces for cell-based assays," *Biomaterials*, vol. 27, no. 16, pp. 3044–3063, 2006.

[85] H. Dan-Qun, Z. Liu, C. J. Hou et al., "Recent advances on optical detection methods and techniques for cell-based microfluidic systems," *Chinese Journal of Analytical Chemistry*, vol. 38, no. 9, pp. 1357–1365, 2010.

[86] A. Kunzmann, B. Andersson, T. Thurnherr, H. Krug, A. Scheynius, and B. Fadeel, "Toxicology of engineered nanomaterials: focus on biocompatibility, biodistribution and biodegradation," *Biochimica Et Biophysica Acta*, vol. 1810, no. 3, pp. 361–373, 2011.

[87] C. F. Jones and D. W. Grainger, "*In vitro* assessments of nanomaterial toxicity," *Advanced Drug Delivery Reviews*, vol. 61, no. 6, pp. 438–456, 2009.

[88] P. Kuhn, K. Eyer, S. Allner, D. Lombardi, and P. S. Dittrich, "A microfluidic vesicle screening platform: monitoring the lipid membrane permeability of tetracyclines," *Analytical Chemistry*, vol. 83, no. 23, pp. 8877–8885, 2011.

[89] H. V. D. Andersson and A. Berg, "Microfabrication and microfluidics for tissue engineering: state of the art and future opportunities," *Lab on a Chip*, vol. 4, no. 2, pp. 98–103, 2004.

[90] J.-H. Lee, H. Wang, J. B. Kaplan, and W. Y. Lee, "Microfluidic approach to create three-dimensional tissue models for biofilm-related infection of orthopaedic implants," *Tissue Engineering*, vol. 17, no. 1, pp. 39–48, 2011.

[91] W. Tan and T. A. Desai, "Layer-by-layer microfluidics for biomimetic three-dimensional structures," *Biomaterials*, vol. 25, no. 7-8, pp. 1355–1364, 2004.

[92] D. G. Anderson, S. Levenberg, and R. Langer, "Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells," *Nature Biotechnology*, vol. 22, no. 7, pp. 863–866, 2004.

[93] N. S. Hwang, S. Varghese, and J. Elisseeff, "Controlled differentiation of stem cells," *Advanced Drug Delivery Reviews*, vol. 60, no. 2, pp. 199–214, 2008.

[94] P. Boisseau and B. Loubaton, "Nanomedicine, nanotechnology in medicine," *Comptes Rendus Physique*, vol. 12, pp. 620–636, 2011.

[95] X. Chi, D. Huang, Z. Zhao, Z. Zhou, Z. Yin, and J. Gao, "Nanoprobes for *in vitro* diagnostics of cancer and infectious diseases," *Biomaterials*, vol. 33, no. 1, pp. 189–206, 2012.

[96] T. S. Hauck, S. Giri, Y. Gao, and W. C. W. Chan, "Nanotechnology diagnostics for infectious diseases prevalent in developing countries," *Advanced Drug Delivery Reviews*, vol. 62, no. 4-5, pp. 438–448, 2010.

[97] A. Pope-Harman, M. M. C. Cheng, F. Robertson, J. Sakamoto, and M. Ferrari, "Biomedical nanotechnology for cancer," *Medical Clinics of North America*, vol. 91, no. 5, pp. 899–927, 2007.

[98] C. Kaittanis, S. Santra, and J. M. Perez, "Emerging nanotechnology-based strategies for the identification of microbial pathogenesis," *Advanced Drug Delivery Reviews*, vol. 62, no. 4-5, pp. 408–423, 2010.

[99] G. Birnbaumer, S. Küpcü, C. Jungreuthmayer et al., "Rapid liposome quality assessment using a lab-on-a-chip," *Lab on a Chip*, vol. 11, no. 16, pp. 2753–2762, 2011.

[100] A. Jahn, S. M. Stavis, J. S. Hong, W. N. Vreeland, D. L. Devoe, and M. Gaitan, "Microfluidic mixing and the formation of nanoscale lipid vesicles," *ACS Nano*, vol. 4, no. 4, pp. 2077–2087, 2010.