CHAPTER 1

General Introduction & Aim of this Thesis
1.1 Cell and material interfaces

The acceleration of aging population and human pursuit of health and longevity have stimulated society's demand for the development of tissue engineering and regenerative medicine\(^1\). With the vigorous development and major breakthroughs of regenerative medicine technology, biomaterials occupy a very important position for providing a suitable therapeutic option with a reduced risk of disease transmission, infection, and immunogenicity, and limitless availability\(^2,3\). Although biochemical factors (e.g., growth factors\(^4\), hormones) regulate cells and tissue functions, the cell-materials interactions, especially the biophysical effect of materials, determines cell functions in development, physiology, and pathophysiology and remains a central endeavor in tissue engineering\(^5\).

1.1.1 The cellular microenvironment

Cells are known to reside in a highly dynamic and extremely complicated three-dimensional (3D) microenvironment, which not only serves as structural support but also provides diverse biochemical and biophysical cues, that regulate cell functions and development\(^6\). The cell microenvironment (Figure 1) are normally including neighboring cells with highly structured and heterogeneous mix, soluble factors, extracellular matrix (ECM), and biophysical stimuli, e.g. mechanical property, 2D topography, and 3D geometry strongly influence cell behaviors such as cell adhesion, spreading, proliferation, cell alignment, migration, and the differentiation or self-maintenance of stem cells\(^7\).

Figure 1. Schematic illustration of the mean components of cellular microenvironment. Adapted with permission from ref\(^5\).

The interaction between neighboring cells plays an important role to determine the physiology and cell behaviors of living cells\(^8\). For instance, Ding et al. have demonstrated that matrix stiffness and cell-cell contact interplay in the mesenchymal stem cells (MSC) differentiation decision\(^9\). In addition, others illustrate that co-culture of MSC and osteoblasts could change the osteogenic differentiation\(^10\). What's more, some recent work has used peptide nanofibers to mimic the cell-cell interaction...
indicator N-cadherin, which can facilitate MSC differentiation into chondrogenic lineage. Nowadays, ECM has attracted extensive interest for the regulation of cell behaviors. As it is well known that cells can sense and respond to surrounding ECM in which they reside, the components of ECM and ECM-like components such as collagen, laminin, gelatin, and fibronectin are crucial for the regulation of cell spreading, proliferation, migration, and differentiation. For instance, fibronectin and laminin are binding to their neighboring cells and other ECM proteins on the nanoscale, thus initiate various of intracellular signaling pathways. Some interesting work has shown that mechanical properties such as changing ECM stiffness is needed for reprogramming normal cells into tumor precursors.

It is generally accepted that chemical or biochemical factors like –COOH, –NH₂, growth factors, or hormones are critical for a number of biological activities. For instance, the platelet-derived growth factors can recruit dermal fibroblasts crusting to the wound site of injury. And some studies demonstrate that collagen hydrogel incorporated with graphene oxide (GO) absorbed transforming growth factor β (TGF-β) regulating MSCs differentiation into chondrogenic lineage. Except for the chemical factors, physical stimuli like strain and stress, magnetic, electrical, thermal, light, stiffness, and topography also serve as an important signal for regulating cell shape, elongation, migration, proliferation, and stem cell differentiation. In this section, we will mainly focus on cell-materials interactions especially the topography influence of materials determines cell behaviors, like migration, macromolecular crowding, and gene-deliver efficiency.

1.1.2 Topography stimuli
Researchers increasingly highlight the essential role of nano-/micro-scale topographic structure on the profound regulation of cell behaviors. Cells in vivo experience, sense, and respond to their surrounding physical cues from few nanometers to hundreds of micrometers by contact guidance, and translate these physical stimuli into intracellular signals through mechanical transduction. The mechanical signals modulated mainly through direct interactions of integrin clustering, activates focal adhesion and RhoA/ROCK pathway, further induce cell skeleton tension and cell morphology change, thereby altering relative gene expression to regulate cell functions (e.g., cell adhesion, alignment, proliferate, migrate and differentiation).

The natural tissues like bone, tendon, and nerve have anisotropic hierarchical structures with nano-/micro-sized features. To better mimic the natural structure of extracellular matrix and prepare substrate with different topographic cues, numerous fabrication methods have been developed. For example, microcontact printing, photolithography, photopatterning, electrospinning, microfluidics-assisted patterning, plasma oxidation for the purpose to developed the 2D substrates or hydrogels for mimicking the more natural 3D geometry, etc. Understanding interaction between topographic cues and cells, as well as the modulation of specific biological functions is still a challenge for materiobiology and tissue engineering.

Extensive research has fabricated different kinds of structures, normally categorized into two classes: isotropic structure (e.g., roughness, porosity) and anisotropic ones (e.g., grating, pillar, fibers, wrinkle), as well as 3D geometry cues. The mechanical properties of substrates can regulate the mechanical transduction of cells and further have an influence on the cell adhesion, cell shape and cytoskeletal architecture that are altered by micro/nanopatterns on the surface of a substrate. The nano- and micro-sized architecture is crucial for cell functions (adhesion, migration, proliferate and differentiation) modulation. For instance, previous studies have indicated that the anisotropic architectures of grooves endow cell alignment to the substrate, and the alignment of nanofibers can promote neuron and myoblasts maturation and differentiation. In Figure 2 some typical cell adhesion distribution and cell skeletal alteration are shown that have been identified previously.
In addition, in heart tissue engineering, the nanoscale cues of hydrogels can induce anisotropic cell behavior and contractility characteristics as observed by cells in their native environment, which gives guidance information for heart tissue repair. Others works have shown that in an \textit{in vitro} wound healing procedure the fibroblasts showed elongated shape along the nanogroove and cell migration rate was regulated by the substrate features, thus nano-groove size and density are important considerations for tissue engineering scaffold design. What’s more, the 3D geometry developed by changing the pores or cross-link ratio the geometry is able to regulate stem cell differentiation behaviors. Taken together, the topography is essential for cell function modulation, learning cell and materials-interfaces is the key point for tissue engineering and disease therapy. In this thesis, we mainly focus on the topography influence on cell behaviors, like migration, macromolecular crowding, and gene-deliver efficiency.

**Figure 2.** (A),(B) Micro-/nanopatterned substrates regulate the distribution of integrin-mediated adhesions, cell shape, cytoskeletal architecture and multicellular organization, (C, D) Further controlled by adjusting the material stiffness, (E, F) Nano-topological features to regulate cell–matrix adhesions for the manipulation of the size and geometry of cells cultured on them. Adapted with permission from ref\textsuperscript{7}.

### 1.1.3 Cell migration

Recent studies focus more on the cell migration behaviors, for the reason that cell movement is essential for numerous physiological and pathological processes such as embryonic development, angiogenesis, immune surveillance, cancer metastasis, tissue regeneration, and wound healing. The topography plays a key role in affecting cell behaviors like cell adhesion, alignment, proliferation, migration, and differentiation. The interfaces between cell and topographic cues is essential for the modulation of cell migration in wound healing procedure. Wound healing is a complex biological process involving a series of events including hemostasis, inflammation, proliferation, and differentiation.
process and the typical wound healing procedure mainly including four phases as shown in Figure 3, including: (1) Hemostasis phase, (2) Inflammation phase, (3) Cell migration/proliferation, and (4) Remodeling phase. In the hemostasis phase, the platelets modulate the process by interacting with subendothelial matrix proteins and tissue factor-bearing cells to move fast to the wound site, and clustering to the platelet plug and releasing various growth factors and matrix remodeling enzymes. In the inflammation phase, the quick movement of immune cells like macrophages or neutrophils is the key factor for the clean-up of dead cells and bacteria and then release growth factors promoting the fibroblasts cell migration. In the cell migration/proliferation procedure, the fibroblasts move to the wound site and proliferate in the wound site to remodel the tissues, afterward, epithelial cells migrate on the wound edge to cover the defect.

Learning about cell migration is critical for the wound healing procedure. The cell migration has been shown to be directed by chemical factors, cell density, molecular signals, stiffness, and topography. The design and manipulation of topographic biomaterials play a pivotal role in controlling cell migration and avoid scar formation. For instance, C2C12 cells grown on suspended fiber networks showed higher migration speed on the attached and aligned adhesion site. Others showed that fibroblast migration speed is influenced by the density of the nano-topographic pattern as well as on the width or depth of the groove. In addition, the collective migration of osteoblast-
like cells (MG-63) and human mesenchymal stem cells were modulated by smaller groove depth in an in vitro fracture healing model\textsuperscript{69}. What’s more, some interesting work demonstrate that not only the topographical density but also the orientation of the nanogroove distinctly regulate NIH-3T3 cell migration speed, cell division, and ECM production in dermal wound healing procedure\textsuperscript{70}. Taken together, the design of topographic material is crucial for understanding cell-matrix interactions and provide guiding information for tissue repair.

1.1.4 Macromolecular crowding

Learning about the cell-material interfaces is critical for many processes and the interface greatly influences intracellular mechanisms and phenomena. The cytoplasm is always heterogeneous and highly volume-occupied with biomolecules, like various nutrients, proteins, nucleic acids, enzymes, intermediate metabolites, and other macromolecular monomers/components. The concentration of macromolecular components that can be reached as high as 50-400 mg ml\textsuperscript{−1} in the crowded and confined spaces\textsuperscript{71}. Accordingly, the high concentrations of macromolecular components inside the cell affects the function of molecular chaperones, polypeptide chains and oligomeric proteins folding\textsuperscript{72}, improve enzyme reaction rate\textsuperscript{73}, and metabolic activity\textsuperscript{74}. Recently, numerous studies focus on the addition of some natural or synthetic polymers like Ficoll\textsuperscript{75}, dextran, poly (N-vinylpyrrolidone) (PVP)\textsuperscript{76}, polyethylene glycol (PEG)\textsuperscript{77} or bovine serum album in the culture media to artificially control and enhance the macromolecular crowding inside the cell and study how it affects cell behaviors. For instance, the addition of carbohydrate-based macromolecules can dramatically enhance stem cells extracellular matrix production and further influence the cell skeleton alignment, proliferation and differentiation\textsuperscript{78}. Also, the macromolecular crowding is applied to material synthesis, and molecular self-assembly\textsuperscript{79}.

For the purpose of exploring macromolecular crowding inside the cell, some excellent work has been done on developing a sensor to directly determine the crowding inside the cell\textsuperscript{80,81}. For instance, Boersma and coworkers developed a FRET-based sensor, which has the FRET pair m-Cerulean (cyan fluorescent protein) and m-Citrine (yellow fluorescent protein) positioned at the N terminus and C terminus, respectively, for directly measuring the crowding inside living cells. The schematic picture of the crowding sensor is shown in Figure 4. The changes in fluorescence are a result from FRET efficiency adjustment, which increases with the increase of macromolecular crowding. The sensor readout is reversible, and only sensitive to the macromolecular crowding induced excluded volume\textsuperscript{82}. Topography has an impact on various cell behaviors like cell adhesion, alignment, proliferation, migration, and stem cell differentiation. Therefore, we believe that the spatiotemporal readout of crowding is a compelling tool for better understanding cell and biomaterials interactions and allow us to investigate the role of macromolecular crowding of the cytoplasm during the cell development stages.
1.1.5 Gene delivery

Nowadays, gene delivery has gained much attention for the purpose of delivery therapeutic genes or introduce artificial modified genes into cells for modifying the cell function or for specific clinical purposes. In recent decades, efforts have mostly focused on applications of biosensors, diagnostic devices, cancer therapy, tissue regeneration, and vaccine development therapy. It is well established that traditional gene delivery systems like electroporation, magnetofection, ultrasound, or viral vectors need expensive hardware, are time consuming, have high toxicity problems or possibly induce immunogenicity. Exploring high-efficiency gene carriers with low toxicity is still a challenge for gene delivery applications.

Due to the remarkable development in nanotechnology, considerable excellent works have been devoted to constructing non-toxic delivery systems with the basic concepts of low toxicity and high transfection efficiency. For instance, gene carriers like polyethyleneimine (PEI), lipofectamine, poly-amidoamine (PAMAM), silica-based nanoparticles (SNPs), and poly-lysine (PLL) are commonly employed for systemic administration. For instance, spikey nanoparticles demonstrate higher transfection efficiency than the hemisphere- and bowl-type subunit nanoparticles and avoids enzymatic cleavage. The vehicles enter the cell membrane by mimicking functions of viral agents that enable stronger binding affinity but avoid the immune potential and toxicity risks of viral vectors. It remains challenging to develop highly active polymers to achieved high transfection efficiency with lower toxicity.

It is important to highlight that present research suggest that substrate-mediated gene delivery plays a critical role in gene delivery systems owing to diverse physiochemical properties and good biocompatibility. For instance, vertical silicon nanowires, silicon nano- to microscale pillars, aligned hollow carbon nanotubes, and nano-grooves play critical roles in gene delivery. For example, some work observed that 200 nm nanopillars can improve human mesenchymal stem cells (hMSCs) transfection efficiency. Some other interesting work found that different shape of pillars showed different gene transfection behaviors and the pillars can penetrate into cell membrane while maintaining cell viability. In another study it was found that nanogrooves influence gene transfection by controlling cytoskeleton organization and nuclei morphology. Taken together, the substrate-mediated gene delivery is promising for non-viral gene transfer in which topography as physicochemical stimulus is able to drive and influence cell function, which is crucial for understanding how material interfaces can be applied to enhance gene delivery.
1.2 Aim of this thesis

The general aim of this thesis is to explore topography-mediated alterations to cell behavior using cell-materials interfaces, and investigate subcellular behaviors like cell morphology alteration, cell migration in wound healing procedure, inner cell macromolecular crowding induced by nano-/micro-patterns, and topography modulated gene delivery of stem cells. For this purpose, wrinkle-gradient substrates were fabricated with diverse wavelength and amplitude parameters to make it possible for investigating the topographic cues as well as direction of wave-like topography on the cell migration behavior in wound healing. In addition, uniform wrinkle surfaces were developed with diverse wavelength and amplitude parameters to study the topography influenced cell macromolecular crowding and identifying the possible related signaling of cell shape alterations, mechanical transduction, metabolic activity and protein expression. Furthermore, the uniform wrinkle substrate were investigated as possible stimulation to control or even enhance gene delivery capacity of stem cells. Topography-induced improvement of transfection efficiency and endocytic capacity were investigated.

1.3 Outline of this thesis

**Chapter 1** gives a basic introduction of the cell micro environment and the essential factors for cell modulation, like ECM, chemical signals, cell-cell contact, physical stimuli (stiffness and topography). And the cell behaviors like migration, macromolecular crowding and gene delivery.

In **Chapter 2**, we report on recent progress of the gradient platforms, as also partly used in this thesis, to study bio-interfaces and the effects of physicochemical stimuli on e.g., cell adhesion, cell morphology, and migration.

In **Chapter 3**, wrinkle topography gradients were developed where especially the wavelength and amplitude were decoupled as such that the topographies, the wavelength and amplitude, both function as separate parameter and direction-induced cell migration, proliferation and adhesion was investigated.

In **Chapter 4**, uniform wrinkle surfaces were developed to investigate the role of topography on cell macromolecular crowding and identifying the possible mechanisms responsible for altered macromolecular crowding inside the cell.

In **Chapter 5**, topography-mediated gene delivery of stem cells is explored and it is investigated how topography may induce improvement of transfection efficiency and endocytic capacity.

This thesis finalizes with a general conclusion and discussion on how this thesis impacts the current scientific knowledge (**Chapter 6**).
1.4 References


(89) Kotnik, T.; Rems, L.; Tarek, M.; Miklavcic, D. Membrane Electroportation and


