Topography-mediated Control of Cellular Response: Migration, Intracellular Crowding, and Gene-delivery
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CHAPTER 6

General Discussion & Future Perspective
6.1 Discussion & Future perspective

Cell-material interfaces occupy a very important position in tissue engineering and regenerative medicine for regulating cell response and tissue functions\(^1\). Especially, the biophysical effect of materials that determines cell functions in development, physiology, and pathophysiology, remains a central endeavor in tissue engineering\(^2\). With the vigorous development and major breakthroughs of regenerative medicine technology, increasing amounts of information have been generated for understanding bio-interface complexity in regulating cellular responses. It remains a significant challenge to understand biophysical cues like topography to modulate cell development and subcellular behaviors\(^3\). The general aim of this thesis is to explore topography-mediated alterations of cell behaviors and investigate cell-material interface-induced subcellular behaviors like cell morphology alteration, cell migration in wound healing, intracellular macromolecular crowding, and topography-modulated gene delivery of stem cells.

In this thesis, we discussed the role of high-throughput screening (HTS) in regulating cell spreading, proliferation, and migration. Wrinkle-gradient substrates were fabricated with diverse wavelength and amplitude parameters for investigating the topographic cues on the fibroblast cell migration behavior in wound healing approaches. In addition, uniform wrinkle substrates were developed with various wavelength and amplitude parameters to study the topography-induced intracellular macromolecular crowding and identifying subcellular behaviors like cell morphology alterations, mechanical transduction, metabolic activity, and protein expression. Additionally, the uniform wrinkle substrates were also used for investigating the modulation of gene-delivery capacity. The main finding is that topography and its sub-parameters such as direction, wavelength, and amplitude have an important influence on fibroblast migration in wound healing procedure. Different wrinkle features induce different intracellular macromolecular crowding phenomena that is associated with other subcellular activities. In addition, wave-like topography-mediated enhancement of non-viral gene delivery of stem cells was investigated. The obvious influence of topography on cell spreading, proliferation, migration, macromolecular crowding, and gene expression highlights its importance as a design parameter for the application of biomaterials.

Considerable research has been devoted to using high-throughput screening methods to identify how to regulate cell behaviors\(^4-7\). Chapter 2 discussed that the high-throughput screening platforms serve as an important tool for determining cell adhesion\(^8\), spreading\(^9\), orientation\(^10\), proliferation\(^11\), migration\(^12\), and cell fate decision\(^13\). In this chapter the high-throughput screening platforms with physical cues (e.g. mechanical properties, topography, wettability), chemical or bio-chemical stimuli (e.g. material composition and proteins) and multiple parameter combinations were discussed for the manipulation of cell behaviors. Unlike the independent substrates or the randomly chosen degrees of biomaterial properties, the high-throughput screening platform combines multiple factors in a single system, which are timesaving, expedite analysis procedures, and minimizes systematic or methodological errors. To take the advantage of high-throughput screening methods as mentioned above, Chapter 3 indicated that the HTS developed enables efficient investigation for the modulation of fibroblasts migration in wound healing procedures\(^12\). The PDMS based topographical gradient with wave-like features were fabricated by decoupling the amplitude and wavelength gradually differ in wavelength and amplitude to explore the role of topographic direction, structure repetition, and feature size of the substrate on fibroblast migration. The approaches developed are combined with multiple parameters to make it possible to investigate cell migration behaviors in a high-throughput way. In addition, the PDMS used have the properties of cost-efficiency, non-toxic and approved by FDA for implantable engineering scaffold\(^14\). The results indicated that cell movement was guided by topographical properties, with a lower wrinkle wavelength (2 μm) eliciting the fastest migration speed, and the migration speed increased with decreasing amplitude\(^15\). The wavelength and amplitude both play an important role in directing cell migration. The cell migration further depends on the...
topographical orientation with respect to the anisotropy of the topography and the cell migration speed is regulated by focal adhesion expression. These results demonstrate that anisotropic gradient platform can serve as an effective system to obtain the optimum parameter for specific cellular behaviors, which could improve regenerative medicine.

Cell movement is also essential for numerous physiological and pathological processes such as embryonic development, angiogenesis, immune surveillance, cancer metastasis, tissue regeneration, and wound healing. Except for the topography stimuli, the cell migration is regulated by chemical factors, stiffness, growth factors, electrical signals, molecular signals, and cell-cell contact. For instance, the growth factors regulates cell migration by controlling the cell focal adhesion and contractility. However, some research demonstrates that growth factors like PDGF, bFGF, TGF-β2, and TGF-β3 can stimulate fibroblasts to excessively produce ECM, which may induce scar formation. The current investigations in this chapter focus more on the biomaterial scaffolds to mimic the ECM topography enabling cells to be guided by ‘contact guidance’ or ‘topotaxis’ leading to less scar formation. Compared to the normaly used nano-grooves with right angles and sharp ridges, the wave-like substrate we used with a semicircular shape, better mimic the ECM fibers and therefore represent a more biologically relevant approach to study the natural wound recovery procedure. Additionally, the topographic orientation significantly affects the cells migration capacity in the wound healing procedure, which provides important instructions for material design.

This high-throughput screening approach which was used to investigating the fibroblasts migration with various surface feature parameters can provide a superior data collection with fewer experiments. It can be further used to mimic the complexity of in vivo conditions for other tissues or be used as a model to control tissue properties and cell behaviors. For instance, Zhou et al. prepared directional wrinkle gradients to investigate the osteoblast attachment and cell orientation. And the authors translated PDMS-based wrinkle gradients to inorganic surface (SiO₂, TiO₂, CrO₃, and Al₂O₃) to investigate the hBM-MSC orientation and focal adhesion assembly. Furthermore, Yang et al. used the high-throughput screening approach for investigate the influence of mesenchymal stem cells differentiation towards osteogenic and neuronal lineage, respectively. Artificial ECM-mimicking scaffolds that are designed according to the special features of a tissue (e.g., its composition, mechanical properties, topography, and 3D geometry) have been determined to provide biological activity clues to regulate cell functions for tissue regeneration. What is more, the mechanical properties of the substrate also play a crucial role in modulating various cell behaviors. For example, substrates of about 30–35 kPa are beneficial for osteogenic differentiation, softer substrate (<1 kPa) enhance neurogenic differentiation, and substrate with moderate mechanical properties improve myogenesis or adipogenesis. Therefore, it would be a promising strategy for combining several parameters on single substrate and find the promising parameter in an efficient way. Therefore, the HTS platforms can be combined with other bio-factors (like soluble factors), mechanical stimuli (like stiffness and wettability), 3D scaffolds (like collagen scaffold and hydrogels) and the anisotropic geometry to regulate cell functions of tissues (e.g. the muscle, tendon, bone, heart, skin, and nerve) in vivo.
Figure 1. Materials with tissue-mimetic physical properties (e.g., compositional, mechanical, and structural features) offer specific stimulations to accelerate tissue regeneration. Reprinted with permission from ref36.

Except for the high-throughput platform, the influence of uniform wrinkle topography on macromolecular crowding and gene delivery were explored. In Chapter 4 the topography induced macromolecular crowding alteration in living cells were discussed. The macromolecular crowding components inside the cytosol has a profound impact on polypeptide and oligomeric proteins generation, folding, diffusion, enzymatic reactions37, and metabolic activity38. For instance, the addition of some natural or synthetic polymers can enhance the extracellular matrix deposition and metabolic stimulation of MSCs39. It is well established that topography has an impact on various cell functions, thus investigating the macromolecular crowding of cells that are cultured on topographic surfaces is important for understanding cell-material interfaces and illustrate what occurs naturally inside the cell. The HEK293T cells were transfected with a fluorescence resonance energy transfer (FRET)-based sensor for direct evaluate the macromolecular crowding inside living cells that are stimulated by wave-like surface topographies. The substrates used are uniform wrinkles with different wavelengths that is 0.5 μm, 2 μm, 10 μm, 25 μm and the Flat surface functions as the control. The main findings is that, increased macromolecular crowding was observed for cells cultured on 0.5 μm and 2 μm topographies, and the 2 μm induced a larger cell area and nucleus formation, higher
metabolic activities, proliferation rate, and more protein expression, correlated with increased focal adhesion and myosin tension but not YAP-TAZ transduction.

These findings illustrate that the spatiotemporal readout of crowding is a compelling tool for understanding cell-biomaterials interactions, thereby giving direction to identify specific mechanisms and allow us to investigate the role of macromolecular crowding of the cytoplasm during the cell development. Previous studies demonstrated that topography-induced acceleration of osteogenic differentiation is caused by focal adhesion, RhoA/ROCK signaling pathway. Some work in our group determined that topography induced enhancement of stem cell differentiation is correlated with more focal adhesion formation, myosin tension and YAP-TAZ localization into the nucleus. Chapter 4 shows that higher crowding is correlated with increased focal adhesion and cell contractility but not YAP-TAZ transduction. This is probably due to the different cell type and cell signalling pathways. For instance, osteogenic differentiation is also mediated by other pathways like mitogen-activated protein kinase (MAPK) pathway, integrin-linked kinase (ILK)/β-catenin pathway, FAK/MAPK pathway, and extracellular signal-regulated kinase 1/2 (ERK1/2) pathway. Therefore, further investigations are necessary to completely identify the mechanisms for macromolecular crowding enhanced by topography.

It should be noted that this crowding sensor can be used in Escherichia coli and eukaryotic cells. Additionally, it is sensitive only to the excluded volume induced by macromolecular crowding and the sensor readout was reversible. In other research, different types of sensors were used for in vitro and in vivo studies. For example, the sensor arrays to monitor cell adhesion and spreading, biomimetic sensor for detecting nitric oxide molecules, synthetic fluorescent sensors to detect metals ions, and metabolic sensor that couples nutritional availability. Therefore, it would be a promising strategy to study cell-materials interfaces using crowding sensor correlated with other sensors. Furthermore, as stem cell is essential for tissue engineering, we have tried to import the crowding sensor into hBM-MSCs but due to the low transfection efficiency, the crowding effect was not achieved. Expectantly, in the future, we can further extend this crowding sensor combined with other intracellular sensors into stem cells with high-throughput screening approaches that can provide more information about cell-materials interfaces and many new insights for tissue engineering and regenerative medicine.

In addition to the uniform wrinkle surfaces that modulated macromolecular crowding, in Chapter 5 the aligned nano- and micro-patterned PDMS substrates were used to investigate the topography influence on gene delivery in hBM-MSCs and myoblast cells. Gene delivery on purpose to introduce therapeutic genes or artificially modified genes into cells for modifying the cell function, which is essential for applications like biosensors, cancer therapy and tissue regeneration. In comparison to the traditional deliver systems that need expensive hardware, time consuming, possibly induce immunogenicity, or have high toxicity problems, substrate-mediated gene delivery system is promising owing to its diverse physicochemical properties and good biocompatibility. We found that a 55% percent improvement of transfection efficiency was identified for hBM-MSCs grown on 2 µm wrinkles as compared to hBM-MSCs cultured on Flat controls. The highest gene-expression efficiency was observed on the 10 µm topography of V49 fibroblasts, which enhanced the transfection efficiency by 64% as compared to the Flat control. The results are in line with our initial hypothesis that mechanical stimuli of topography induced substrate-mediated gene delivery of stem cells. The induced gene transfection efficiency highlights the importance of topography-mediated gene delivery.

In Chapter 5, the hBM-MSCs spreading area and elongation was altered by topography. Correspondingly, other works of our group have explored the influence of topography on cell morphology change and stem cell differentiation. Similarly, others demonstrated well spread area
and elongated morphology promote mesenchymal stem cells gene transfection. Another study showed that nanogrooves influence gene transfection by controlling cytoskeleton organization and nuclei morphology. The gene transfection efficiency of hBM-MSCs was enhanced on W2 more than on the other wrinkle features. As well as our previously work reported, the wrinkle topography has an influence on single cell stiffness, stem cells growth on the W0.5 and W3 and showed higher stiffness than on the other topographies and stem cells differentiation behaviors concomitantly changed. However, the reason that induces the diverse of stem cells tansfection are still not clear. The intracellular processes such as internalization, endosomal escape, cytosolic trafficking and nuclear entry play a central role in gene delivery. Some researchers showed that cellular uptake of cationic complexes mainly rely on clathrin-mediated endocytosis, which is modulated by cell division control protein (Cde42) from the Rho family of GTPases. RhoGTPases activation is essential for focal adhesion assembly and disassembly, focal adhesions anchor actin stress fibers in turn facilitate intracellular trafficking that presumably improved transfection. Additionally, the proliferation and cellular metabolism is often shown to enhance gene delivery. Therefore, further investigations (e.g. specific mechanotransduction signal pathways and metabolic activities) are necessary to completely identify the mechanisms for gene delivery enhanced by topography.

**Figure 2.** Niche interactions known to modulate stem cell phenotype. Reprinted with permission from ref64.
As it mentioned in the beginning of this thesis, cells are residing in a highly dynamic and extremely complicated three-dimensional (3D) microenvironment (Figure 2), which provides diverse biochemical and biophysical cues, that regulate cell functions and development. The interaction with neighboring cells, soluble factors, extracellular matrix (ECM), and biophysical stimuli (e.g. mechanical property, 2D topography and 3D geometry) strongly influence cell behaviors (e.g. cell adhesion, spreading, proliferation, cell alignment, migrate and the differentiation or self-maintenance of stem cells). This thesis provides evidence that topography works as a useful tool for understanding cell-material interfaces and investigate cellular behaviors like mediated alterations of cell migration, intracellular macromolecular crowding and gene delivery. However, it is demonstrated in other research that the cellular behavior can be obviously different in 2D and in 3D culture models. For instance, Burdick et al. found that MSCs displayed enhanced cell spreading and more YAP/TAZ translocated into the nucleus when cells are grown on the surface of stiffer hydrogels; however, the complete reverse trend was detected when cells were seeding within hydrogels with 3D structure, highlighting the important role of 3D structure for in vitro investigations. In the future work, it would be fascinating to couple topography with 3D micro niches for better mimicking natural tissue structure to explore the influence on cell functions and translation towards commercial uses and clinic application.
6.2 References


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