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EDITOR:

Donglu Shi

Department of Chemical and Materials Engineering,
College of Engineering, University of Cincinnati,
Cincinnati, OH 45221, USA

The Institute for Advanced Materials and Nano Biomedicine,
Tongji University, Shanghai 200092, China

Research Institute of Micro/Nano Science and Technology,
Shanghai Jiao Tong University, Shanghai 200240, China

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Preface

The research on nanomaterials and nano biomedicine has been advancing rapidly in recent years, particularly in the development of unique nanostructures for specific biomedical applications. The research addresses the critical issues in medical applications including in vivo imaging, cell targeting, local drug delivery and treatment, bioactivity, compatibility, and toxicity. In the biomedical applications, traditional materials science and engineering have to deal with new challenges in the areas of synthesis, structure development, and biological, chemical, and physical behaviors, since medical needs place new demands in these respects.

The novel nanotechnologies included in this book are of great importance for biomedical applications. Based on these new developments, it is possible to alter the intrinsic properties of nanomaterials that cannot be achieved by conventional methods and materials. A key aspect of being able to manipulate the properties of the nanomaterials is the nanoscale architecture and engineering by various processing techniques. Some of the novel approaches introduced in this book can provide multi-functionality for a variety of substrates, be it biological, physical, or chemical, which can then be engineered for particular biomedical applications. For instance, novel surface functionalization methods have been developed for bio assays and cell targeting. In these approaches, a thin coating of polymer can be applied to the nano species and to provide various functional groups for passive or covalent coupling to biological molecules, such as antigens, antibodies, and DNA/RNA hybridization. However, the conventional synthesis of materials has only resulted in a single functionality which is generally not suitable for the complex procedures required for medical applications. The novel concept introduced in this book can be used to develop multiple functionalities, particularly suitable for medical diagnosis and treatment. The enhancement of properties is based on the study of the new nano structures and interfacial mechanisms.

This book summarizes the most recent research and development in nano biomedicine and addresses the critical issues in nanomaterials synthesis, structure, and properties. In particular, the major topics in nano biomedicine are covered in

this book. The book devotes three parts of 25 chapters to various aspects of nanomaterials and their medical applications. Detailed experimental procedures are presented at a level suitable for readers with no previous training in these areas.

The first part of the book concentrates on the research works of design, synthesis, properties, and applications of nano scale biomaterials. Chapter 1 is on the topics of stem cells and related nanotechnology. In Chapter 2, an overview is documented on the recent progress of polymer nanofibers, mostly electrospun in biomedical applications, along with a brief description of history, principle, and operating parameters of electrospinning process. Chapter 3 introduces new concepts in assembly of biomaterials. In view of the emerging importance of bio-inspired materials in medical applications, this chapter is focused on describing the fundamentals of intermolecular interactions and their applications in biomaterials science. The particular focus will be on processes and structures that mimic the natural ECM. Chapter 4 is on the fabrication and assembly of nanomaterials for biological detections. In Chapter 5, the authors first introduce the peptide design strategies for the construction of nanostructured materials. It then gives a brief tutorial of amino acid structure and function. It further describes higher-order assemblies of peptides and peptidomimetics. Chapter 6 introduces an important category of nanomaterials: quantum dots. Chapter 7 focuses on the phosphate ceramics for applications in bio-related fields. In this chapter, the authors briefly review the progress made in the last decade on the microwave-assisted synthesis and processing of biomaterials both in nanometer- and micrometer-size range. Chapter 8 introduces the characterization of biointerfaces and biosurfaces in biomaterials design. In Chapter 9, the authors bring the focus of the discussion to one of the important nanomaterials: carbon nanotubes and their applications in biosensing. Chpater 10 discusses the issues on heparin-conjugated nanointerfaces for biomedical applications.

The second part of the book is on the new nanotechnologies in biomedicine. In Chapter 11, the authors introduce some of the novel technologies in drug delivery. Chapter 12 reports unique experimental results on nano metal particles for biomedical applications. Chapter 13 is on the micro- and nanoscale technologies in high-throughput biomedical experimentation. Chapter 14 introduces delivery system of bioactive molecules for regenerative medicine. Chapter 15 gives an overview on modification of nano-sized materials for drug delivery. Chapter 16 is another chapter on drug delivery, however via a different approach. Chapter 17 is about most recent developments in DNA nanotechnology. A major objective in this chapter deals with the creation of ordered nanostructures for executing complex operations. Chapter 18 provides an overview on the nanoscale bioactive surfaces and endosseous implantology. Chapter 19 gives an overview of potential applications of carbon nanotube smart materials in biology and medicine.

The last part of the book concentrates on some of the most recent experimental results on the nanomaterials synthesis and structure developments. These include

the synthesis, properties, and application of intrinsically electroconducting nanoparticles of polypyrrole and pyrrole/sulfonic diphenylamine (20/80) Copolymers. Some studies focuse on the fracture processes in advanced nanocrystalline and nanocomposite materials. Unique nano properties such as field emission of carbon nanotubes are also introduced. Finally, the book concludes by introducing some theoretical aspects of the nanomaterials. In this chapter, the authors develop microscopic modeling of phonon modes in semiconductor nanocrystals.

Chapter 20 is on the physical origins of phonon behaviors in nanocrystals. Chapter 21 gives an overview on computer simulations and theoretical modeling of fracture processes in nanocrystalline metals and ceramic nanocomposites. Chapter 22 describes a detailed experimental study on the fabrication, structures, unique properties, and wide application potential of novel conducting polypyrrole (PPY) nanoparticles and nanocomposites. Chapter 23 introduces some of the most recent developments in the fascinating carbon nanotubes. Chapter 24 reviews the progress in the flexible dye-sensitized nanostructured thin film solar cells (DSSCs). Chapter 25 presents recent results on the synthesis of magnetic nanoparticles (MNP) and various types of magnetic nanofluids (MNF) or ferrofluids, their structural properties and behaviors in an external magnetic field.

We hope that these chapters will provide timely and useful information for the progress of nanomaterials and their applications in biomedicine.

Contents

1 Stem Cells and Nanostructured Materials

Vince Beachley[1] and Xuejun Wen[1,2,3]

[1] Department of Bioengineering, Clemson University, Charleston, SC 29425, USA
[2] Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC 29425, USA
[3] Department of Orthopedic Surgery, Medical University of South Carolina, Charleston, SC 29425, USA

Abstract Stem cells and nanomaterials are currently two of the most promising technologies for tissue regeneration and the treatment of degenerative disease. Because of their ability to self-renew and differentiate into any cell type, stem cells offer the potential to regrow all types of damaged or degenerated tissues that are unrepairable by currently available treatment methods. Nanomaterials may prove to be ideal growth substrates for tissue regeneration as well as an ideal delivery vehicle for the diagnostic markers, growth factors, and drugs that are required to promote tissue regeneration and treat degenerative disease. Despite their great potential, stem cell behaviors such as proliferation and differentiation must be tightly regulated in order for this technology to be practical in a clinical setting. Experimental evidence has shown that the interactions of nanomaterials with stem cells can have a significant effect on many types of stem cell behaviors. In addition, nanomaterials can be used to provide targeted delivery of various agents in a controlled manner that allows for regulation of the chemical environment. Regulation of the chemical environment is critical for controlled guidance of stem cell behavior and for the treatment of degenerative disease. A precise understanding of the interactions between stem cells and nanomaterials is an important step toward unlocking the great potential of these two technologies.

Keywords nano, stem cell

1.1 Introduction

Stem cells and nanotechnology, two exciting and rapidly growing fields, have received extensive attention during the last decades. Stem cells and precursors

(1) Corresponding e-mail: xjwen@clemson.edu

bring new hope to regenerate functional tissue with native histological structures and properties, as opposed to simple replacement with artificial structures alone. The two main advantages of stem cells are the ability to self-renew, which means they can reproduce themselves, and the ability to potentially differentiate into all the possible cell types (Pedersen, 1999; Solter and Gearhart, 1999). Stem cells may be harvested from two different sources. Embryonic stem (ES) cells may be harvested from embryos and can be derived from germ cells as well. If problems such as immune rejection and the high possibility of tumorgenicity can be solved, ES cells may serve as a good source of cells for tissue regeneration. Their potential for the study of human developmental biology is always promising (Good, 1998). Stem cells can also be harvested from adult tissue, such as from muscle, cartilage, bone, nervous system, liver, pancreas, tooth, adipose tissue, etc. (Good, 1998). Like stem cells, precursor cells can differentiate into more than one cell type, but these cells have undergone some degree of differentiation (Weissman, 2000). For example, glial-restricted precursors (GRP) can differentiate into type Ⅰ and type Ⅱ astrocytes and oligodendrocytes, but not neurons (Foster and Stringer, 1999). Precursor cells can be harvested from adult tissue as well (Rizzoli and Carlo-Stella, 1997). Knowledge of stem cells can also bring profound insight to cancer research due to the fact that many cancer cells possess the characteristics of stem/progenitor cells and many cancer cells originated from stem cells. It is known that two key chemical signals, Hedgehog and Wnt, are active in the stem cells that repair damaged tissue. These signals also have been found in certain hard to treat cancers, supporting an old idea that some cancers may start from normal stem cells that have somehow gone bad. Therefore, a section about cancer treatment using nanostructured biomaterials is included in this chapter as well.

Nanostructured materials refer to certain materials with delicate structures of ‘small’ sizes, falling in the 1 – 100 nm range, and specific properties and functions related to the ‘size effect’ (Niemeyer, 2001; Safarik and Safarikova, 2002; Whitesides, 2003). Dramatic development of nanotechnology in material science and engineering has taken place in the last decade (Gao et al., 2004; Niemeyer, 2001; Whitesides, 2003). This does not come as a surprise considering that nanostructured materials have the capability to be adapted and integrated into biomedical devices, since most biological systems, including viruses and membrane and protein complex, are natural nanostructures (Laval et al., 1999). Currently, medicine and biomedical engineering are among the most promising and challenging fields involved in the application of nanostructured materials (Desai, 2000; Ziener et al., 2005). Rapid advancements of nanostructured materials have been made in a wide variety of biomedical applications, including novel tissue engineered scaffolds and devices, site-specific drug delivery systems, non-viral gene carriers, biosensor and screening systems, and clinical bio-analytical diagnostics and therapeutics (Mazzola, 2003; Ziener et al., 2005). For example, nanocomposites have been used to stabilize and regenerate bone matrices (Bradt

et al., 1999; Du et al., 1998; Kikuchi et al., 2004; Kikuchi et al., 2004); biosensing with nanotubes and nanowires has demonstrated unprecedented sensitivity for biomolecule detection (Alivisatos, 2004; Drummond et al., 2003; Penn et al., 2003); and nanoscale assemblies and particles have been used to deliver high concentrations of therapeutic drugs and/or biomolecules, possessing high bioaffinity to specific host sites for precise drug administration (Moghimi and Szebeni, 2003; Muller et al., 2001; Takeuchi et al., 2001).

The combinational use of stem cells and nanostructured materials may help us to understand many scientific questions and also may bring many practical applications that promote the use of either or both components in biomedical research and clinical applications. In this chapter, the interactions between stem cells and nanostructured materials are discussed. In order to better present the contents, nanostructured materials are classified into two categories, one is nanotopographic substrate, which includes nanofibers and surface nano-textures, and the other is nanoparticles.

1.2 Interaction of Stem Cells with Nanotopographic Substrates

Cells in their natural in vivo surroundings are exposed to a complex chemical and structural environment. The natural extracellular matrix (ECM) is made up of structural components that are of nanoscale dimensions. Major fibrous extracellular molecules are in the nano-scale range, fibers such as collagen fibers, elastin fibers, keratin fibers, etc. are nanofibers. Mimicking the natural environment when culturing cells in vitro is highly important because cell behavior is determined by both genetic make up and the surrounding environmental cues. Cellular behaviors such as proliferation, differentiation, morphology, and migration are commonly controlled in culture by modulation of the chemical environment, cells also respond to different morphological cues that can be determined by the growth substrate in vitro and in vivo. Four components may be involved in the growth, differentiation, and morphology of cells on biomaterial surfaces: (1) adsorption of serum components, (2) extracellular matrix components secreted by the cell, (3) cell adhesion molecules, and (4) cytoskeleton mechanics (Matsuzaka et al., 1999). It has been shown that the structural substrate property of surface roughness can cause selective protein absorption, and that higher surface roughness increases total protein absorption (Deligianni et al., 2001). Increased protein adsorption could be attributed to an increase in surface area for rough surfaces and thus could be important in relation to nanotopographical materials because these exhibit extremely high surface areas. In relation to nanostructure and stem cell interactions, the cytoskeleton mechanics component is of importance because cells cultured on substrates with nanoscale features can take

on different shapes in response to the specific features that are encountered. It is very apparent that nanotopography effects cellular behavior through the regulation of morphology, but it is likely that there are unknown effects associated with nanotopographies as well. Cells can react to objects as small as 5 nm (Curtis, 2001) and it is possible that nanostructures, especially those of similar dimensions to the natural ECM, can influence cell behavior through mechanisms other than determination of cell morphology, cytoskeletal mechanics, and protein absorption. It has been shown that stem cell behavior can be highly dependent on the substrate that they are cultured on and the understanding of stem cell interactions with nanosurfaces could provide valuable information about stem cells that could be utilized for desirable in vivo applications.

1.2.1 Cell Shape and the Cytoskeleton

While much is known about how various growth factors can regulate differentiation, the significance of cell density on cell differentiation is not well understood. It has been hypothesized that the differences in cell density cause differences in cell shape that in turn may act as differentiation cues (McBeath et al., 2004). The effect of cell shape on the differentiation of stem cells has been investigated. Spegelman and Ginty (1983) found that differentiation of an adipogenic cell line could be inhibited when it was allowed to attach and spread on fibronectin coated surfaces. The inhibitory effect on cell differentiation was reversed by keeping the cells rounded and by disrupting the actin cytoskeleton. On the contrary, cell spreading has been shown to cause an increase in osteoblast differentiation by osteoblastic progenitor cells as measured by increased osteocalcin expression (Thomas et al., 2002). It has been shown that regulation of cell shape can influence the differentiation of multipotent human mesenchymal stem cells (hMSCs) into adiogenic or osteoblastic fate (McBeath et al., 2004). hMSCs allowed to flatten and spread expressed osteoblastic markers, such as alkaline phosphatase, while constrained cells that remained unspread and rounded expressed adiogenic lipid production. In addition, more alkaline phosphatase activity was found at low hMSC plating density and more lipid staining was observed at high cell plating density. One proposed mechanism for the transduction of cell shape information into gene expression is by the transmission of mechanic forces directly from the myosin actin cytoskeleton to the nucleus (Maniotis et al., 1997). While cytoskeletal organization is related to cell shape, the cytoskeleton can influence gene expression independently of cell shape. In the study above, the inhibition of myosin-generated cytoskeletal tension in hMSCs caused decreased alkaline phosphatase activity and increased lipid production without changing cell shape (McBeath et al., 2004).

Stem cells have been cultured on a variety of nanotopographies including

nanofibers, nanoparticle films, and etched nanosurfaces. Stem cells are highly responsive to nanotopagraphies for morphology, attachment, proliferation, and differentiation. Stem cells cultured on nanofibrous scaffolds can introduce two mechanical cues to cultured cells when compared to traditional culture methods. Nanofiber scaffolds present cells with a nanoscaled fibular microstructure and in many cases a three-dimensional 3-D growth environment. It is important to take both of these variables into consideration when analyzing the results from these studies. Because of differences in dimensionality, nanosurface interactions and nanofiber interactions will be described in separate sections.

1.2.2 Morphology, Attachment and Proliferation

1.2.2.1 Nanosurfaces

Several investigations have observed the effects that surface nanotopography can have on the attachment, spreading, and orientation of cultured stem cells. Nanoparticle films made by layer-by-layer assembly can be used to create nanotopographies of increasing surface roughness. Mouse mesenchymal stem cells (mMSCs) seeded on TiO_2 films of increasing particle desposition and surface roughness attached and spread better on the rough surfaces (Kommireddy et al., 2006).

Photolithographic techniques have been used to create nanotopographies of pits, bumps and grooves on polymer surfaces. Rat bone marrow mesenchymal stem cells (rBMCs) cultured on grooved surfaces with an applied groove depth of 0.5, 1.0 or 1.5 μm and a groove width of 1, 2, 5 or 10 μm induced alignment of the cells, matrix, actin filaments, and focal adhesion points to the surface grooves (Matsuzaka et al., 2000, et al., 1999). hMSCs were also cultured on wide grooves of 50 μm width and 327 nm depth and narrow grooves of 5 μm width and 510 nm depth (Dalby et al., 2006). In this case, cells cultured on narrow grooves developed stress fibers that were highly aligned in the direction of the grooves, while cells cultured on wider grooves only approximately aligned to the axis of the grooves. This would be expected, as grooves of 50 μm width are larger than the diameter of a cell. Bone marrow stem cell derived osteoblast-like cells cultured on 150 nm wide 60 nm deep grooves also directed cell orientation and actin fiber alignment with features of a much smaller scale(Zhu et al., 2005). hMSCs have also been cultured on pits of 30 μm and 40 μm widths and 310 nm and 362 nm depths respectfully and on bumps 10 – 45 nm in height(Dalby et al., 2006a, 2006b). For all sizes of pits and bumps significant increases in cell area and defined cytoskeletal fibers were observed.

Conflicting results on nanosurface effect on proliferation have been observed. Proliferation was enhanced for TiO_2 nanoparticle surface and 150 nm wide 60 nm deep grooved surfaces versus smooth surfaces (Kommireddy et al., 2006; Zhu et

al., 2005). In contrast, in another study using 1 – 10 μm wide, 0.5 – 1.5 μm deep grooves, the differences in cell proliferation were not significant (Matsuzaka et al., 2000).

1.2.2.2 Nanofibers

Nanofiber meshes seeded with different types of stem cells have demonstrated the ability to promote cell adhesion, directional guidance and morphological changes. Osteoprogenitor cells cultured on electrospun polymer fiber meshes with diameters ranging from 140 nm to 2100 nm responded to fibrous nanotopography and osteogenic growth factors (Badami et al., 2006). Cells on fibers had a smaller projected area than cells on smooth surfaces, but cells on 2100 nm fibers had a higher aspect ratio. Proliferation was also effected by the fibrous nanotopography. Cells cultured on fibers exhibited a lower cell density than those on smooth surfaces in the absence of osteogenic factors, but when osteogenic factors were added the cell density of fiber surfaces was equal to or greater than that on smooth surfaces. In both cases cell density increased with fiber diameter. In contrast, osteoblast cells grown on carbon nanotubes of various diameters proliferated at much higher rates on smaller fibers with three times as many cells on 60 nm fibers than 125 nm fibers after 7 days in culture (Elias et al., 2002). Mouse (ES) cells cultured in a nanofibrullar network also greatly enhanced proliferation in comparison with the growth of tissue on culture surfaces without nanofibers (Nur et al., 2006). Another investigation observed hematopoietic stem cell proliferation to increase at a similar rate for both polymer films and nanofiber polymer meshes (Chua et al., 2006). Cell adhesion properties of nanofibers were also tested on hematopoietic stem cells grown on polymer nanofiber meshes and polymer films. After ten days of expansion culture, cells were gently washed three times and approximately 40% of total cells on nanofiber meshes were adherent as opposed to 25% of total cells on film substrates.

Similar to linear oriented etched surfaces, aligned nanofibers are able to promote directional guidance in stem cell culture. Neuronal stem cells seeded on random and aligned 300 nm and 1.5 μm nanofibers attached well and changed their shape from rounded to elongated and spindle-like for all fiber scaffolds. In addition, cells turned through large angles in order to grow parallel to the fiber alignment independent of fiber diameter (Yang et al., 2005).

1.2.3 Differentiation

1.2.3.1 Nanosurfaces

Nanotopography has been shown to have an effect on differentiation as measured by increased osteogenic gene expression in bone marrow cells. Rat bone marrow cells cultured on grooved polymer surfaces 500 – 1500 nm deep had greater

alkaline phosphatase activity than cells cultured on smooth surfaces (Matsuzaka et al., 1999). The osteoblastic markers osteocalcin and osteopontin were expressed by human bone marrow stem cells (hBMCs) that were cultured on bumps 10 – 45 nm in height, while the same cells cultured on smooth surfaces displayed negligible positive staining (Dalby et al., 2006a). Increases in osteocalcin and osteopontin versus negligible staining in controls were also observed for hBMCs cultured on nanoscale pits 40 μm in width and 310 nm in depth (Dalby et al., 2006b).

1.2.3.2 Nanofibers

Different types of stem cells have been observed to differentiate in a variety of nanofiber scaffolds. hMSCs were induced to differentiate into adipogenic, chondrogenic, and osteogenic lineages in electrospun nanofibrous polymer scaffolds when cultured in specific differentiation media (Li et al., 2005). Neuronal stem cells were able to differentiate into neurons with sprouting neurites in a nanofibrous polymer scaffold made by liquid-liquid phase separation (Yang et al., 2004). Mouse embryonic fibroblasts cultured in 3-D peptide scaffolds were observed to undergo strong osteogenic differentiation after osteogenic induction while cells cultured in 2-D conditions did not differentiate (Garreta et al., 2006). Furthermore, mouse embryonic fibroblasts cultured in 3-D systems without osteogenic induction still maintained an adult stem cell-like phenotype and expressed the early stage markers of osteoblast differentiation.

Beyond having the capability to support stem cell differentiation, nanofibrous topography has been shown to selectively influence differentiation based on fiber diameter. Differentiation of neural stem cells cultured on aligned and random nanofiber meshes with fiber diameters of 300 nm and 1500 nm were observed to be highly dependent on fiber diameter (Yang et al., 2005). When the neural differentiation was evaluated on the basis of shape change it was found that the quantitative differentiation rates were ~80% and ~40% for 300 nm and 1500 nm, respectively. Fiber size dependent differentiation results were consistent for both randomly oriented and aligned nanofibers.

The ability of nanofibrous scaffolds in preventing differentiation has also been explored. Hematopoietic stem cells cultured in polymer nanofiber meshes for 10 days showed a slightly higher percentage of $CD34^+$ $CD45^+$ cells when compared to polymer film (Chua et al., 2006). Nanofiber meshes also mediated a lower monoblastic phenotype and greater number on primitive progenitor cells compared to films. Mouse ES cells also proliferated in 3-D polymer nanofiber meshes while maintaining their pluri-potency (Nur et al., 2006). While proliferation with self-renewal was allowed to continue in nanofiber topography, cells were observed to maintain their ability to differentiate when exposed to differentiation factors. In a separate study, a small fraction of mouse ES cells isolated during embryoid body development or after osteogenic induction appeared to develop into small ES cell-like colonies (Garreta et al., 2006). It was

also found that the frequency of these colonies was remarkably higher in 3-D peptide nanofiber cultures than in 2-D culture, suggesting that 3-D microenvironment promoted the generation of a stem cell-like niche that allows undifferentiated stem cell maintenance.

1.2.4 Self-Assembling Peptide Nanofibers

The self-assembling peptide method used to create nanofibrous scaffolds for in vitro culture can be utilized for in vivo tissue engineering as well. When these peptides are injected into the body, the interaction with the physiological environment induces peptide nanofiber assembly. Peptide solution injected into the myocardium was able to assemble a 3-D nanofiber mesh and did not induce a major inflammatory response (Davis et al., 2005). This 3-D microenvironment recruited endothelial progenitor cells, smooth muscle cells, and myocyte progentitor cells and promoted vascularization. Implantation of matrigel as control resulted in few numbers of endothelial cells and no myocyte progenitors. In addition, the injection of neonatal myocytes with the peptide solution into the microenvironment increased the density of endogenous cardiac progenitors recruited and injected ES cells were able to differentiate into cardiac myocytes in the nanofiber microenvironment. Self-assembling nanofiber peptide networks have also been used as drug delivery vehicles. Improved differentiation of neural progenitor cells was observed when they were cultured in peptide nanofibers incorporated with isolucine-lysine-valine-alanine-valine (IKVAV) epitope found in laminin (Silva et al., 2004). Nanofiber scaffolds incorporating the IKVAV epitope promoted rapid and selective differentiation of NSCs into neurons, with about 35% of cells differentiating after only 1 day. Neural stem cells cultured on 2-D laminin coated surfaces differentiated at a much lower percentage that did not exceed 15% even after 7 days. It was shown that the increase in differentiation was not due to 3-dimensionality when NSCs cultured in non-bioactive nanofiber meshes with soluble IKVAV did not promote differentiation, and was further shown when a 2-D substrate coated with IKVAV incorporated fibers promoted differentiation at the same level as 3-D IKVAV nanofiber meshes. The hypothesis for the success of this approach was that IKVAV nanofiber meshes could amplify the density of epitope presentation to the cells by a factor of 10^3 when compared to a laminin monolayer.

1.2.5 Summary

The results of nanofiber cell culture in relation to oriented cell guidance and improved attachment agree well with results from nanosurface culture; however, cell area was increased versus control for nanosurfaces and decreased on

nanofibers. This could result from differences in the structure or dimensions, but it could also be a result of the three dimensionality of the nanofiber structure. Results for cell proliferation vary between similar studies for nanofiber scaffolds and for nanosurfaces. This discrepancy could be due to the differences in cell types and structures used in the individual studies, but it is certainly an indication that there may not be a direct relationship between topography and proliferation or that this relationship can be outweighed by other factors.

Nanofibrous topography has been shown to have a very strong effect on the differentiation of stem cells. Stem cells have been able to readily differentiate in nanofiber meshes and in some case the nanofiber mesh itself has been a requirement for differentiation. The influence of nanofibrous structures on stem cells differentiation lies in both its structural properties, such as fiber diameter, and its three dimensionality. It is important to note that there is evidence that dimensionality plays a role in maintaining stemness in proliferating ES cells and that nanofibrous structures could be the bioengineering tool used to exploit this role. It has been demonstrated that cells cultured on nanofiberous 3-D meshes experienced a loss of actin containing stress fibers and the absence of classic focal adhesions (Schindler et al., 2005). Stem cells cultured on nanosurfaces experience increased adhesion and formation of stress fibers that usually coincide with increased differentiation; therefore, it could be hypothesized that the loss of actin and focal adhesions could in fact be the reason for the ability of 3-D nanofiber networks to maintain stemness. The ability of nanostructures to affect stem cell behaviors such as attachment, proliferation, and differentiation shows the value of understanding and utilizing these special structures in advancing the applications of stem cells.

1.3 Stem Cell Interactions with Nanoparticles

Nanoparticles can be used for a variety of applications with stem cells and cancer cells. Magnetic or fluorescent nanoparticles are attached to the surface of stem cells in order to separate them from larger groups of cells by flow cytometry. Nanoparticles can also be internalized in stem cells and cancer cells after which the internalized nanoparticles can be exploited for a variety of functional purposes, such as gene delivery or transfection. For example, nanoparticles are used to deliver substances that need to be protected from the outside environment such as DNA to stem cells or drugs to cancer cells. Nanoparticles can also be used in vivo as markers to track transplanted stem cells or to locate tumor cells with selectively properties in vivo. In relation to stem cell applications, nanoparticles can be used as contrast agents or vehicles. Contrast agents such as magnetic nanoparticles are the target substance for delivery to the cell and are usually encapsulated by another substance before applied to the cell. These nanoparticles can be used as vehicles for delivery of target substance to the cell.

1.3.1 Nanoparticles as Contrast Agents

1.3.1.1 Super Paramagnetic Nanoparticles

Paramagnetic materials are materials that do not normally have magnetic properties, but become magnetic when exposed to an external magnetic field. Superparmagnetic nanoparticles are small particles that can act as imaging probes in magnetic resonance (MR) images. The most commonly used superparmagnetic nanoparticle is iron oxide (FeO_2), which is biocompatible and inert. Gadolinium (Gd) is another paramagnetic nanoparticle that can be visualized by MR imaging. Gd is strongly toxic as a free ion so it is nessecary that it be combined with ligands to form very stable chelates when used for biological purposes. Iron oxide nanoparticles usually consist of a FeO_2 core and an outer polymer shell and acts primaraly as a negative T_2 constrast agent producing dark spots in MR images. Gd acts to enhance T_1 MR images.

1.3.1.2 Quantum Dots

Quantum dots are inorganic semi-conductor nanoparticles that have been explored as fluorescent labeling agents for cells for biological imaging. Quantum dots are typically less than 10 nm in size. Quantum dots are advantageous over conventional organic probes because they can be excited by a wider range of wavelengths and they exhibit narrower emission bandwidths (Dubertret et al., 2002). Quantum dots (QDs) are coated with ligand shells for incorporation into cells. (Dubertret et al., 2002) CdSe/ZnS-core/shell quantum dots are of special interest because of their uniquely strong luminance and high photostability (Hoshino et al., 2004).

1.3.1.3 Nanoshells

Nanoshells are composed of a dielectric core and surrounded by a thin metal shell. Nanoshells cores and shells are typically silica and gold respectfully. Nanoshells can be designed with specific optical emission absorption properties. Nanoshells tunable optical resonance has also been exploited to generate heat (Cuenca et al., 2006).

1.3.2 Nanoparticles as Vehicles

1.3.2.1 Silica Nanoparticles

Organically modified silica nanoparticles surface functionalized with amino groups have been shown to bind and protect plasmid DNA from enzymatic digestion during the tranfection process. Silica nanoparticles are used as an immobilization matrix rather than in tracking. Silica nanopartilces have been used to deliver DNA to stem cells in vivo (Bharali et al., 2005). Mesoporous silica nanoparticles could be internalized by cells without modification (Huang et al., 2005).

1.3.2.2 Polymer Nanoparticles

Polymer nanoparticles can encapsulate substances to provide protection from the outside environment and add specificity for targeted cell delivery. Through controlled biodegradation, polymers can assure a controlled rate for sustained drug release. An important use of polymer nanoparticles is as a carrier for gene therapy. Catatonic polymers bind and condense plasmid DNA to protect it during intracellular transport. Polymer surfaces are also easy to modify which raises the prospect of targeting specific cellular receptors to avoid side effects resulting from expression of the genes in sites other than those intended (Corsi et al., 2003). Some polymers used as gene carriers are poly-*L*-lysine (PLL), polyethyleimine (PEI), chitosan, and poly (lactide-*co*-glycolide) (PLGA). Polymer nanoparticles show evidence of varying levels of cytotoxicity and transfection efficiency and can be modified to optimize these characteristics (Corsi et al., 2003).

1.3.3 Effect of Internalized Nanoparticles

1.3.3.1 Toxicity

The toxicity of commonly used nanoparticles is manner of debate. There is significant evidence for the toxicity of commonly used non-nanoparticle cationic liposome transfection agents (van den Bos et al., 2003). The limitation of cationic liposome is one of the reasons that the use nanoparticles as carriers for genes, and tracking agents is of such interest. Quantum dot tracking agents exhibit concentration dependent toxicity, but have not appeared to cause cytotoxicity at lower, but still functional levels and encapsulation can alleviate this effect (Dubertret et al., 2002; Hoshino et al., 2004). Polymer nanoparticles have also contributed to increased cytotoxicity at varying levels depending on conditions such as the type of polymer or the molecular weight (Corsi et al., 2003). FeO_2 particles can be toxic to cells at high concentration as well, but can be internalized at applicable concentration without apparent toxicity. The toxicity of nanoparticles is a however a subject of debate and there are conflicting reports on the toxicity of nanoparticles, but the overwhelming majority of published experiments reported negligible or minimal effects on cell viability for the specific type and concentration of nanoparticles that were transfected (Aime et al., 2004; Bulte et al., 2001; Corsi et al., 2003; Huang et al., 2005; Jendelova et al., 2004; Lewin et al., 2000; Miyoshi et al., 2005; Vuu et al., 2005; Zhao et al., 2002). Unfortunately, most viability tests conducted on nanoparticles are done over relatively short periods of time and with little detail. It has been stated that there is a serious lack of information concerning the impact of nanostructured materials on human health and the environment (Braydich-Stolle et al., 2005). An important issue in the assessment of the safety of nanoparticles may be the in vivo effects at the level of the organism and the long-term effects. Because of its sensitivity to

environmental changes, a mouse spermatogonial stem cell line was used as a model to test the effect of different nanoparticles in vitro (Braydich-Stolle et al., 2005). Silver, aluminum, and molybdenum nanoparticles added into the spermatogonial cell line culture all demonstrated a concentration-dependent toxicity whereas the corresponding soluble salts had no effect. The effect of nanoparticle internalization was dependent of the material, as silver nanoparticles were very toxic and molybdenum did not affect metabolic activity at low concentrations.

1.3.3.2 Differentiation

The effect of internalized nanoparticles on differentiation is also a phenomena that is not well understood. Stem cells have been reported to differentiate normally with internalized iron oxide, quantum dots, and silica nanoparticles, but there are relatively few cases in which were this effect has been investigated (Bulte et al., 2001; Huang et al., 2005; Jing et al., 2004). In addition, many of the conclusions made about the normal differentiation of cells with internalized nanoparticles have been made based on morphological observations and gross analysis on the growth properties of cells without detailed characterization of molecular activities such as gene expression (Hsieh et al., 2006). Investigation of the differentiation properties of bone marrow stem cells (BMSCs) cultured with internalized CdSe/ZnS quantum dots showed that cells with quantum dots exhibited impaired linage specific gene expression for chondrogenesis and osteogenesis (Hsieh et al., 2006; Hsieh et al., 2006). The presence of quantum dots did not affect the proliferation of BMSCs or the size of chondrospheres after chondrogenesis induction, but mRNA, protein of type Ⅱ collagen and aggrecan was significantly inhibited. In a separate experiment, BMSCs induced to differentiate to an osteogenic lineage displayed lower alkaline phosphatase activity and significant inhibition of osteopontin and osteocalcin expression when compared to control cells. These results raise concerns about the effect of using quantum dots to label stem cells as well the possibility that internalization of other types of nanoparticles could have similar effects on stem cell differentiation.

1.3.3.3 Cell Internalization of Nanoparticles and Cell Tracking

In order to utilize nanoparticles to track stem cells or deliver agents to them, the nanoparticles must first be internalized in vitro or in vivo. Methods of labeling cells by surface attachment that are commonly used in cell sorting are not suitable for in vivo conditions because of the rapid reticuloendothelial recognition and clearance of cells thus labeled (Lewin et al., 2000). The most common modes of internalization are pinocytosis, which deals with the ingestion of fluid by means of small vesicles and endocytosis, which is a process where substances bound to the cell membrane and molecules present in the extracellular fluid are entrapped in endosomic vesicles. The high incubation concentration for absorption