Exploring combined influences of material topography, stiffness and chemistry on cell behavior at biointerfaces
Zhou, Qihui

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 31-12-2018
CHAPTER ONE

GENERAL INTRODUCTION
Faced with an ever-increasing burden of disease, congenital abnormalities and accidents each year, tissue engineering and regenerative medicine (TERM) hold great promise to repair or replace tissues or even entire organs on demand for a better quality of life [1,2]. Undoubtedly, biomaterials play an increasingly pivotal role in the development of biomedical devices and tissue engineering scaffolds [3], which seeks to unlock the mechanism underlying cell-material interactions named as materiobiology [4]. Harnessing the cell-material interactions to trigger the tissue regenerative potential to ensure normal tissue formation and maturation during healing is still a huge challenge. For that reason, numerous research groups have been trying to design and develop the fourth generation of biomaterials with smart or biomimetic features (Figure 1) based on recent discoveries about the complex cellular microenvironment and its interaction with cells [5–7].

Figure 1. Evolution of biomaterials. Materials that originally were meant not to interact with surrounding biological systems (inert) to materials that not only interact but also determine the fate of surrounding tissues and cells.

1.1. THE CELLULAR MICROENVIRONMENT

*In vivo*, cells are known to reside in a highly dynamic and extremely complicated system surrounded by multiple biochemical and biophysical signals, defined as “cell microenvironment”, that significantly mediates cell behavior and fate [8]. Particularly, stem cells localize within a specialized microenvironment, termed “niche” [9–11], a concept that was originally formulated by Schofield in 1978 to describe the hematopoietic stem cell microenvironment [12]. The stem-cell niche provides the specific physicochemical features to renew themselves and govern their survival, stemness (maintenance of their pluripotency), and the phenotype of differentiation for tissue repair and regeneration [2,10]. In general, there are the four key components within the cell microenvironment (Figure 2), including neighboring cells, soluble factors, biophysical stimuli and the surrounding extracellular matrix (ECM), which synergistically influence cellular responses, e.g., adhesion, proliferation, migration, self-renewal, differentiation, and so on [8]. Herein, neighboring cells, soluble factors and biophysical stimuli are briefly introduced together and then the surrounding ECM is highlighted in this section.
1.1.1. NEIGHBORING CELLS, SOLUBLE FACTORS AND BIOPHYSICAL STIMULI

 Cells in vivo do not reside in isolation but rather communicate with both similar and diverse kinds of cells. Cell-cell interactions play a critical role in cell function and tissue development. Generally, cell junctions (e.g., tight junctions, anchoring junctions, and gap junctions) are induced by specific adhesion proteins, such as cadherins or related proteins (e.g., desmogleins and desmocollins) or ions (e.g., Ca$^{2+}$) \cite{8,13}. Many studies have demonstrated the pivotal role of direct cell-cell interactions in governing cell responses and tissue development \cite{14}. Recently, owing to integrated organ-on-a-chip or microfluidic co-culture platforms that were developed specifically to detect these interactions, the direct cell-cell communication and interaction are relatively well understood \cite{15,16}. The features of microfluidic systems include high-throughput, miniaturization, high resolution, and integration, which can mimic complex microenvironment and simplify their complexity for studying different cell types, such as adult cells, stem cells, immune cells, and cancer cells \cite{17,18}.

 In addition to direct cell-cell contact, indirect interaction between cells mediated by soluble factors also plays a key role in cell behavior and tissue function. In vivo, there are many soluble factors within the cellular microenvironment, including diverse nutrients (e.g., glucose, glutamine and amino acids) and signaling factors (e.g., growth factors, mitogen, hormones and cytokines) \cite{19,20}. Among soluble factors, growth factors are the most important and widely investigated cues for constructing the functional and biomimetic microenvironment \cite{21}. Generally, these growth factors dissolving in aqueous
media or immobilized on the ECM are dynamic in space and time and in a concentration gradient. The specific growth factors, local concentration, and their time-dependent and spatial distribution play key roles in controlling different cell responses. Therefore, the controlled release of growth factors on demand in the cell microenvironment is an important frontier.

Besides the cell-cell interactions described above, cells in vivo are often stimulated by numerous physical parameters, such as stress, strain, and thermal fields [8]. Particularly for the mechanical properties, cells may experience various stress and strain stimuli depending on their locations. They can sense and transduce mechanical cues into intercellular signals that modulate their cytoskeleton and respond through a process known as mechanosensing and mechanotransduction [22,23]. In general, the mechanical stimulus needs the mediation of the ECM to work on cells.

1.1.2. THE ECM

In human tissues and organs, every cell is exposed to an intricate 3D network of fibers named the ECM which is composed of proteins (e.g., collagens, elastin, laminin, and fibronectin) and glycosaminoglycans (e.g., hyaluronic acid) [24]. These interlocked ECM macromolecules are secreted and assembled by the resident cells of each tissue. As a result, the biochemical composition and specific physical properties of ECM are distinctive between tissues [25]. Interestingly, ECM exists in a dynamic state due to their neighboring cells and biophysical stimulus, which can mediate cell behavior dynamically and reciprocally [26]. Interactions between cells and their ECM have been extensively studied over the past few decades, yielding specifics on binding partners, motifs, and strengths [27]. Previously, the ECM has been considered as a passive supporting substrate in which the resident cells were regarded as the major actors. Recently, the ECM is undoubtedly increasingly recognized as a bioactive structure, which offers structural, mechanical, and compositional signals that can direct cell activities and functions during the natural tissue regeneration process [4,8]. The ECM is well-known to be essential for the physiology of living cells and tissue functions, while the aberrant ECM can induce unexpected cell behaviors and then result in some diseases (e.g., cancer, fibrosis) [28]. Therefore, mastering the communication between cells and ECM is one of the most important tasks in order to mimic the structural and biologic features of the native ECM within biomimetic materials for instructing cells with specific cues. Although the physicochemical properties of the ECM can be changed over time and in space, understanding their basic features will be beneficial to the design of desired functional and biomimetic materials. The effect of biochemical cues on cells has long been recognized, but the importance of the biophysical features from the ECM were neglected. Recently, it was increasingly appreciated that the ECM offers many specific cues to cells including the structural and dimensional presentation, the mechanical stiffness, and the spatiotemporal variations. Most tissue extracellular matrixes (e.g., bone, tendon, nerve, myocardium, etc.) have hierarchically organized and anisotropic structures consisting of well-aligned micro/nano-scaled fiber [29–31]. The ECM fiber orientation not only induces the orientation/migration of many cell types, but also directs cell function and stem cell differentiation [29,32,33]. Another important structural characteristic is the pores generated in the interstitial space of ECM networks [34]. The
porosity and pore size of the ECM mesh are crucial factors for determining the available space for cell penetration and exchange of nutrients. In addition, ultrastructural analysis of the native ECM fibers tend to have feature diameters from a few nanometers to ~150 nm and actual fiber bundles have feature diameters from several hundred nanometers to ~400 µm, depending on the tissue type [35–37]. The ECM biomechanical properties named as stiffness (elastic or Young's modulus) vary significantly between tissues and organs, and may play a critical role in tissue homeostasis and function [38]. It is reported that native tissues have mechanical features spanning orders of magnitude, from static or compliant (soft) in brain or lung tissue, to hard bone tissue owing to mineralized fibers [8,39]. Cells can sense and respond to different stiffness parameters through mechanosensing and mechanotransduction [40,41]. Importantly, changes of tissue stiffness are often considered a representative prognostic factor for diseases [42]. For instance, tumorigenesis and pathological fibrosis are closely related with an increase in matrix stiffening, because abnormal mechanical changes in ECM enhance tumor cell invasion and myofibroblast differentiation [38,43]. Taken together, the ECM biophysical properties can have a great impact on basic cell responses and tissue developmental processes.

1.2. CELL AND BIOMATERIAL INTERACTION

Biomaterials e.g. implants or scaffolds similar to native ECM macro-environment have been used widely to repair or engineer tissues, which are biocompatible, enable nutrient transport, and offer structural integrity [6,44]. In order to maximize the full potential of cell-based therapies in TERM, it is vital to engineer and understand artificial ECM signals that can regulate cell behavior. Recently, inspired by the complex cellular microenvironment mentioned above, researchers found that cells are inherently sensitive to surrounding static/dynamic microenvironment of biomaterials, especially physical cues (e.g., mechanical properties, wettability, 2D topography, and 3D geometry), substrate-immobilized insoluble/soluble biochemical signals (e.g., material component, fixed proteins or diffusible factors), or other stimuli [2,4,8]. It is well-documented that these biomaterial properties can regulate cell adhesion, morphology (e.g., cell shape, spreading, elongation, and alignment), function and stem cell differentiation [1,8].

1.2.1. CELL ADHESION AND MORPHOLOGY

Adhesion is the first step and critical requirement for anchorage-dependent cells to survive, proliferate, and consequentially functionalization or differentiation on a substrate [45]. Poor adherence of these cells to substrates causes cell quiescence or even apoptosis. Therefore, cell adhesion is regarded as the initial indicator of cell interplay with its surrounding microenvironments, which precedes all other cellular behaviors, e.g., spreading, migration, proliferation, and differentiation. Following cell adhesion, they start to conform to the microenvironments, which could result in changes in cell morphology, shape, spreading, elongation, alignment, and eventually cell differentiation. During embryonic development and through life, stem
cells stimulated by different cellular microenvironments are directed into specific cell
types that have different morphologies, e.g., branched neurocytes, circular adipose cells,
spindle shaped fibroblast, etc. Numerous researches have demonstrated that the
opposite is also true: cell morphology (e.g., shape, spreading, elongation and alignment)
acts as a potent regulator of cell fate [46].

For cell shape, Ding and co-workers cultured single mesenchymal stem cells (MSCs)
from rats on patterned hydrogels with the same adhesive area but different shapes. It
was found that the aspect ratio (AR) of the shape regulates the direction of MSC
differentiation. Comparing square and rectangular cells, the optimal adipogenic
differentiation appears at AR=1, but the optimal osteogenic differentiation was
identified when AR ≈ 2. Among the isotropic shapes, i.e., circular, square, triangular and
star shaped cells, the optimal adipogenic and osteogenic differentiations were found in
circular and star shaped cells, respectively [47]. Apart from studying shape-factors that
affect the phenotype direction of stem cells, Wang and co-workers also proved that the
multipotency of MSCs (stemness) decreased with increasing aspect ratio and spreading
areas [48].

For cell spreading, it was restricted by decreasing the area of substrate islands, which
leads to DNA synthesis inhibition and then induce the expression of involucrin, a
marker of terminal function [49]. Also, larger cell spreading on square patterned islands
causd cell survival and growth whereas smaller islands resulted in cell apoptosis [50].
Moreover, capillary endothelial cell shape was changed by ECM molecules, which can
regulate cell growth and differentiation [51]. Further, it is logical to extend this
mechanism to stem cells. McBeath and colleagues reported that highly spread MSCs are
more contractile, which leads to osteogenic differentiation, whereas MSCs were
bywardly restricted, resulted in adipocytes [52]. In addition, Wagner and co-workers
found that the elongated MSCs might drive the stem cells towards osteogenic lineage
whereas the round morphology enhanced adipogenesis [53].

Cell orientation or alignment, which refers to unidirectional organization of cell body,
plays a key role in various cell responses, e.g., cytoskeleton reorganization, membrane
protein relocation, migration direction, proliferation, ECM remodeling, and
differentiation [29,34,53]. In addition, cell alignment mimics the hierarchical structure,
provides mechanical property and special biological functions on tissue regeneration,
including neuron, skeleton, cardiac muscle and tendon [29–31]. Therefore, it is necessary
to build cell alignment in vitro for exploring biomechanics and cell biology, as well as
regenerating structured and functional tissue.

1.2.2. BIOMATERIAL TOPOGRAPHY AND STIFFNESS

Both biomaterial topography and stiffness serve as an important indirect signal, which
can be sensed by cells through mechanosensing and mechanotransduction. Both
material physical cues can be transduced into intracellular biochemical information, and
vice versa the intercellular signals can be transformed back to dynamic mechanical
signal (e.g., traction forces) [4]. The information communications between cell and
biointerface can activate the integrin-focal adhesion cytoskeleton actin transduction
pathway, stimulating cytoskeletal tension and inducing cell morphology deformation
and associated signaling cascades that thereby alter gene expression to regulate cell
functions and promote tissues regeneration [56]. More interestingly, cells present directed
migration behavior toward topography and stiffness gradients, which can contribute to wound healing and tissue repair [57,58]. Cell adhesion complexes (focal adhesions) and cytoskeletal generated forces play a significant role in transducing these signals into genetic events that eventually dictate cell fate and functions. Generally, biomaterial topographical structures can be divided into two types, i.e., isotropic and anisotropic. As mentioned in Section 1.1.2 about the ECM of anisotropic tissues, directional topography and its interaction with cells are of particular interest. Numerous micro and nanofabrication technologies have been developed to prepare aligned topographical features, such as electrospinning [32], plasma oxidation [55], photolithography [59], direct laser writing [60], etc. As is reported, aligned micro/nanotopography (e.g., wrinkle, groove, grating, aligned pores, etc.), which are comparable to the natural anisotropic ECM in vivo, can guide the orientation/elongation/migration of many cell types through the contact guidance and the structure-associated organization of cell adhesion ligands [29,33,61]. In addition, the dimension of aligned topography can significantly affect cell responses. Kim and co-workers observed the area of ventricular myocytes on hydrogel nanogratings ranging from 150 nm×50 nm×200 nm to 800 nm×800 nm×500 nm (width×gap×height) [62]. They found that the biggest features induced larger cell area and longer perimeter. Also, the cells on the hydrogel gratings were more elongated than those on the planar substrate. The majority of cell types has been demonstrated to significantly change their alignment on aligned topographies over the dimension range from nanoscale [29,62–64] to microscale [32,55,65]. For instance, MSCs were oriented and elongated along the nanogratings (250 nm width) whereas cells on planar substrate displayed an isotropic morphology [66]. Similarly, osteoblasts on the nanograting substrates were oriented when the dimensions down to 75 nm in width and 33 nm in depth [67]. On the other hand, Bashur and colleagues described that the diameter (1 µm) of electrospun PLGA microfibers also can induce the alignment of fibroblasts [68]. Even, the directional microtopographies with the size from tens to hundreds still showed effects to cell alignment [69]. Microtopography similar to the length-scale of a mammalian cell has effects on the whole cell body, while nanotopography mediated the signals to subcellular organelles and structures. Therefore, the optimization of topographical structures, particularly their sizes ranging from nanoscale to microscale, is critical to obtain the best cellular performance in TERM.

As mentioned in Section 1.1.2, tissues in the human body have different mechanical features ranging from soft (brain, ~0.1 kPa), to moderately stiff (skin and muscles, ~10 kPa) and stiff (precalcified bone, >1 GPa). As demonstrated in vitro, organic and inorganic biomaterials build mechanically defined microenvironments that can have a significant effect on cell adhesion, morphology, proliferation, and differentiation, resulting in tissue morphogenesis and maturation [70,71]. Yoshikawa and colleagues reported that myoblast cells showed significant stress fiber generation and flattening with increasing the hydrogel elasticity from 1.4 kPa to 40 KPa [72]. Engler and co-workers found that MSCs exhibited various morphologies when cultured on polyacrylamide (PAAm) substrates with different stiffness ranging from 1 to 40 kPa. MSCs grown on a soft sample (1 kPa) expressed specific neuronal cytoskeleton markers (β-3-tubulin), whereas cells on the substrates (11 kPa and 34 kPa) displayed expression of early myogenic and osteogenic transcription factors, such as MyoD and CBFα-1, respectively [73]. Importantly, cell responses on different stiffness materials depend on
cell types. Fibroblasts, epithelial cells and endothelial cells showed increased proliferation on stiffer substrates \[74\text{-}76\], whereas neural stem cells displayed better proliferation on softer substrates \[77\], probably because of their natural ECM features and softness of brain tissue. Interestingly, controlling the substrate stiffness could affect non-invasive gene delivery, regulating a cell’s ability to uptake exogeneous signalling molecules \[78\]. Taken together, engineered topographical and mechanical cues on substrates are powerful tools for directing cell interactions with the ECM.

1.2.3. BIOMATERIAL CHEMISTRY

Biomaterials chemistry can influence significantly the mass and conformation of the adsorbing proteins and then regulate cell response, which play a critical role on the subsequent cellular behaviors \[79,80\]. When biomaterials are placed into a biological environment, cells will not directly respond to the material surface but always via a protein conditioning film that originates from either the culture medium supplemented with fetal bovine serum (FBS) or proteins from biological fluids such as blood, saliva etc. This protein adsorption is generally much faster than the cell adhesion events and hence any alterations in this film will directly affect cellular behavior \[81\].

Generally, different materials have various chemical properties such as wettability, solubility, reactivity, charge and so on. Surface wettability, indicating interface energies of biomaterial surface (quantified as water contact angle, WCA), has previously been correlated with protein adsorption and cell behavior \[79,80\]. Wei and co-workers investigated the effect of surface wettability on competitive protein adsorption (albumin: Alb; fibronectin: Fn) and found that Fn adsorbed more on hydrophilic surfaces, whereas Alb predominantly adsorbed on hydrophobic surfaces. The initial attachment of osteoblastic cells increased with increasing surface wettability, which correlated well with Fn adsorption in the competitive mode \[82\]. In addition, surface wettability is critical for cell spreading and differentiation. Generally, cells have good spreading, proliferation and differentiation on hydrophilic surfaces. Mouse osteoblasts on hydrophilic surface ranging from 24 to 31° WCA, showed higher metabolic activity and expressed more osteogenic proteins (alkaline phosphatase and osteocalcin) than those on unmodified counterparts (WCA 72°) \[83\].

Surface wettability originating from material chemical functionalities (e.g., positive, negative or neutral) can affect protein adsorption and then mediate cell response. For instance, Lee and co-workers described how alkylsilane self-assembled monolayers with different functional groups (OH, COOH, NH\textsubscript{2} and CH\textsubscript{3}) affected \(^{125}\text{I}\)-labeled fibronectin adsorption where at pH 7, COOH is actually COO\textsuperscript{-} and NH\textsubscript{2} becomes NH\textsubscript{3}\textsuperscript{+} while OH remains unaffected. They found the adhesion constant and binding efficiency of the adsorbed Fn for the \(\alpha_5\beta_1\) integrins (CH\textsubscript{3} \(\approx\) NH\textsubscript{2} \(<\) COOH \(\approx\) OH). Fibronectin interacted more strongly with \(\alpha_5\beta_1\) integrins when adsorbed on COOH vs. OH surfaces suggesting that negative charge may be a critical component of inducing efficient cellular adhesion \[84\]. The above studies indicate that the specific biomaterial chemistry plays a major role in protein/cell-material interaction and this chemistry is reflected by the numerous different synthetic polymers and inorganic materials used as biomaterials. Also it illustrates that relative simple concepts such as wettability, is far more complex with large consequences for cell-material interactions.
1.3. CHALLENGES

Although biophysical and biochemical cues located on biomaterial surfaces proved to profoundly affect (stem) cell behavior, subsequent investigations has raised more questions than they answered, especially about the complex microenvironment of the ECM and its interaction with cells. Understanding ECM complexity in regulating cellular responses is vital for optimizing biointerface design and advancing biomedical material development. How to unlock the code between biomimetic materials and cell interactions still remains relatively unknown with eminent challenges to be met such as:
1) Most physicochemical properties of biomaterials were studied individually and identifying single parameter stimulation which is not appropriate to direct cell function, since cells always interact with multiple cues simultaneously. Therefore, it is vitally important to understand and explore the combined effects of material features on the (stem) cell behavior.
2) Initially, most of these studies used independent substrates with different and randomly selected degrees of biomaterial properties, which provided interesting yet limited information. As an alternative to the traditional experimental approaches using uniform substrates, surface gradient platforms offer an ideal tool to address this challenge in vitro and in vivo, enabling the identification of optimal conditions of cell-material interactions in a high-throughput screening (HTS) strategy. The conventional approaches are incapable of screening the huge amount of potential material parameter combinations to identify the optimal cell responses, and also involves a combination of serendipity along with many series of trial-and-error experiments. For advanced TERM, high-efficient and complex bioanalysis platforms are needed to explore complex microenvironments and study healing, development, and homeostasis.

1.4. AIM OF THE THESIS

The general aim of this thesis is to develop advanced biomaterial interfaces to explore and elicit their interactions with (stem) cells for improving and accelerating the development of functional and biomimetic materials. Key features in this study are combined physicochemical stimuli and high-throughput screening platform. In order to better direct cell response, complex interfaces with different material physicochemical parameter combinations are developed and utilized for elucidating how multiple cues influence (stem) cell response. Due to the unpredictable fashion between cell and topography interaction, single surface gradients with different materials are developed to identify optimal surface characteristics for directing stem cell response in a high-throughput screening (HTS) manner. Further, surface gradients with double linear or orthogonal features are designed and used to identify optimal surface parameter combinations for directing stem cell response in a HTS fashion.
1.5. OUTLINE OF THE THESIS

Chapter 1 gives an overview of the recent great discoveries regarding the native cellular microenvironment, particularly the ECM, and the current progress and challenges with respect to the investigation of cell-biointerface interaction for engineering the functional and biomimetic materials.

Based on the challenges and objectives mentioned above, advanced material interfaces are designed and developed using the novel preparative techniques. In Chapter 2, complex interfaces with stiffness and topography combinations are developed and applied for illustrating the different behavior of the cell-types originating from tissues with different intrinsic stiffness. In Chapter 3, Au nanowire-patterned array platforms with multi-scale design from the macroscale to the nanoscale are developed for regulating human bone marrow-derived mesenchymal stem cell (hBM-MSC) organization through the synergistic effects of surface micro/nanotopography and chemical cues.

In Chapter 4, a novel approach was developed for preparing directional nanotopographic gradients on polydimethylsiloxane (PDMS) substrates which allow us to determine optimal topographical dimension towards osteoblast adhesion and alignment more efficiently and accurately as compared to uniform wrinkle substrates. Chapter 5 presents a novel strategy translating wrinkled topography gradients from Chapter 4 to clinically relevant inorganic biomaterials (SiO$_2$, Ti/TiO$_2$, Cr/CrO$_3$, and Al$_2$O$_3$) via the combination of masked plasma oxidation and metal deposition oxidation methods. The optimal interface parameters are identified for promoting hBM-MSC alignment, elongation, filopodia development as well as cell adhesion. In Chapter 6, orthogonal stiffness and wettability gradients within a single substrate are developed for elucidating combined physical parameter influences on stem cell behavior and thereby gaining insights in desired cellular responses. hBM-MSC behavior is non-linearly regulated by surface stiffness and wettability. The optimal combined properties for promoting cell adhesion, nucleus size and vinculin expression are identified based on the orthogonal double gradients.

Finally, in Chapter 7 and Summary, the general discussion and summary of the above chapters are presented and placed into a broader perspective and provide an outlook for any potential future work.
REFERENCES
