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Stem Cell Instruction via Appropriate Geometry and Local Curvature of Microstructured Polymeric Substrates

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Hereby, I certify that the work presented in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Date and Signature

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Coming together is a beginning;
keeping together is progress;
working together is success.
— Henry Ford

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3. Xu, X., et al., Microwell Geometry Modulates Interleukin-6 Secretion in Human Mesenchymal Stem Cells. MRS Advances, 2017. 2(47): p. 2561-70.

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4. Li, Z. #, Wang, W. #, Xu, X. #, et al., Integrin β1 activation by micro-scale curvature promotes pro-angiogenic secretion of human mesenchymal stem cells. J. Mater. Chem. B, 2017. 5(35): p. 7415-25. # *Authors contributed equally to this work.* .

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1 Introduction

1.1 Stem Cells-Extracellular Matrix (ECM) Interaction

1.1.1 Hallmarks, Hierarchy and Classification of Stem Cells

The rigorous definition of modern stem cell currently is the cell having the capacity of self-renewal and give rise to differentiated specialized cells regardless of their source, which can be found throughout the embryo development and entire adult life (**Figure 1**).

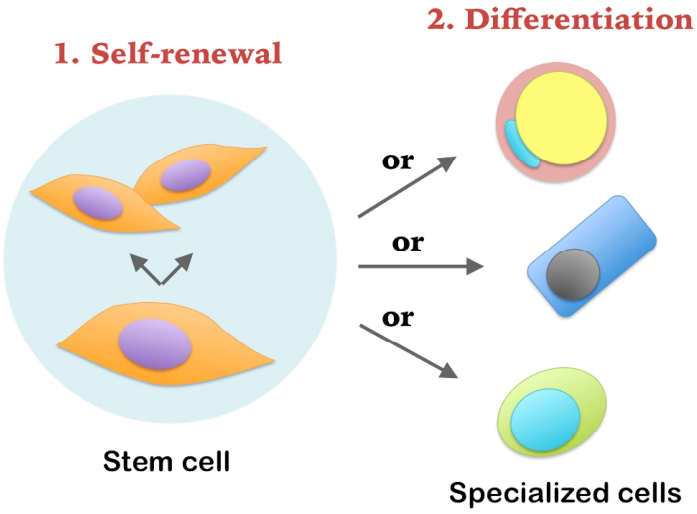

Figure 1. Modern definition and hallmarks of stem cells

Stem cells may be of embryonic or postnatal origin [1]. Based on the stem cell source, the cells can be generally divided into two main categories:

(1) Embryonic stem cells (ESCs): Stem cells, which are derived from blastocyst in an early-stage embryo;

(2) Postnatal stem cell or adult stem cells (ASCs): The undifferentiated cells that occur in a postnatal differentiated tissue, such as bone marrow or the brain, in the adult body.

As above-mentioned, ESC belongs to pluripotent stem cell, which holds a great potential in regenerative therapies. ASCs have been found in numerous tissues through

the body including blood, skin, hair, tooth, muscle, adipose tissue, bone marrow, liver and brain. Studies suggest that some adult stem cells are multipotent that each give rise to only a few derivative types and the others are oligopotent and unipotent such as lymphoid stem cells and muscle stem cells [2]. Mesenchymal stem cells (MSCs) as one well-studied type of ASCs were firstly identified at 1974 when Friedenstein et al. isolated the tightly plastic adherent cells from bone marrow. They were typically showing fibroblast-like spindle morphology (**Figure 2**) and were able to form single cell-derived colonies, termed as 'colony forming units-fibroblastic' [3]. The name 'mesenchymal stem cells' were given mostly due to the mutipotent differentiation potential and the derived germ layer of these cells [4]. It has been reported that MSCs can be successfully differentiated *in vitro* into several mesoderm cell types such as osteocytes [5, 6] and chondrocytes [7].

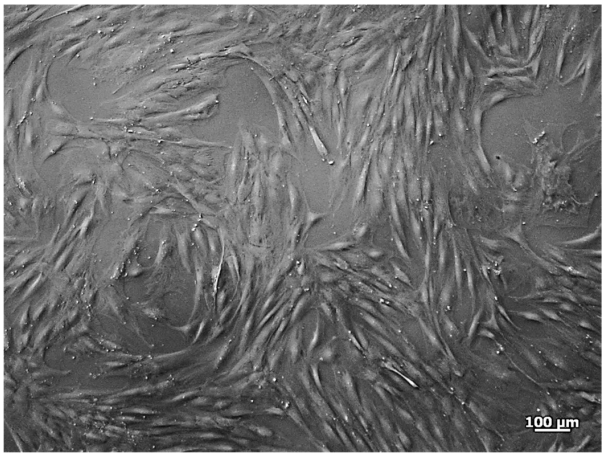

Figure 2. A monolayer of MSCs isolated from human bone marrow in culture

The capability of differentiating into other cell types can be defined as 'plasticity' or 'differentiation potency'. Stem cells can be divided into series of different categories according to their multi-lineage differentiation potential. The hierarchy (**Figure 3**) of the differentiation potency of stem cells defines the following terms:

(1) Totipotency: The ability of a single cell to giving rise to an entire organism, which places it at the top of the hierarchy (e.g. fertilized eggs before 16-cell stage during blastocyst phase);

(2) Pluripotency: The capacity to differentiate into all cell types from ectoderm, mesoderm and endoderm germ layers (e.g. embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs));
(3) Multipotency: The potential of cells, which can specifically produce a limited range of differentiated cells or progenitor cells from a particular germ layer (e.g. hematopoietic stem cells and mesenchymal stem cells (MSCs));
(4) Oligopotency: The ability to differentiate into limited cell types (e.g. lymphoid stem cells);
(5) Unipotency: The lowest level of the hierarchy, it has been defined as the ability to differentiate into only one specific cell type (e.g. progenitor cells or precursors).

Totipotency is lost rapidly during development at the zygote and early blastocyst stages. After the so-called segregation phase, ESCs can be isolated from the inner cell mass, which is the major component of the blastocyst. ESCs are defined as pluripotent stem cells since they can give rise to all three germ layers but are incapable of giving rise to an entire organism [8].

In June 2006, Yamanaka et al. identified an "ESC-like cell" by genetically reversion of specialized somatic cells to assume a pluripotent stem cell-like state. These "ESC-like cells", termed induced pluripotent stem cells (iPSCs). Genes such as Oct3/4, Sox2, c-Myc, and Klf4, which are important for maintaining the essential properties of ESCs were introduced [9]. Traditionally, this kind of cells requires ESC qualified serum and feeder cells to sustain their pluripotency. Nowadays, iPSCs can be adapted and maintained under the chemical defined serum- and feeder-free culture condition (**Figure 4**).

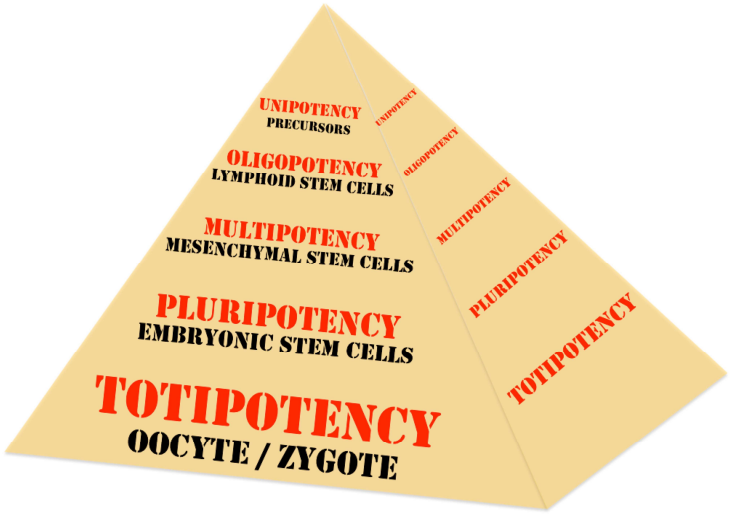

Figure 3. A hierarchy of varying degrees of stem cell differentiation potential

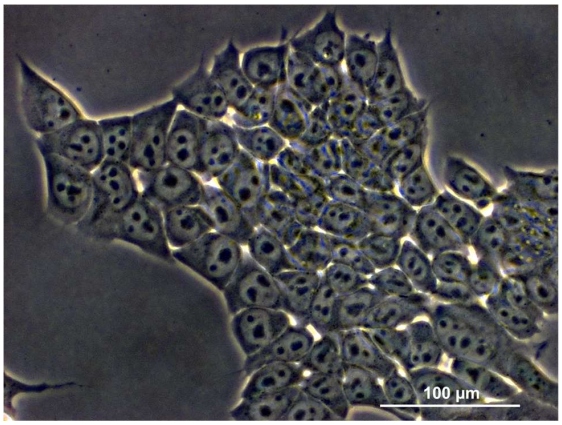

Figure 4. Xeno-free culture of mouse iPSCs on laminin coated tissue culture plate.

1.1.2 A Promising Tool for Regenerative Medicine

Although many issues remain to be addressed before novel cell therapy can become regular bedside treatments, cell-based therapy for regenerative medicine consists of injecting or transplanting healthy and functional cells into the lesion sites and eliciting endogenous stem cell-based regeneration introduced by biomaterials, which offers a promising way to effectively influence the recovery course of patients with severe injuries, chronic and degenerative diseases.

Either differentiated specialized cells or undifferentiated stem cells that capable of differentiate according to their circumstances can be chosen for cell therapies. On one hand, there are numerous multicellular complexes in human body with several types of

cells specialized in their particular functions. The specialized cells can be collected from specific tissues of patient. The advantage of this strategy is these cells are ready to implant without any manipulations. However, it is difficult to get a considerable number of these cells for therapy, as the cells lose the innate microenvironment required in proliferation [10]. On the other hand, the autologous, allogenetic and xenogenetic stem cells can be obtained from the patient himself, the human and the animal donors. Stem cells can extensively proliferate and capable of maintain their undifferentiated state, until they are induced to differentiate into a specific cell type for their potential therapeutic applications [11].

Currently, varieties of severe and degenerative diseases cited as potential targets of regenerative therapy, for instance cardiovascular disease, autoimmune disease, diabetes, osteoporosis, cancer, Alzheimer's disease and Parkinson's disease. The development of stem cell studies may provide an extraordinary scientific breakthrough that almost all tissues of the human body principally can be reconstructed. It is not too idealistic to announce that stem cell-based therapy has the potential to extend the lifespan and improve the quality of life.

Today, regenerative medicine has much more focused on the aspect of stem cell therapy and seeks to understand why and how stem cells, either derived from human embryos or adult tissues, are able to differentiate into specialized tissues. Moreover, it is necessary to keep on expanding the knowledge on whether the differentiation can be realized in a controllable way and what determinates the stem cell fate before and after therapeutic applications.

1.1.3 Therapeutic Potential of Stem Cells

Still, there are several distinct biological differences between ESCs and ASCs, and among various ASCs derived from different tissues. The implication of these differences for efficiency and effectiveness of regenerative therapy has not been clarified yet. In addition, characteristics and therapeutic potential of stem cells especially the ESCs have been presented at the center of scientific debate [12].

Pluripotent ESCs exhibit the capacity to become any type of specialized cell in the body and therefore have great potential for experimental and therapeutic purposes. Besides such a favorable potential, there are numerous issues concerning the use of ESCs, which

cannot be ignored. At first, there is an ethical dilemma regarding to the issue that the human embryos at which moral and legal status can be used for clinical applications [12]. Later, people are realized that the therapy with ESCs is largely hampered not only by the ethical issues, but also by the insufficient control of the great plasticity once implanted or transplanted, which may induce tumor formation [13]. Last but not least, any cell type derived from an ESC will be allogeneic. Therefore, upon transplantation, the rejection may occur without immunosuppressive drugs [14].

Multipotent ASCs, as a less controversial alternative with low tumor formation risk to ESCs, emerged later in development have been identified in many postnatal tissues. They retain plasticity and in response to biochemical and biomechanical signals, they can give rise to more differentiated cells with functions specific to the target organ. Hence, ASCs may provide a more direct route to clinical translation [15].

So far, the most favorable ASCs provided successful therapies with animal models and clinical trails for replacement of tragic diseased and tissue defects for regenerative medicine are bone marrow derived MSCs [16].

Rationales of employing MSCs as one of the most promising therapeutic strategies for regenerative medicine applications are listed as following:

(1) MSCs are naturally capable of differentiating to generate the connective tissues mainly derived from mesoderm (e.g. bone, cartilage, adipose, muscle);
(2) MSCs are able to migrate and accumulate locally to the injured tissue or other defect sites in the body [17, 18];
(3) MSCs have been widely used in various animal models and clinical trials no matter they are harvested from autologous, allogeneic or xenogeneic origin. There is no indication that these cells may induce tumor formation upon transplantation [19, 20];
(4) MSCs can support and accelerate tissue regeneration predominantly due to their paracrine activity on anti-inflammation and pro-angiogenesis processes [21-23];
(5) Strikingly, MSCs are able to avoid rejection when transplanted mainly because of their secretion of the immunosuppressive products such as prostaglandin E2 and indoleamine 2,3-dioxygenase and nitric oxide [24, 25]. Based on this immune modulation system, MSCs can induce local immune suppression while the overall immune responsiveness of the body is preserved [26, 27].

Despite the enthusiasm raised by recent clinical reports and the greater differentiation potential than previously thought, the mechanism of MSCs differentiation and the potential of MSCs to differentiate fully into cell types in other germ layers are still poorly understood and remain to be clarified [19, 28, 29]. In 2006, the successful reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) by introducing transcription factors provides a new vison of stem cell-based regenerative therapy [9]. The iPSCs have become the most promising cell source due to their wide differentiation capacity to generate all cell types derived from three germ layers [8, 30] and the avoidance of ethical debates.

1.1.4 Maintenance of Stem Cell Homeostasis by ECM

Naturally, all cell types including stem cells reside in their local ECM, a dynamic and multi-functional compartment of elastic fibers embedded gel-like material with unique chemical composition, structural organization and mechanical properties. Importantly, the ECM comprises over hundreds of different proteins, glycoproteins and approximately 36 proteoglycans, and continuously undergoes controlled remodeling [31]. Collagens, the main structural components, provide tensile strength to the ECM. Glycoproteins, such as laminin and fibronectin, play a key role in ECM assembly, trapping and releasing growth factors, and present as ligands to interact with the cell transmembrane receptors such as integrins. Proteoglycans, especially the heparan sulfate fills the inter-space of ECM, confers the hydrated state and offers the binding site of several growth factors [32]. Therefore, this multi-functional complex not only provides structural support, but also directs tissue homeostasis in health and regeneration in disease [33, 34]. In the past decades, more and more ECM-like biomaterials have been designed and fabricated as an ECM replacement to improve endogenous tissue regeneration [35].

As a constitutive part in stem cell local environment, there is great diversity in ECM components and properties peculiar for each tissue to reflect the specific in situ function requirements. Indeed, the development, function, repairing and restoring of organs and tissues are strongly supported by modulating the relative amounts, organization and degradation of the different ECM components, which mediated by the constant ECM rebuilding and remodeling from cells including regulation on ECM component gene expression and proteinase secretion profile (e.g. matrix metalloproteinases) of cells [34,

36, 37]. On the basis of such reassembly and three-dimensional reorganization of these extracellular macromolecule complexes, the factors and the signals derived from the interface of stem cell and ECM can be efficiently turned to their functional forms for the preservation of the stem cell homeostasis [38].

ECM is widely involved in major cellular processes such as migration and morphogenesis as well as proliferation, differentiation and apoptosis of stem cells [39]. Numerous studies have illustrated that ECM gene played as a crucial regulator for tuning stem cell function [40-42]. An *in vivo* study has demonstrated that the reduced ability of aged ECM, which provides an inadequate local environment for stem cell maintenance, can strongly influence the endogenous regeneration and stem cell-based therapy [43]. It has been verified that the decellularized kidney ECM represented their native architecture and components successfully guided the in situ cell differentiation towards the original type of cells which were resided in the kidney [44]. Nowadays, decellularized ECM has been extensively applied and considered as a promising tool for tissue repair and regeneration especially in the cardiovascular system [45, 46].

The instruction effect of ECM on stem cell behavior can be mediated by either indirect or direct actions. In an indirect manner, the ECM interacts with cells mainly based on the storing and releasing bioactive morphogens and growth factors [47]. Enzymes trigger ECM remodeling and thereby releasing functional fragments to stimulate cells [37]. Moreover, the ECM engagement was considered as a potential feedback approach and consequently regulate the stem cell fate in response to local condition changes [48]. In addition to indirect manner, the direct signaling mechanism presents instructive signals to cells mainly through binding to their receptors that expressed on the cell membrane [49]. The structural, biochemical and biophysical properties including wettability, porosity, elasticity and topography are able to affect variety of cell anchorage-associated biofunctions [50]. Although an increasing number of different transmembrane receptors has been recognized for ECM–stem cell direct interaction, integrins is still defined as the main receptors for supporting or interfering the communication between ECM and stem cells and highly involved in cell adhesion, migration and homing process in contact with ECM. Integrins are heterodimeric receptors presenting α and β subunits that physically connect the ECM component (e.g. collagen, laminin and fibronectin) to the intracellular cytoskeleton and transduce external signals generated from ECM via activate its downstream signaling pathway

such as focal adhesion kinase (FAK) and Rho-associated protein kinase (ROCK) [50, 51]. Among different types of integrins, integrins with β1 subunit are indispensable for the balance between self-renewal and differentiation of stem cells mostly via controlling of symmetric and asymmetric cell divisions [52-54]. Certain interplay of β1 integrins and other signaling molecules such as Notch, EGF and Hedgehog receptors were established in mammary stem cells [55-57].

Recently, the ECM mechanical property is considered as a critical determinant of stem cell fate. On one hand, concerning the highly dynamic nature of ECM, the mechanical stimuli may arise during dynamic assembly or remodeling of ECM [58]. On the other hand, the viscoelasticity, elasticity, ligand density and even the topography can be defined as the static passive aspects of mechanical property of ECM [59]. Cells are able to sense, transduce and respond to both of the static or dynamic external force by applying traction forces through mediating cell-ECM attachment and further altering the downstream signaling pathways in an appropriate manner. This capacity and the process for transmission of mechanical stimuli into a biochemical cellular response is termed as mechanotransduction. It is a matter of fact that cell behavior alteration in response to mechanostimulation is generally dependent on endogenous tension forces generation through physical ECM/transmembrane receptor binding, actomyosin cytoskeleton, nuclear matrix, nuclear envelope and chromatin reorganization [60, 61].

1.2 Steering Stem Cell Fate with Biomaterial Surface Cues

1.2.1 The Requirements of Biomaterial for Stem Cell-based Regeneration

Although stem cell research is on the cutting edge of biomedical investigation, in fact, cell-based therapy is still in its infancy and there are substantial gaps to the realization of novel therapies via stem cell applications.

Biochemical and physical signals are thought to be the major triggers and regulators of stem cell proliferation and differentiation. Although full differentiation of stem cells into different cell types via the cocktail of soluble biochemical signals has been widely applied [62-65], the efficiency and purity of differentiated cell populations are vary and hard to control.

Examining on all the aforementioned limitations or challenges appeared in cell-based therapy, gives a strong indication that the microenvironment including ECM, supporting cells and neighbor cells in the origin place and in the target tissue mainly determines the stem cell behavior and fate before and after transplantation.

As a concrete example, although the bone marrow transplantation, which has been clinically applied without fully understanding of the underlying mechanisms, the outcome of the therapy has improved markedly as the understanding has grown slightly [66].

To fulfill the promise of stem cells to meet the need for regenerative medicine, particularly in skeletal tissue regeneration, it is unnecessary to fully understand how the *in vivo* stem cell development works, but scientists definitely need to gain the knowledge of the fundamental and meaningful issues:

(1) The reason why stem cells are able to maintain themselves in a self-renewal state;
(2) An undifferentiated or a differentiated stem cells can be implanted;
(3) On the basis of genetic, environmental and soluble cues, the exact factors engage in instructing stem cells to start or stop dividing and differentiation;
(4) Whether and how physiological properties guide the functional cell-based therapy.

Therefore, based on the known and underlying mechanisms, finding a right way to culture stem cells *in vitro* is a high priority of cell-based regenerative medicine applications for upgrading the therapeutic efficacy.

Under these circumstances, biomaterial, as another major pillar of regenerative medicine, can potentially not only provide the substrate for cell growth but also regulate the stem cell fate.

1.2.2 Control of Stem Cell Fate via Substrate Surface Modification

1.2.2.1 The Intrinsic and Extrinsic Factors

Scientific breakthroughs in stem cells and biomaterial science have provided a unique opportunity for tissue regeneration from combination of stem cells and the engineered biomaterial based scaffolds. In fact, specialized cell-loaded scaffold has already been widely applied in many tissue engineering processes [67] including nerve [68], arteries [69], liver [70], cartilage [71], bone [71] and skin [72].

Generally, for stem cell based therapy, the procedure is divided into four main steps:

(1) Harvest the stem cells from the patient itself, a close relative or an allogeneic donor;
(2) Stem cell seeding into an biomaterial based implant, which serves as mainly a physical support and culture environment for cells;
(3) *In vitro* culture and differentiate the stem cells to a desired lineage with defined differentiation induction medium, cytokine and growth factors or expand the self-renewing stem cells to a desired number;
(4) Apply the stem cell-loaded biomaterials for *in vivo* tissue and organ regeneration.

Significantly, challenges to this approach include the design and fabrication of a suitable scaffold which is able to promote cell adhesion both *in vitro* and *in vivo*, to support cell growth, migration, proliferation, differentiation and induced formation of natural tissue.

Stem cell behavior is strongly regulated by both intrinsic programs and extrinsic factors, including the physical, chemical and biochemical cues provided by their microenvironment, especially their ECM components [73, 74].

Inspired by the natural embryogenic process of stem cells, the following aspects for regulation of stem cell behavior need to be considered (**Figure 5**):

(1) Structured scaffold, a supporting system for cell growth and differentiation;
(2) A physiological milieu such as temperature, oxygen and pH;
(3) Supplement of biochemical factors including nutrients, cytokines and growth factors;
(4) Application of physical regulatory signals.

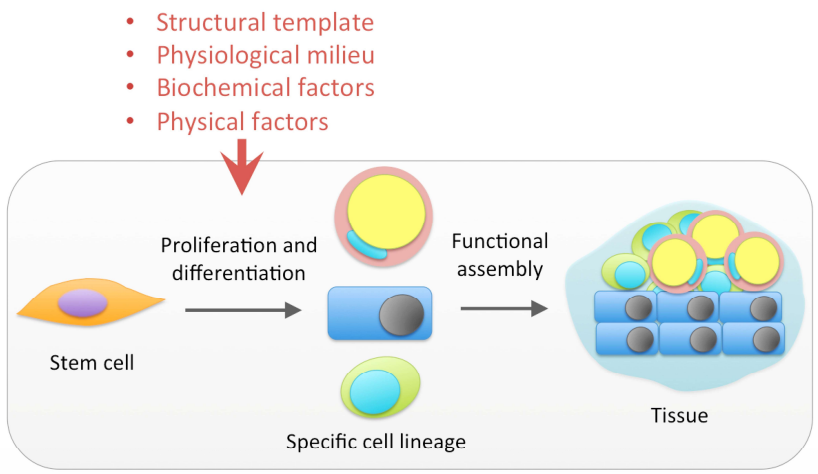

Figure 5. Factors regulate stem cell function and the progression of tissue assembly

In vivo, the individual cell fate during the processes of a functional tissue assembling is affected by multiple factors with a precise determined spatial and temporal sequence. There are several critical components involves including the matrix cues, signaling molecules such as soluble factors, local biomechanical and physical forces. For regenerative medicine applications, all the above-mentioned multiple cues are integrated together and exert a combinatorial influence to the fate and function of stem cells. However, the mechanisms are poorly understood (**Figure 6**).

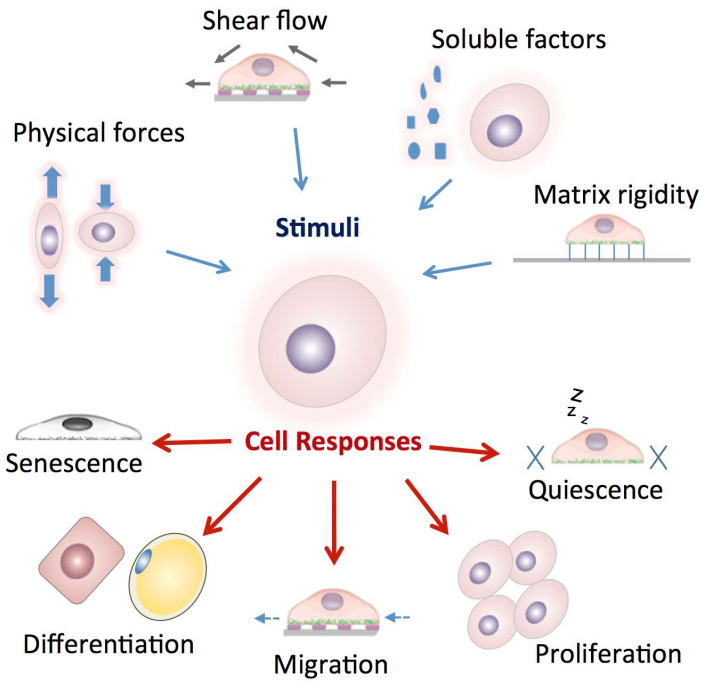

Figure 6. Critical stimuli and stem cell responses in tissue assembling and remodeling procedure

In vitro, similarly, controlled proliferation and differentiation of stem cells can be achieved by using biochemically or biophysically modified surface of biomaterials particularly the polymeric scaffolds.

First of all, the polymeric biomaterial surface for stem cell culture should be biocompatible and cell instructive which contains the following aspects:

(1) Sterilized;
(2) Nontoxic;
(3) Low endotoxin burden (<0.5 endotoxin unit/ml)
(4) Adhesive for stem cells;
(5) Achieve large scale of stem cells for therapy;
(6) Keep safety (e.g. No tumor risk);
(7) Mechanical compatibility with stem cells and/or target tissue;
(8) Maintain self-renewal or differentiation potential of stem cells in a controllable manner.

1.2.2.2 Surface Roughness Modulate Stem Cell Fate

Recent study has revealed that even in the absence of osteogenic supplements, MSC osteogenesis was successfully induced by polycaprolactone structured with a proper surface roughness [75].

1.2.2.3 Stiffness as a Regulator of Stem Cell Lineage Commitment

The contractile force will present once the cells attached to any natural or artificial substrate. Such force can generate the tensile stress via various cytoskeleton elements [76]. It has been reported that contractile forces generated from different stiff and elastic substrates are able to influence major cell behaviors such as proliferation[77], apoptosis [78] and migration [79, 80].

Hence, it is not surprising that the stiffness can also regulate stem cell differentiation. Engler et al., has reported that by culturing on variably compliant polyacrylamide gels that mimicked the stiffnesses of neural tissue, muscle and bone, MSCs could differentiate accordingly into neurogenic lineages on soft substrates (0.1-1 kPa), myogenic lineages on stiffer substrates (8-17 kPa), and osteogenic lineages on relatively rigid substrates (25-40 kPa) [81].

Additionally, compared with the relatively soft substrates, the stiffer one can activate MSCs to re-enter into the normal cell cycle from the quiescent state, and thus preserve the self-renewal [82].

In fact, multiple tissues present the same or a similar elasticity *in vivo*. Therefore, applying a single set of stiffness may not be adequate to make the MSC differentiation controllable [83].

1.2.2.4 Influence Stem Cell Fate via Surface Immobilized Biosignals

When loading any adherent cells on the polymer surface or into the polymer scaffold, in the presence of protein-containing culture medium, cell attachment is initiated through a complex of protein layer, which forms immediately on the synthetic polymer surfaces. Simultaneously, the cell-polymer interface appears and the communication between cells and polymeric biomaterials are established. The mediator for cell-polymer interaction is the proteins layer, which normally contains the same or similar adhesive domains in ECM (such as Arginyl-glycyl-aspartic acid (RGD)). On one hand, since the protein adsorption and interaction of different types of mammalian cells on

polymer surfaces are highly depended on the surface charge, charge distribution, wettability, hydrophilicity/hydrophobicity ratio and bulk chemistry, the chemogradient surfaces, whose wettability or other aforementioned properties are changed gradually, have been designed for screening the optimal conditions of protein adsorption migration and proliferation of target cells [84, 85]. On the other hand, scientists have attempted to enhance or interfere the cell-material interactions by adsorption, physical immobilization or covalent binding of various bioactive or inactive peptides on the polymer surfaces [86-88].

It was observed that 140 nm spacing of surface coupled RGD ligand would be enough to promote anchorage dependent fibroblasts adhesion via formation of focal adhesion points, spreading and cytoskeleton reorganization [89]. Along this direction, to control the differentiation of stem cell, Ding et al. has printed RGD nanoarrays on the polymer surfaces for human MSCs induction. The study has demonstrated that the nanoscale spacing of RGD could strongly modulate the adipogenic and osteogenic differentiation of MSCs [90].

Microcontact printing technology, which allows creation of on-demand patterns on polymer surface via create the adhesive and non-adhesive areas, has been applied for the spatiotemporal control of cell-substrate interactions [91]. It has been revealed that the size and shape of geometric micropatterns could steer cell lineage commitment of MSCs. At the single-cell level, micropatterns with larger size were found to facilitate the osteogenic differentiation but suppress the adipogenic differentiation of MSCs [92-94]. Cell shape is a potent regulation target of cell growth and many events related to embryonic development and stem cell differentiation [95]. A number of studies have shown that cell fate can be influenced artificially through controlling of their shape by geometric substrates. For example, culturing MSCs on nanopits with 120 nm diameter and 100 nm depth induces osteogenesis in the absence of osteogenic induction media [96]. Long-term *in vitro* maintenance of MSC phenotype and multipotency can be achieved by the influence of nanoscaled geometry cues [97].

In addition to geometric features at the nanoscale, a scale larger than the cell size at micro-level also affects the cell behavior [98, 99]. Preosteoblast cells seeded on the millimeter level porous structured scaffold surfaces were growing and produced ECM

conponenets to form a tissue layer. Cells at the corner of the square pores showed similar growth kinetics as round pores but higher proliferation rates [100].

Kilian et al., have demonstrated that osteogenesis of MSCs increased with the aspect ratio of the coated rectangles. Further, by comparing the cells growing on different micropatterns, they found that MSCs prefer to undergo adipogenic differentiation on flower-shaped, and osteogenic differentiation on star-shaped micropatterns, respectively, while cells on pentagon-shaped micropatterns exhibited similar differentiation capacities to adipocytes and to osteoblasts [101, 102].

Moreover, growth of chondrocytes in flattened shape in 2D culture leads to a shift irregularly from a chondrocyte phenotype to a fibroblast-like phenotype [103] while maintaining of chondrocytes in a 3D shape with pellet culture can preserve their normal phenotype [104].

1.2.2.5 Mechanical Sensing and Intracellular Signal Transduction of Stem Cells

Focus on the interface between cells and substrates, different ECM proteins adsorbed on the substrate surface can be recognized by multiple cell receptors or sensors presented on the cell membrane. As aforementioned, the major receptors that mediate cell adhesions on the substrates with the dynamic presentation of ECM proteins are from integrin family [105, 106]. Integrins feel the extracellular signals via forming a dimer of α and β subunits. The specificity of integrins in response to different motifs of ECM proteins is mainly based on the differing combination of α and β subunits [102].

The mechanical signals can be transduced into cell nucleus directly or indirectly. The direct mechanotransduction can be processed by directly manipulating chromatin and altering gene expression from adherent stimuli to cytoskeleton organization and nuclear membrane stretching into nuclei [107]. The indirect mechanical signal transduction is described as a signal cascades from outside integrin sensing to cell nuclei, which mainly based on Rac, Cdc42 or Rho/ROCK mediated activation of focal adhesion kinase and mitogen-activated protein kinase [108].

The differentiation of stem cells into a chondrocyte phenotype requires a more rounded cell shape compared to osteogenesis. This shape change alters the organization of actin

cytoskeleton, focal adhesions assembly and involves Rho/ROCK signaling pathway [109] (**Figure 7**).

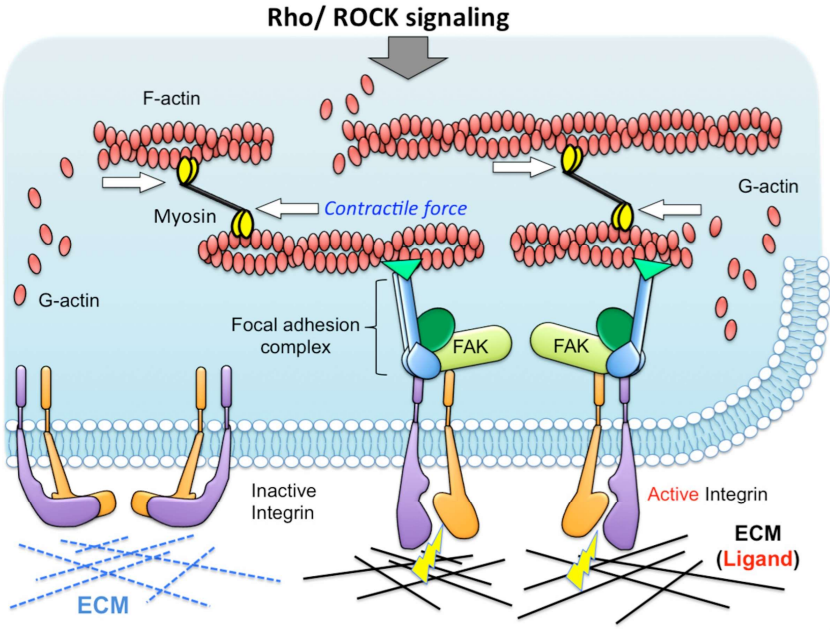

Figure 7. Rho/ROCK signaling pathway involves in focal adhesion and cytoskeleton reorganization

The mechanisms involved in elasticity or geometry guided stem cell differentiation remain to be further determined. However, inhibition of Rho activity can prevent these effects, suggesting that changes may be initiated in the F-actin cytoskeletal architectures. These findings are highly supported by studies showing significant changes in the F-actin cytoskeleton and cell tension during differentiation of MSCs into chondrocytes, adipocytes and osteoblasts [92, 101, 110, 111].

1.2.2.6 Mechanosensing of Topography Cues in Pluripotent Stem Cells

Growing evidence suggests that culture substrate properties, especially physical cues, are involved in transgene-free determination of pluripotent stem cell (PSC) fate [112-118]. Increased cell spreading caused by strongly adhesive substrates promoted mouse PSC differentiation [112]. This phenomenon was accompanied by increased levels of phosphorylated FAK and focal adhesions around the periphery of highly spread cells [113, 119]. Using conventional rigid tissue culture plastic, Chowdhury et al. observed that those mouse PSCs in tight contact with the substrate at the edge of colonies, were

easier and faster to differentiate than the cells located in the center. They further established that cells cultured on soft substrates (~1 kPa) exerted lower cell-matrix traction than on stiff substrates and formed homogeneous colonies even in the absence of LIF [114]. The same group revealed that mouse PSCs themselves were softer and more sensitive to local force alteration than somatic cells. A local cyclic stress generated by RGD-coated magnetic beads through focal adhesions induced the myosin-dependent spreading in PSCs. Moreover, the applied stress led to reduced OCT4 expression in these cells [115]. To date, numerous studies have explored the role of mechanotransduction in fate decisions and epigenetic regulation of PSCs. For example, to screen the effects of a broad range of substrate mechanics on neural induction of human pluripotent stem cells, the group of Fu developed a microengineered substrate system consisting of micropost arrays with tunable rigidities. The differentiation of functional motor neurons was found to be accelerated on soft substrates (~ 5 kPa) in comparison with glass coverslips and rigid substrates [116]. Microtopography was shown to improve reprogramming efficiency and modulated the histone H3 acetylation and methylation markers and epigenetic state of cells during the reprogramming procedure [117]. The reprogramming was boosted via spatial confinement of cells in the 3D microenvironment of poly(ethylene glycol)-based hydrogel system. In addition, the acceleration of mesenchymal-to-epithelial transition and epigenetic remodeling was also observed [118]. Mechanical force generated from cell-cell and cell-material interfaces has been reported as a crucial factor for spatial organization of germ layers during differentiation [120]. Most recently, Fu et al. have demonstrated that biophysical cues were indispensable for triggering amnion-like tissue self-organization *in vitro* [121]. These studies highlight the importance of dynamic or static biomechanic stimuli generated from substrate topography, and of mechanotransduction in general, in the regulation of stem cell pluripotency, somatic-stem cell reprogramming efficacy and morphogenesis.

In summary, the interaction of stem cell-ECM plays a key role for endogenous tissue regeneration with polymeric implants. Most of the currently used polymeric biomaterials are not initially designed for biomedical applications. The precise spatial and temporal regulation of biochemical and biophysical factors directing stem cell fate is critical during embryogenesis and development events. Accordingly, there is an urgent need to modify or upgrade the biomaterials to control or tune stem cells

differentiation for tissue regeneration in the proximity of implants. It has been demonstrated that the chemical and physical properties of scaffold materials strongly influence cell-ECM interactions, and regulate cellular behaviors [122, 123]. Particularly, a series of physicochemical properties of cell culture substrates such as the surface chemistry [124, 125], elasticity [81, 126], roughness [127] and anisotropy [128-130] were shown to influence stem cells by affecting their adhesion, spreading, morphology, proliferation, apoptosis, gene expression and differentiation. Besides, geometrically micropatterning provides a new insight of the influence of cell adhesion and spreading on cell functions and inspire the design of biomaterials to process in an effective manner for cell fate manipulations [131] (**Figure 8**).

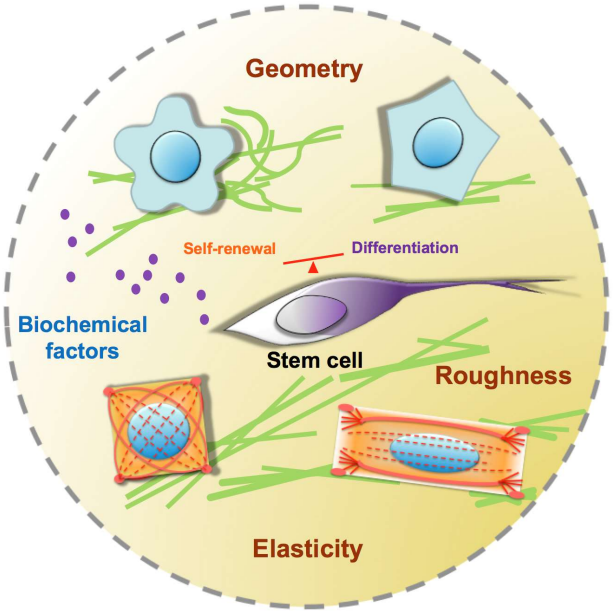

Figure 8. Physicochemical regulators well known to control the stem cell self-renewal and differentiation

2 Motivation, Aims and Hypotheses, Strategies

2.1 Motivation

Stem cells and ECM are essential components for endogenous tissue restoring. The precisely controlled stem cell cellular functions including adhesion, growth, migration and differentiation play a critical role for efficient tissue regeneration as well as the avoidance of inflammation and tumorigenesis. Besides the soluble factors, a series of physicochemical properties of polymeric implants was shown to regulate the stem cell-substrate interaction and instruct stem cells fate and consequently alter their regenerative effectiveness. For instance, the bulk chemistry, surface charge, charge distribution, wettability, hydrophilicity/hydrophobicity ratio, surface geometry, elasticity and roughness. Thus, there is need for expand the knowledge on whether and how the polymer systems with appropriate physicochemical and biophysical cues drive stem cells towards their desired functional stages.

Traditionally, the most widely used system for biophysical especially the geometry study is the flat substrates coated with ECM molecules in a distinct pattern, namely 'adhesive islands'. Although this 2D model is simple to apply and easy to visualize, the overall cell movement and cell-cell communication are restricted. Cell-cell contact and cell migration is a vital cell behavior naturally occurred in tissues. The process will be accelerated in injured sites during wound healing, tissue restoring and regeneration. In order to gain insight into cellular response from *in vitro* approaches, introduction of the third dimension — cell cross talk/motility would therefore be important. Microcavities have demonstrated their capability to regulate cellular behavior. They have been widely used to modulate the growth of pluripotent stem cells to generate homogenous cell colonies with defined sizes and shapes. However, there is limited understanding on the effect of local curvature change of geometric microcavities on the behavior of MSCs. In addition, regulating and controlling the major cellular behavior of MSCs using the microcavities is of great clinical value as the traditional applied surface with smooth polystyrene could not fully satisfy the clinical requirement of MSC expansion. Hence, it is necessary to identify and develop a microcavity system to support MSCs growth without impairing their motility and maintain their multipotent state for therapeutic applications as well as directing MSCs towards a desired differentiation process by utilizing the structural geometric cues.

Cardiovascular diseases is the number one lethal issue globally because of the irreversible damage of myocardium together with interrupted local blood supply. Both active angiogenesis and functional cardiomyocytes generation are essential for an effective cardiac regeneration. Besides direct applying bioactive molecules, pro-angiogenesis effect of MSC paracrine can also be achieved by modulation of biophysical properties of material such as elasticity and micro-nano-hybrid surface topographies. Cardiomyogenic differentiation from multipotent stem cells such as MSCs remains controversial so far. However, the differentiation of pluripotent stem cells towards the cardiomyocyte linage has been well demonstrated by generating beating cardiomyocytes with comparable structure and function to neonatal myocadium via self-organization of pluripotent stem cell-derived embryoid bodies (EBs). Among pluripotent stem cells, iPS can serve as the most promising therapeutic cell sources for cardiogenesis since it can be generated from various somatic cell types and avoid ethical issues. Although certain geometric pattern, ridges, grooves, roughness, convex and concave structures with different landscape of curvature have been applied for efficient steering of stem cell behavior, the role of local curvature on geometric architecture-based cell instruction remains elusive. Therefore, uncovering the underlying mechanisms is of great clinical relevance to utilize the beneficial effects.

2.2 Aims and Hypotheses

Within this thesis, the fate of the MSCs in response to physicochemical cues of different polymeric substrates, the influence of micro-scale geometry and local curvature on stem cell expansion, differentiation, paracrine profile and transmembrane mechanosensor (e.g. integrin) as well as the key downstream intracellular signaling should be illustrated.

The research work bases on following hypotheses: (1) We hypothesize that the survival, self-renewal and differentiation potential of MSCs on polyetherurethane (PEU) and poly(ether imide) (PEI) surfaces can be tuned, and compared to human MSCs, the *in vitro* long-term maintenance of rat MSCs may differ in response to the same material surface. (2) The major cellular behaviors, secretion profile and differentiation potential particularly the osteogenesis of human MSCs can be instructed by geometric signal from microcavities on polystyrene (PS) substrates. (3) Approperiate local micro-curvature of polymeric substrates can promote pro-angiogenic secretion of human

MSCs and mouse iPSCs, and enhance cardiomyogenesis of continuously cultured or preconditioned mouse iPSCs.

2.3 Strategies

For all studies using polymeric substrates with and without microstructures, we employed the polymeric cup system (**Figure 9**), which can exclude the fixative materials influence with biocompatible polymers that have been widely applied for medical supplies and devices to evaluate their tuning function on the autologous behavior and lineage commitment potential of rat bone marrow-derived MSCs, human adipose-derived MSCs and mouse iPSCs.

For evaluation of physicochemical property on regulation of MSC behavior, we selected the biocompatible and hemocompatible polymer PEI, which allows to tailor the surface chemistry and an elastic PEU, which allows adjusting the mechanical properties by variation of the hard to soft segment ratio. Cells were seeded directly to such substrates, multiple parameters of MSCs were then examined including cell viability, adhesion, morphology, apoptosis, senescence, proliferation and spontaneous differentiation to monitor the MSCs behavior.

We introduced PS-based microstructured substrates comprising arrays of microcavities in either square or round shape. The fabrication procedure was illustrated in **Figure 9**. In brief, the silica waffle with microstructures were designed, polydimethylsiloxane (PDMS) mold was prepared using such waffle. Then the PS films were put on top of PDMS mold and placed in between two glass plates to make structures. Afterwards, the structured films were embedded to polymeric cups via injection molding [132, 133].

The microstructured substrates were designed to allow MSCs to freely migrate on the substrates without confinement by the surface structures and distribution of cell-adhesive molecules, and the microcavities are sized to accommodate at least one individual cell. Vitronectin was coated on the entire surfaces to enhance the cell attachment.

The major cellular response of human MSCs to different geometic microcavites was probed as following:

(1) Initial interaction with microcavities including cell adhesion and distribution;

(2) Cell morphology, gene profiling and migration;
(3) Phenotype of cells, proliferation and osteogenic differentiation;
(4) Paracrine profile;
(5) The underlying mechanism of the regulatory effects and the potential intra-cellular signaling transduction.

To create the substrates with different levels of micro-curvatures, another approach by simply using the differently structured metal cylinders for injection-molded PS and PEI inserts was applied. a cylinder with a polished contact surface M00 (PS-000 and PEI R0), and two cylinders with micro-rough surfaces according to the standard of German Institute for Standardization (DIN 16747: 1981-05), M30 (PS-160 and PEI R1) and M45 (PS-320 and PEI R2) were used (**Figure 9**).

A smooth surfaces (PS-000 and PEI R0) were used here as a control. PS-160 and PEI R1 were expected to regulate the stem cells at the single cell level via its local curvature, while PS-320 and PEI R2 might be capable of modulating cell clusters.

Fibronectin was coated on the surfaces of PS-000, 160 and 320 for initiation of human adipose-derived MSC adhesion.

The human MSC response to different local micro-curvatures of PS substrates was investigated:

(1) Initial interaction with curvature including cell size, adhesion, spreading and distribution;
(2) Cell morphology, focal adhesion and cytoskeleton organization, fibronectin reorganization;
(3) Phenotype of cells and proliferation;
(4) Integrin activation level;
(5) Paracrine profile;
(6) Function of paracrine (conditioned medium from MSCs) on pro-angiogenesis;
(7) The potential intra-cellular signaling pathway.

The cardiomyogenesis of mouse iPSCs on PEI substrates was studied:

(1) Stemness phenotype of cells and proliferation;
(2) EB formation capacity in regulation with different local curvatures;

(3) Proliferation and cell cycle of iPSCs in EBs;

(4) Characterization of EB-derived cardiomyocytes including cell beating frequency;

(5) Functional assay of paracrine (conditioned medium from EBs) on pro-angiogenesis.

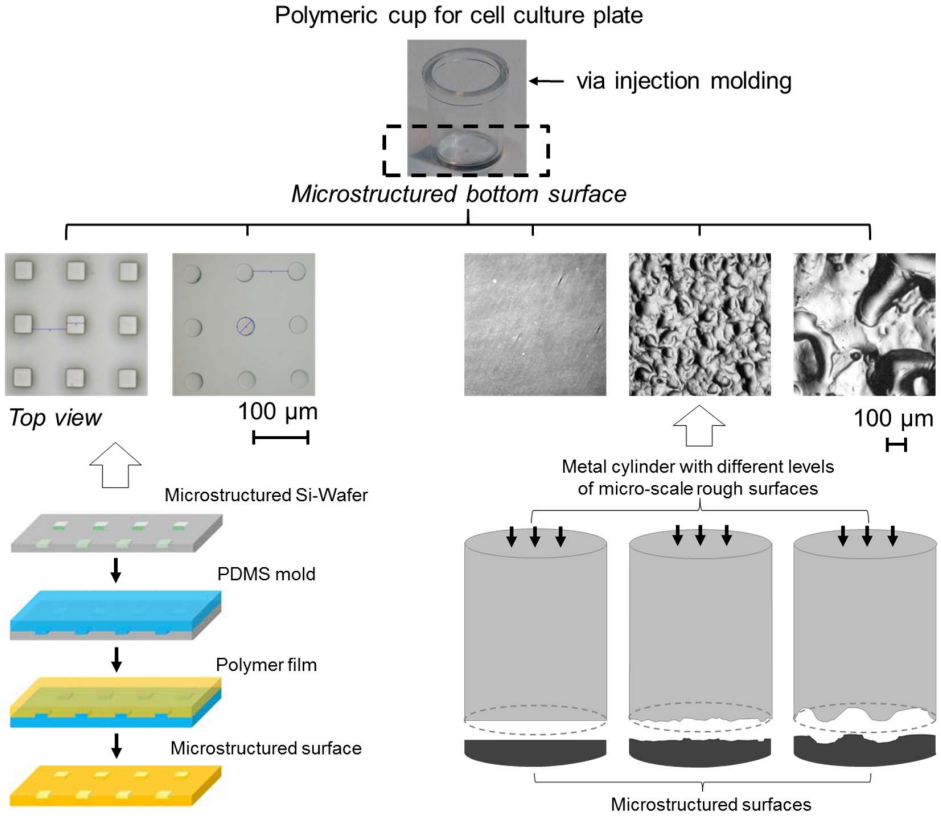

Figure 9. Fabrication of microstructured polymeric inserts

3 Publications

3.1 Cultivation and Spontaneous Differentiation of Rat Bone Marrow-derived Mesenchymal Stem Cells on Polymeric Surfaces

Xun Xu, Karl Kratz, Weiwei Wang, Zhengdong Li, Toralf Roch, Friedrich Jung, Andreas Lendlein and Nan Ma

Clin Hemorheol Microcirc. **2013**, 55, 143–156

DOI: 10.3233/CH-131698

The original article including the supplemental information is included on the following pages and online available at:

https://doi.org/10.3233/CH-131698

3.1.1 Author contribution

1) Literature study
   a) Characterization and spontaneous differentiation of rat MSCs
   b) Influence of polymeric substrate on morphology, survival and expansion of MSCs
   c) Effects of senescence on MSC-based therapy
2) Study design (with discussion and advice from co-authors Dr. Kratz, Dr. Wang, Prof. Ma and Prof. Lendlein)

a) Methods of rat MSC phenotype characterization
b) Methods of cytotoxicity, cell division assay
c) Methods for monitoring morphological change and cytoskeleton alteration of MSCs
d) Methods for MSC senescent detection based on beta-gal activity analysis

3) Experimental work
   a) Maintenance of rat MSC, phenotyping of MSCs
   b) Measurement of MSC viability, adhesion and cell expansion
   c) Detection of apoptosis and senescense of MSCs
   d) Morphological study and rat MSC spontaneous adipogenic differentiation
4) Analysis and interpretation of experimental data
   a) The frequency of positive and negative markers of rat MSCs
   b) Comparison of cell adhesion rate, cell density between PEU and PEI
   c) Apoptotic and senescent levels of MSCs on PEU and PEI
   d) Comparison of lipid droplet formation in MSCs from PEU, PEI and TCP
5) Manuscript
   a) Manuscript structure (discussed with co-authors)
   b) First manuscript draft
   c) Revising and finalization of the manuscript according to the comments of Prof. Lendlein

3.1.2 Publication (Appendix I)

3.2 Controlling Major Cellular Processes of Human Mesenchymal Stem Cells using Microwell Structures

Xun Xu #, Weiwei Wang #, Karl Kratz, Liang Fang, Zhengdong Li, Andreas Kurtz, Nan Ma, Andreas Lendlein

# *Authors contributed equally to this work.*

Adv. Healthcare Mater. **2014**, 3, 1991–2003

DOI: 10.1002/adhm.201400415

The original article including the supplemental information is included on the following pages and online available at:

https://doi.org/10.1002/adhm.201400415

3.2.1 Author contribution

1) Literature study
   a) Bottleneck of low therapeutic efficiency of MSC based therapy
   b) Instruction of stem cells via biophysical cues
   c) Microwell system for stem cell culture
   d) Signaling pathway in stem cell osteogenesis
2) Study design (with discussion and advice from co-authors Dr. Wang, Prof. Ma and Prof. Lendlein)

a) Methods of ECM protein coating on polymeric substrates
b) Methods of human adipose-derived MSC phenotyping
c) Methods of Time-lapse tracking of cell movement and distribution on structured surfaces of polymeric substrates
d) Methods of immunostaining of focal adhesion and cytoskeleton
e) Quantification or semi-quantification methods of the alteration at gene and protein levels
f) Methods of cell proliferation and cell cycle sub-phase detection
g) Semi-quantification methods of osteogenic differentiation
h) Application method of ROCK activity inhibitor

3) Experimental work
a) Vitronection coating on microstructured substrates
b) Selection of the size of microwell for single cell accommodation.
c) Maintenance and phenotyping of human adipose-derived MSCs
d) Focal adhesion and microfilament F-actin staining
e) Short- and long-term time-lapse tracking of moving MSCs.
f) Focal adhesion and migration related gene PCR array and detection of ROCK protein via Western blotting
g) Examination of cell viability, proliferation and cell cycle
h) ALP and ARS staining for early and late phases of osteogenesis of MSCs.

4) Analysis and interpretation of experimental data
a) The frequency of MSC marker before and after 10-day culture
b) Vitronection distribution and coating density
c) Cell distribution (inside and outside the microwell) post seeding
d) 3D reconstruction of focal adhesion, cytoskeleton structures of MSCs inside the microwell.
e) Velocity of MSC migration
f) Gene expression and protein level of ROCK
g) Distribution of proliferating cells
h) Proliferation and cell cycle analysis with and without ROCK signaling inhibitor
i) Quantification of ALP and ARS level during osteogenesis

5) Manuscript

a) First manuscript draft
b) Revising and finalization of the manuscript according to the comments of Prof. Lendlein and other co-authors.

Cover picture was designed by Xun Xu, Weiwei Wang, Nan Ma and Andreas Lendlein, and illustrated by Xun Xu:

3.2.2 Publication (Appendix II)

3.3 Surface Microwell Geometry Modulates Interleukin-6 Secretion in Human Mesenchymal Stem Cells

Xun Xu, Weiwei Wang, Zhengdong Li, Karl Kratz, Nan Ma and Andreas Lendlein

MRS Advances, **2017**, 2, 2561-2570.

DOI: 10.1557/adv.2017.487

The original article including the supplemental information is included on the following pages and online available at:

https://doi.org/10.1557/adv.2017.487

3.3.1 Author contribution

1) Literature study
    a) Microwell system for stem cell culture
    b) Signaling pathway in stem cell cytokine secretion

2) Study design (with discussion and advice from co-authors Prof. Nan Ma and Prof. Lendlein)
    a) Methods of detection and quantification of IL-6
    b) Methods of evaluation of the IL-6/ STAT3 signaling
    c) Blocking of ROCK pathway
3) Experimental work
    a) Human MSC characterization
    b) IL-6 secretion level
    c) Total protein isolation of MSCs and quantification of STAT3 protein
    d) IL-6 secretion in the presence of ROCK inhibitor
4) Analysis and interpretation of experimental data
    a) Human adipose derived MSC markers expression level
    b) IL-6 level of MSC in the absence and presence of ROCK inhibitor
5) Manuscript
    a) First manuscript draft
    b) Revising and finalization of the manuscript according to the comments of Dr. Wang, Prof. Ma and Prof. Lendlein.

3.3.2 Publication (Appendix III)

3.4 Integrin β1 activation by micro-scale curvature promotes pro-angiogenic secretion of human mesenchymal stem cells

Zhengdong Li #, Weiwei Wang #, **Xun Xu** #, Karl Kratz, Jie Zou, Liudmila Lysyakova, Matthias Heuchel, Andreas Kurtz, Manfred Gossen, Nan Ma and Andreas Lendlein

# *Authors contributed equally to this work.*

J. Mater. Chem. B, **2017**. 5, 7415-7425

DOI: 10.1039/C7TB01232B

The original article including the supplemental information is included on the following pages and online available at:

https://doi.org/10.1039/C7TB01232B

3.4.1 Author contribution

1) Literature study
    a) Topography and stem cell secretion

b) Integrin activation of MSCs
c) Signaling pathway in stem cell secretion

2) Study design (with discussion and advice from co-authors Prof. Ma and Prof. Lendlein)
   a) Methods of migration test
   b) Methods of human adipose-derived MSC phenotyping
   c) Methods of Time-lapse tracking of cell movement and distribution on structured surfaces of polymeric substrates
   d) Methods of precondition
   e) Methods of integrin activation
3) Experimental work
   a) Migration level of HUVEC cells in conditioned medium from MSCs growing on polymer substrates with microcurvatures.
   b) Quantification of integrin activation via Flow cytometry
   c) FAK and ROCK expression level by ELISA and Western blot
4) Analysis and interpretation of experimental data
   a) Analysis of HUVEC cell migration velocity
   b) Activated Integrin level
   c) VEGF expression levels in the presence of FAK and ROCK inhibitors
5) Manuscript
   a) First manuscript draft
   b) Revising and finalization of the manuscript according to the comments of Prof. Lendlein and other co-authors.

3.4.2 Publication (Appendix IV)

3.5 Surface geometry of poly(ether imide) boosts mouse pluripotent stem cell spontaneous cardiomyogenesis via modulating the embryoid body formation process.

Xun Xu #, Weiwei Wang #, Zhengdong Li, Karl Kratz, Nan Ma and Andreas Lendlein

# *Authors contributed equally to this work.*

Clin Hemorheol Microcirc. **2016**. 64, 367-382

DOI: 10.3233/CH-168107

The original article including the supplemental information is included on the following pages and online available at:

https://doi.org/10.3233/CH-168107

3.5.1 Author contribution

1) Literature study
    a) Topography and stem cell angiogenesis
    b) Embryoid body formation of iPSCs
    c) Stem cell memory
    d) Spontaneous cardiomyogenesis from pluripotent stem cells
2) Study design (with discussion and advice from co-authors Dr. Weiwei Wang, Prof. Nan Ma and Prof. Lendlein)
    a) Methods of characterization of mouse iPSCs
    b) Methods of embryoid body formation
    c) Methods of preconditioning
    d) Methods of characterization of iPSC-derived cardiomyocytes
    e) Methods of pro-angiogenesis function of conditioned medium and tube formation assay
3) Experimental work
    a) Phenotyping of mouse iPSCs
    b) Formation of CFSE labeled embryoid bodies
    c) Expression level of cardiomyocyte markers
    d) Beating frequency of iPSC-derived cardiomyocytes
    e) Cell memory effect on cardiomyocyte differentiation and pro-angiogenesis of mouse iPSCs
4) Analysis and interpretation of experimental data
    a) Speed and size of embryoid body formed on polymeric substrates with different surface geometries

b) Comparison of cardiomyocyte marker expression of cells directly cultured or preconditioned with polymeric substrates with different surface geometries
c) Beating frequency of embryoid bodies directly grown or preconditioned with polymeric substrates with different surface geometries

5) Manuscript
a) First manuscript draft
b) Revising and finalization of the manuscript according to the comments of Dr. Wang, Prof. Ma and Prof. Lendlein.

3.5.2 Publication (Appendix V)

4 Discussion

Polymeric materials were widely applied in cell maintenance, medical disposable supply and sterilization pouches mainly based on their low immunogenicity and supporting properties. Nowadays, they have been designed for mimicking the desired ECM components and structures, and further served as a functional regulator for cell-based regenerative therapies [134-137]. The effect of physicochemical properties of polymeric materials on stem cell fate has been established in the absence of complex growth factors [97]. However, the materials applied for most of these studies are placed in commercially available tissue culture plate (TCP), which might introduce the interference from the fixation materials or TCP itself. To avoid such interference, in the present study, a polymeric insert system was introduced for restricting cell interaction to the target polymer substrates while excluding the influence of TCP or other materials.

The long-term expansion of MSCs to a clinical relevant number is still a challenge since they can only be expanded *in vitro* to a certain passage and undergo growth arrest and replicative senescence, which can be characterized by flat enlarged cell shape and up-regulation of senescence-associated beta-galactosidase [138]. Consistently, in our study, flat cell morphology and increased senescence-associated beta-galactosidase positive cells as well as higher senescence ratio with low cell expansion were observed in MSCs on PEU, compared to cells on PEI substrates. Among several physicochemical features that may trigger cell senescence [139, 140], low hydrophobic polymeric surface was demonstrated to boost the cell senescence of fibroblasts [140]. However, no statistical alteration on wettability between PEI and PEU substrates was revealed in the present study. We anticipate that senescence of MSCs may react as a reflector of protein adsorption and reorganization of ECM components in their local environment triggered by the alteration of chemical composition of polymers. Therefore, it is necessary to consider cell senescence level in addition to traditional cytotoxicity assays for the evaluation of polymeric substrates. Interestingly, compared to our previous study on PEI and PEU substrates with human MSCs [141], spontaneous adipogenic differentiation of rat MSCs was observed on both substrates. The released bioactive factors that produced from senescent cells might modulate local environment and induce adipogenesis [142] Moreover, the morphological change and cytoskeleton rearrangement during senescence process might also result in the outcome of spontaneous adipogenic commitment.

Stem cells perceive not only physicochemical factors but also geometric cues in their microenvironment [101]. Cell size relevant structure dimension needs to be well concerned when design the microstructures, as cell size and spreading area can strongly influence cell adhesion, apoptosis, proliferation rate and differentiation potential [98]. Here, the microcavities and micro-curvatures on polymeric substrates with an area larger than 75 μm^2, the reported threshold regarding apoptosis induction [98], were used. The microcavity size was selected to fully accommodate one single cell of MSC. An optimal inter-microcavity space was designed for single MSC spreading to exclude the coverage of multiple microcavities with one single cell. For micro-curvature study, a smooth surface (PS-000) was used as a non-structured control. PS-160 with the peak distance of micro-curvature comparable to MSC dimension (~ 100 μm in adhesion) were expected to regulate the stem cells at the single cell level via its local curvature, while PS-320 surpassing single MSC size might be capable of modulating cell clusters. It was reported that the MSCs exhibited a lower attachment on the titanium substrate with the mean peak/valley distance relevant to the single cell size [143]. In contrast, the initial cell attachment was similar on our fibronectin-coated PS substrates with different levels of micro-curvature. This might be due to the protein adsorption and chemical properties of the substrates employed. In this study, we demonstrated that the cell spreading, morphology, actin cytoskeleton and pro-angiogenesis secretion were strongly modulated via integrin activation and its down stream signaling by sensing local curvature at single cell level on PS-160 substrates. Unlike MSCs, the pluripotent stem cells including iPSCs always grow as tight colonies wherein individual cells strongly attach to each other. The study on micropatterning of ESCs on Matrigel with controlled colony diameters from 200 to 800 μm verified that large colonies with high local cell density sustained the self-renewal state of ESCs, while small colonies were found to dramatically lose pluriopotency-associated phenotypes within 48 hours [144]. However, Onishi et al. reported that the micropatterning of pluripotent epiblast stem cells in 200 μm colony size and 500 μm spacing could enhance the stem cell pluripotency [145]. Similarly, the robust activation of pluripotency could be observed in mouse ESCs with the colony size at 200 μm [146] and effective distance of cell-cell communication via paracrine signaling can only be reached up to 250 μm [147]. Thus, the colony sizes and local cell densities as well as the distance between colonies are major physical parameters, which need to be considered in controlling their pluripotency and regulating differentiation signalings in pluripotent stem cells. Based

on these findings and considering the size of single iPSC was approximately 10 μm and the 3D sphere of iPSC cluster (EB) was typically 100-200 μm in diameter after initially 48 hours culture on low binding TCP. The PEI substrates with different microscale curvatures applied for our study were designed as three representative levels: R0, the smoothest surface of the PEI substrate; R1, with a microroughness level relevant to single iPSC size (R_q ~ 4 μm) and mean valley spacing below 250 μm was expected to accommodate EBs and allowing paracrine sensing between the cells; R2, with the highest roughness (R_q ~ 23 μm) and a mean peak spacing over 250 μm and larger than the dimension of single initial formed EB was expected to separate the EBs by preventing EB fusion. As a result, the single cell of miPSC was settled on PEI surfaces, and rapidly proliferated and formed tiny cell aggregates especially at the valley area of the R1 and R2. The small aggregates could merge together or migrate along the valley to collect more cells and clusters and became larger and larger to form the 3D spheres with diameters of 200-300 μm. Compared to R1 and smooth surfaces, with a similar diameter, EBs from R2 surface contained more cells, which were compacted firmly in response to the size effect of local curvatures. The strengthened cell-cell adhesion might further result in the promotion of spontaneous cardiomyogenesis and the increase of pro-angiogenesis paracrine.

Naturally, during embryonic development accompanied with morphogenesis, pluripotent embryonic cells usually undergo a process known as epithelial-mesenchymal transition (EMT) with the ability to migrate, and once cells arrive to their destination they revert to the epithelial phenotype to settle, proliferate and differentiate into multiple somatic cell types to form different organs [148, 149]. Thus, cell migration is indispensible throughout the entire embryogenesis. MSCs were found to be unable to preserve a long-term lifespan *in vivo* after transplantation [150, 151]. Additionally, it was observed that only 1% of injected MSCs could reach the injury site [152]. Therefore, the high efficient MSC-based therapies may mainly attribute to the rapid migration of transplanted MSCs to the damaged tissue [153]. To this end, pretreating MSCs with IL-6 or HGF [154] and overexpression of chemokine receptors via transfection [155] have been applied for regulating cell migration capacity prior to application of MSCs. However, they are still infeasible for regenerative therapies due to the introduction of additional artificial bioactive factors and potential safety issue on genetic modification with transfection. To this point of view, biophysical approach

becomes more and more attractive. Traditional applied systems for studying physical especially the geometry cues are 2D biomolecule patterning with distinct shapes or shapes of same area with increased aspect ratio, which is precisely controllable with micrometer scale resolution and simple to apply. Although cell adhesion, plasma membrane, cytoskeleton organization, proliferation, differentiation potential, intracellular signaling, chromatin condensation and histone modification can be monitored and altered via 2D patterning [92, 98, 101, 156-158], the overall cell migration is restricted in this system. 2D micro patterning in combination with hydrogel system representing deformable matrix observed in nature was developed to explore the relationship between geometry and substrate stiffness [159]. The dimension is expanded and additional parameter has been concerned within such 2D system. However, the unlimited cell movement and cell-cell communication and reflection of the true dimension *in vivo* are still missing. Furthermore, the artificial polarity between apical and basal parts of the same cell can be generated by 2D planar surfaces. Recently, more and more investigations on cell encapsulation with 3D hydrogel scaffolds, 3D electrospun architectures, and computational design based 3D printed scaffolds as well as spatiotemporal dynamic 3D matrix (4D system) have been carried out [160-165]. Nevertheless, due to the high complexity and dynamics of 3D and 4D system, precise control between distinct parameters is extremely difficult to achieve. Therefore, to bridge the gap between 2D and 3D systems, in the present study, we introduced another dimension into our polymeric insert system with microstructured bottom surface as a 2.5D transitional model allowing freely cell motion. Within such 2.5D system, we can sustain the ease of 2D culture for fundamental scientific researches while reduce the artificial polarity, restriction of overall cell migration and complexity of undefined multiple cues simultaneously presented in local 3D environment.

In this thesis, the stem cell spreading, cell-cell adhesion, morphology and cytoskeleton were altered on all smooth and microstructured polymeric substrates indicating certain mechanical stress/force may be generated and experienced by stem cells. A major challenge in the field of mechanobiology is mechanotransduction, which is the molecular mechanism of cell responding to mechanical stimuli. Cell membrane exists as the initial barrier at the interface between cell and material surface. Thus, several kinds of macromolecules located at cell membranes are expected to be the candidates of mechanosensors.

As a cell transmembrane receptor, integrin was demonstrated to be responsible for sensing and transducing external mechanical signals such as content, distribution and stiffness of ECM conponents from local environment into cytoplasm as a series of intracellular biochemical signaling events [166, 167]. Increasing the affinity of integrin receptors for their extracellular ligands was defined as integrin activation. Integrin can be bi-directionally activated via binding of various activators to its intracellular tails, termed “inside-out signaling” or via engaging integrin to ECM components, namely, “outside-in signaling”. The “outside-in” signaling regulates major behavior of adherent cells from cell adhesion, migration and survival to the cell fate determination [167, 168]. Conformational change of α and β subunits in integrin and endocytosis of integrin are key regulators for activation. Integrin endocytosis enables turnover and recycling of integrin to facilitate cell adhesion, migration and downstream signaling transduction. The activation of integrin triggers its clustering on the cell membrane and forming the focal adhesion complex. Intracellularly, among the focal adhesion proteins, FAK is the first downstream components to be autophosphorylated at Y397 by activated integrin at cell membrane and internalized integrin. This FAK phosphorylation is connected to RhoA/ROCK, MAPK/ERK and STAT3 signaling [169-171]. Here, such mechanical sensing and transduction cascades were verified as molecular mechanisms for accelerated osteogenic differentiation, secretion of pro-angiogenic IL-6 and decreased migration capacity of MSCs growing on square-shaped microcavities (S50), and promoted functional pro-angiogenic paracrine of MSCs cultured on PS-160 substrate in response to appropriate microcurvate cues. E-cadherin, the crucial mediator of cell-cell adhesion located and highly expressed at plasma membrane of pluripotent stem cells was served as another surface mechanosensor [172]. The cell-cell junction is facilitated by extracellular domain of E-cadherin, while the intracellular tail links with a variety of signaling proteins (e.g. β-catenin) and forms a complex. The initiation of mechanotransduction can be carried out via E-cadherin engagement and the conformational change, phosphorylation status and releasing of certain components of intracellular complex. During *in vivo* cardiac development and *in vitro* cardiomyocyte differentiation from stem cells, phosphorylation of cytosolic β-catenin and nuclear translocation of β-catenin were vastly involved [173, 174]. When stem cells start to form the self-organized EBs, the tightness of cell-cell junction is predominant for determining the behavior and differentiation potential of stem cells. Here, as above-mentioned, EBs from R2 surface contained more amount and compact cells with similar

dimension to other surfaces. Regarding to the dimensions of the designed PEI substrates with microcurvatures, we speculate that this could contribute to the initial growth signals provided by E-cadherin. The strengthened cell-cell junction and condensed E-cadherin are expected to consequently regulate a series of downstream mechanotransducers such as β-catenin to further enhance cardiomyogenic differentiation of EBs derived from R2. Further studies are expected to clarity this mechanism based on the mechanosensing initiated by E-cadherin. Interestingly, it was proved that the MSCs were able to remember their initial state on the stiff substrates and differentiation potential to osteolineage when the tunable substrates were temporally softened. Sustaining nuclear localization of the intracellular mechanosensor YAP and osteolineage marker RUNX2 was introduced as a potential mechanism to this mechanical memory phenomenon [175]. In consistent with the memory effect of this study, we also observed that the EBs cultivated on the substrates with different local microcurvatures kept the mechanical memory of their cardiomyogenic potential and pro-angiogenesis paracrine when they were reseeded to non-structured TCP surfaces for couple of days. As a potential mechanism, we anticipate that YAP and the alteration of epigenetics can be the downstream effectors or targets of E-cadherin and may interact with β-catenin.

Despite the differentiation potential of stem cells, the contribution of stem cell-based therapy is extensively attributed to the paracrine secretion procedure [176-178]. Secretome of stem cells can modulate the immune environment, enhance homing ability and blood vessel formation to achieve therapeutic effects for biomedical applications. Fine-tuning the contact surface of culture devices or implants with their composition and topography has been considered as the most promising, safe and effective strategy of regenerative therapies. Both osteogenesis and cardiomyogenesis requires the supporting from the blood vascular system to acquire enough oxygen and nutrition. Interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) working as regulatory cytokines for inflammation, apoptosis and angiogenesis plays a predominant role in tissue regeneration [176, 179]. To this end, in our study, the pro-angiogenic factor secretion including IL-6 and VEGF in response to the geometric microcavities and micro-curvatures is of great importance and has been intensively investigated. As expected, the alteration of secretion levels of IL-6 and VEGF from MSCs are highly relevant to the modification of geometric cues. This results are

supported by the study using 2D adhesive micropatterns with varying local curvature to stimulate the pro-angiogenic factor secretion from MSCs [180]. Similar effect has been detected during cardiomyogenesis of iPSCs. Interestingly, the status of the pro-angiogenic secretome can be memorized when the EBs were reseeded on TCP in the absence of local micro-curvature cues. These results shed light on design the biomaterials for both preconditioning of stem cells and cell-free implantation with a well-defined topography to enhance their therapeutic efficacy.

5 Conclusion

Stem cells hold a great potential for regenerative therapy due to their properties of self-renewal, differentiation potential, homing ability, pro-angiogenic secretion and immunoregulation. With the help of biomaterials, stem cells can potentially be adapted since the physicochemical properties of biomaterials are able to guide the regenerative therapy towards the desired goals and augment the therapeutic efficacy by modulating cell fate and the regeneration process. Therefore, the understanding of stem cell-material interaction and the underlying mechanism is essential for designing biomaterials that are qualified for regenerative applications.

Different cell types or same type of stem cells derived from different species may differ in response to the physicochemical factors from the different materials and to the same material with different microstructures according to their size, phenotype and sensing approach. Here, rat bone marrow-derived MSCs, human adipose derived-MSCs and mouse iPSCs were used.

The study of bone marrow derived-MSCs (Chapter 3.1) was focused on the intrinsic physicochemical properties of PEI and PEU with similar wettability. They both were applied as polymeric inserts to ensure that the MSCs were exclusively in contact with the target polymers, and served as culture vessels for long-term maintenance of MSCs *in vitro*.

Previously, we have investigated the cell compatibility of different polymers including PEI and PEU with human bone marrow-derived MSCs. The cell shape was altered and the alignment of F-actin cytoskeleton was reorganized in a random way in PEU. Among the tested polymers, PEI could support the long-term maintenance of human MSC self-renewal state. Similarly, for rat MSCs, flat and enlarged shape with rearranged F-actin cytoskeleton was observed for cells growing on PEU, while cells on PEI and TCP shared a typical spindle-shaped morphology and F-actin orientation. Although cells could grow on both polymeric surfaces with the similar adhesion ratio and subsequent division rate, cells cultured on PEU exhibited lower cell density after 5 days culture, which may directly attribute to the higher apoptosis level and senescence ratio. In this

context, PEI provided relatively better cell compatibility for rat MSC survival and growth.

Regardless of implementing a human or rat system, among all the tested polymers, PEI proved to be or was observed to be the most appropriate polymer substrate for bone marrow-derived MSCs culture. Strikingly, compared to the cells growing in traditional culture conditions with TCP and the human MSCs growing in PEU and PEI, both substrates could trigger rat MSCs towards spontaneous adipogenesis. Besides the effects that different chemical compositions of polymeric surfaces have on cells, the stem cell-mediated reflection to the polymer surface or the entire microenvironment at the cell-material interface may also differ due to species or other issues. These issues may play a certain role in differentiation, however, they still need to be further clarified.

In this thesis, for the study of human adipose-derived MSC response to micro-scale geometric cues (Chapter 3.2 and 3.3), microstructured substrates comprising arrays of square-shaped or round-shaped microwells were developed as a transitional model between 2D and 3D systems. Initially after seeding, cells were likely to roll into the microwells instead of adopting a random distribution. Post spreading, MSCs moved freely on both microstructured substrates without physical constraints. In spite of the free cell migration, different migration velocities were observed on different microstructured substrates. The cell morphologies were three-dimensionally modulated by the local curvature of microwell structure due to differences in focal adhesion and alterations of the cytoskeleton. In contrast to the substrate with round-shaped microwells, the substrate with square-shaped microwells promoted the secretion of angiogenic factor IL-6, proliferation and osteogenesis of MSCs. Such microwell shape-dependent modulatory effect was highly associated with ROCK signaling. Following ROCK inhibition, the differences in IL-6 secretion, proliferation and osteogenesis between cells on different substrates were diminished.

These findings allow a better understanding of MSC response to geometric cues and highlight the possibility to control the fate and functions of MSCs through structured features via manipulation of ROCK signaling. This knowledge might help to direct stem cells towards desired orientations and functions through utilization of biomaterial surface structures.

As indicated in Chapter 3.2 and 3.3, the distinct local curvature of square and round-shaped microwells was considered as a potential trigger and regulatory factor involved in modulation of MSC cellular response. In Chapter 3.4 and 3.5, a series of microroughness surfaces with local micro-scale curvature change were created. Surfaces with an appropriate microcurvature relevant to a single MSC size (PS-160) could effectively enhance the VEGF secretion and the pro-angiogenic effect of human adipose-derived MSCs via integrin-activation initiated intracellular signaling cascades (e.g FAK and ERK). The functional cardiomyogenic differentiation together with pro-angiogenic secretion of mouse iPSCs was boosted and mechanically memorized initially via cell compacting and later on via strengthening of cell-cell junctions by local curvatures fitting to EB diamension (PEI R2). We speculated that both mechanosensors E-cadherin and YAP might be the potential determinants. Further studies need to be carried out.

Regardless of various MSC sources (adipose tissue- and bone marrow-derived) and different types of stem cells (MSCs and iPSCs), both 2.5D microstructuring with different local curvatures and polymeric substrates with different chemical components can modulate stem cell morphology and behavior, and provide the basis for development and future commercialization in tissue culture systems. This approach of induced and controlled endogenous regeneration using a polymeric biomaterial may also be applicable to other stem/progenitor cells and to various types of implants, such as microstructured substrates of orthopedic implants, where the integration into relevant tissues could potentially be improved. Further, with the introduction of microtechnological platforms for cell culture, the pace of stem cell research can be dramatically enhanced.

6 Zusammenfassung

Stammzellen zeigen ein großes Potential auf dem Gebiet der regenerativen Therapien aufgrund ihrer Eigenschaften der Selbsterneuerung, Differenzierung, Anwachsungs Fähigkeit, Angiogenetische Sekretion und Immunregulation. Mit Hilfe von Biomaterialien können sich Stammzellen möglicherweise besser an ihre Umgebung anpassen, da sich die physikalisch-chemischen Eigenschaften von Biomaterialien dafür eignen das Verhalten der Zellen im Verlauf der regenerativen Therapie positiv zu beeinflussen und die Effizienz der Regeneration zu verbessern. Hierfür ist ein grundlegendes Verständnis der Wechselwirkungen zwischen den Stammzellen und dem Material notwendig um geeignete Biomaterialien zu entwickeln.

Verschiedene Zelltypen oder dieselbe Art von Stammzellen, die aus unterschiedlichen Arten stammen, können unterschiedliches Verhalten auf die physikalisch-chemischen Faktoren, die auf die verwendeten Material zurückgehen, aufweisen. Auch wenn dieselben Materialien verwendet werden können die Beschaffenheit der Oberfläche, wie zum Beispiel unterschiedliche Mikrostrukturen (entsprechend ihrer Größe), Phänotyp und Sensorik verändern. Für diese Studie wurden, MSCs aus der Knochenmark von Ratten, und – MSCs aus den menschlichen Fettgewebe sowohl als auchMaus iPSCs verwendet wurden.

Die Untersuchung von Knochenmark abgeleiteten-MSCs (Kapitel 3.1) konzentriert sich auf der ähnlichen der physikalisch-chemischen Eigenschaften in Bezug auf die Benetzbarkeit von PEI und PEU. Sie wurden beide als Polymeremulgatoren eingefügt, um sicherzustellen, dass MSCs ausschließlich in Kontakt mit dem Ziel Polymerenkommen wurden, und wurden als Kulturplatformen für die langfristige Wartung von MSCs *in vitro*.

Zuvor haben wir die Zellekompatibilität verschiedener Polymere, darunter PEI und PEU mit menschlichen Knochenmark MSCs untersucht. Die Zellform veränderte sich und eine statistische Ausrichtung des F-Aktin Zytoskelett wurde für PEU beobachtet. Innerhalb der untersuchten Polymeren konnte PEI die langfristige Erhaltung von menschlichen MSC im Erneuerungszustand gewährleisten. Ähnliches Verhalten konnte auch für die MSCs von Ratten beobachtet werden. Hier wurden flache und verbreiterte Formen mit orientierten F-Aktin Zytoskelett innerhalb der auf PEU angesiedelten Zellen gefunden, während die Zellen auf PEI und TCP eine typische

spindelförmige Morphologie und F-Aktin Orientierung aufwiesen. Obwohl die Zellen auf beiden polymeren Oberflächen mit einem ähnlichen Adhäsionsverhalten und anschließender Zellteilungsrate wachsen, weisen kultiviert Zellen auf PEU eine geringere Zelldichte nach 5 Tagen auf, was sich möglicherweise auf die höhere Ebene der Apoptose und Seneszenz Verhältnis zugerechnet werden könnte. In diesem Zusammenhang wurde eine relativ bessere Zellkompatibilität für MSCs von Ratten bei PEI gefunden, wenn man die Faktoren Überleben und Wachstum vergleicht.

Sowohl in Menschen als auch in Ratten weist PEI, im Vergleich zu allen untersuchten Polymersystemen, das verlässlichste Polymersubstrat für Knochenmark MSCs auf. Auffallend ist, dass im Vergleich zu den Zellen, die auf traditionellen Materialien zum Kultivieren mit TCP und den menschlichen MSCs auf PEU und PEI aufgebracht wurden, beiden Substrate MSCs von Ratten eine spontane Adipogenese auslösen. Neben der Tatsache, dass unterschiedliche chemische Zusammensetzungen von polymeren Oberflächen unterschiedliche Effekte auf Zellen haben, Stammzelle–Reflexion auf der Oberfläche muss das gesamte Mikromilieu zwischen der Zelle und dem Material berücksichtig werden. Diese Probleme können eine gewisse Rolle für die Differenzierung spielen, was noch weiter im Detail untersucht werden muss.

In dieser Arbeit, für das Studium der menschlichen Fettgewebe-MSC-Antwort auf Micro-scale geometrische Signale (Kapitel 3.2 und 3.3), wurden mikrostrukturierte Substrate aus Arrays von quadratischen oder runden Microwells entwickelt als ein Übergangsmodel zwischen 2D- und 3D-Systeme. Nach der Aussaat tendieren die Zellen eher in die Microwells zu rollen anstelle einer geleichmäßigen Verteilung innerhalb dieser. Anschließendes verteilen während der die MSCs frei beweglich ohne Einschränkungen auf beiden mikrostrukturierter Substraten. Trotz der freien Migration, wurden unterschiedliche Geschwindigkeiten auf verschiedenen mikrostrukturierter Substrate beobachtet. Die Zellenmorphologie wurde dreidimensional von der lokalen Krümmung der Microwell-Struktur aufgrund der Unterschiede in den fokalen Adhäsionen und Veränderungen des Zytoskeletts moduliert. Im Gegensatz zum Substrat mit runden Mikrowells, hat das Substrat mit quadratischen Microwells die Sekretion von angiogenetischen Faktor IL-6, Proliferation und Osteogenese von MSCs gefördert. Diesemicrowell Form-abhängige modulierende Wirkung war mit ROCK Signalisierung stark verbunden. Folgende ROCK Hemmung, hat zufolge die

Unterschiede in der IL-6-Sekretion, Proliferation und Osteogenese zwischen Zellen auf verschiedenen Substraten verringert.

Diese Erkenntnisse ermöglichen ein besseres Verständnis der MSC Rückmeldung auf geometrische Signale und die Möglichkeit, sich das Verhalten von MSCs aufgrund von strukturierten Begebenheiten mittels Manipulation von ROCK Signale zu beeinflussen. Dieses Wissen könnte helfen Stammzellen zur gewünschten Orientierungen und Funktionen durch die Nutzung der Oberflächenstrukturen auf Biomaterialien zu steuern.

Wie bereits in Kapitel 3.2 und 3.3, wurden die unterschiedlichen lokalen Krümmungen von quadratischen und runden geformte Mikrotiterplatten als möglicher Signale und regulierende Faktoren bei der Modulierung von MSC Reaktion berücksichtigt. In Kapitel 3.4 und 3.5 wurde eine Reihe von Oberflächen mit unterschiedlicher Rauheit und Krümmung erstellt. Oberflächen mit einem geeigneten microcurvature relevant zu einer einzigen MSC-Größe (PS-160) könnte effektiv die VEGF-Sekretion und die pro-angiogenetische Wirkung der aus den menschlichen Fettgewebe gewonnenen MSCs verbessern über Integrin-Aktivierungs initiierte intrazellulären Signalkaskaden (z.b. FAK und ERK). Die funktionale Differenzierung zu Herzmuskelzellen zusammen mit der pro-angiogenetische Sekretion von Maus iPSCs wurde verstärkt und mechanisch eingeprägt anfangs über Zellverdichtung und später über die Stärkung der Zell-zell-Kreuzungen durch lokalen Krümmungen die sich zu EB Diamensionenangepasst haben (PEI R2). Wir spekulierten, dass beide mechano-Sensoren E-cadherin und YAP die möglichen Determinanten sein könnten. Weitere Studien müssten durchgeführt werden.

Unabhängig von verschiedenen MSC Quellen (vom Fettgewebe und Knochenmark) und diversen Arten von Stammzellen (MSCs und iPSCs), die 2.5D Mikrostrukturierung mit verschiedenen lokalen Krümmungen und polymeren Substraten mit verschiedenen chemischen Zusammensetzungen können Stammzellen-Morphologie und Verhalten beeinflussen und die Grundlage für die Entwicklung und die zukünftige Kommerzialisierung in der Gewebekultur Systeme vorantreiben. Dieser Ansatz von induzierten und kontrollierten endogenen Regeneration mittels eines polymeren Biomaterials kann auch für andere Stamm- und Vorläuferzellen und auf verschiedene Arten von Implantaten, wie mikrostrukturierter Substrate von orthopädischen Implantaten verbessert werden könnte. Des Weiteren könnte mit der Einführung von

mikrotechnologischen Plattformen für Zellkulturen, kann das Tempo der Stammzellenforschung erheblich beschleunigt werden.

7 References

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8 Publication List and Conference Abstracts

Publications:

1. Li, Z. [#], Wang, W. [#], **Xu, X.** [#], et al., Integrin β1 activation by micro-scale curvature promotes pro-angiogenic secretion of human mesenchymal stem cells. J. Mater. Chem. B, 2017. 5(35): p. 7415-25. [#] *Authors contributed equally to this work.*
2. **Xu, X.**, et al., Microwell Geometry Modulates Interleukin-6 Secretion in Human Mesenchymal Stem Cells. MRS Advances, 2017. 2(47): p. 2561-70.
3. Wang, W., et al., Polydepsipeptide Block-Stabilized Polyplexes for Efficient Transfection of Primary Human Cells. Biomacromolecules, 2017. 18(11): p. 3819-33.
4. Zou, J., et al., Adipogenic differentiation of human adipose derived mesenchymal stem cells in 3D architectured gelatin based hydrogels (ArcGel). Clinical hemorheology and microcirculation, 2017. 67(3-4): p. 297-307.
5. Li, Z., et al., Modulation of the mesenchymal stem cell migration capacity via preconditioning with topographic microstructure. Clinical hemorheology and microcirculation, 2017. 67(3-4): p. 267-78.
6. Wang, W., et al., Functional Nanoparticles and their Interactions with Mesenchymal Stem Cells. Current pharmaceutical design, 2017. 23(26): p. 3814-32.
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9. Wang, W., et al., The interaction of adipose-derived human mesenchymal stem cells and polyether ether ketone. Clin Hemorheol Microcirc, 2015. 61(2): p. 301-21.
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11. **Xu, X.**, et al., Controlling major cellular processes of human mesenchymal stem cells using microwell structures. Adv Healthc Mater, 2014. 3(12): p. 1991-2003. *(Cover page)*
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15. **Xu, X.**, et al., Cellular-based immunotherapies for patients with glioblastoma multiforme. Clin Dev Immunol, 2012. 2012: p. 764213.
16. **Xu, X.**, et al., Therapeutical doses of temozolomide do not impair the function of dendritic cells and CD8+ T cells. Int J Oncol, 2012. 40(3): p. 764-72.
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18. Wang, W., et al., The influence of polymer scaffolds on cellular behaviour of bone marrow derived human mesenchymal stem cells. Clin Hemorheol Microcirc, 2012. 52(2-4): p. 357-73.

Conference Abstracts:

1. 2017, **MRS Spring Meeting**, Phoenix Convention Center, Phoenix, Arizona, USA (Abstract & poster presentation).
   Title: *Microstructured Substrates Modulate Interleukin-6 Secretion in Human Mesenchymal Stem Cells (Document ID: 2634830, Final ID: SM8.7.18)*

2. 2016, **18th Conference of the European Society for Clinical Hemorheology and Microcirculation (ESCHM) conference**, Lisbon, Portugal. (Abstract & poster presentation).
   Title: *Geometry of microwells modulate IL-6 secretion of human adipose derived mesenchymal stem cells via ROCK signaling pathway*

3. 2014, **Advanced functional polymers for medicine (AFPM) conference**, University of Liège, Belgium. (Abstract & poster presentation).
   Title: *3D geometrical cue regulates stem cell self- renewal, migration and differentiation*

4. 2013, **32th Deutsche Gesellschaft für Klinische Mikrozirkulation und Hämorheologie (DGKMH) Meeting**, Dresden, Germany (Abstract & oral presentation).
   Title:
   (1) Cultivation of rat mesenchymal stem cells on poly (ether imide) surface with different microscaled roughness (No. S5V4);

(2) *The influence of geometrically patterned polymer surfaces on the development of human mesenchymal stem cells (No. S5V2);*

(3) *Surface roughness promotes differentiation of human adipose derived mesenchymal stem cell into endothelial like cells (No. S5V6)*

5. 2012, **14th International Congress of Biorheology, 7th International Conference on Clinical Hemorheology**, Istanbul, Turkey (Abstract & oral presentation).
   Title: *Different polymer surfaces influence in vitro cultivation of rat mesenchymal stem cells.*

6. 2012, **31th Deutsche Gesellschaft für Klinische Mikrozirkulation und Hämorheologie (DGKMH) Meeting**, Universitätsklinikum Halle/ Saale, Germany (Abstract & oral presentation).
   Title: *The influence of materials and topographic roughness of the scaffolds on cellular behaviors of human mesenchymal stem cells (No.S3V7)*

9 Curriculum Vitae

Personal Details

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12/2011-Present Ph.D candidate, Helmholtz-Zentrum Geesthacht, Institute of Biomaterial Science and Freie Universität Berlin, Germany

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01/2017-Present Scientific researcher, Helmholtz-Zentrum Geesthacht, Institute of Biomaterial, Germany. Research field: Stem cell and microstructured biomaterial interaction

02/2009-08/2011 Research assistant, Immunology and Immunotherapy group, Department of Internal Medicine III, University of Rostock, Germany. Research field: Tumor Immunotherapy, Antigen-Specific cell isolation

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10 Appendix

Appendix I (Page 71-90)

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Appendix V (Page 209-245)

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Appendix I

Cultivation and Spontaneous Differentiation of Rat Bone Marrowderived Mesenchymal Stem Cells on Polymeric Surfaces

The original article is online available at:
https://doi.org/10.3233/CH-131698

Appendix II

Controlling major cellular processes of human mesenchymal stem cells using microwell structures

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Appendix III

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Appendix IV

Integrin β1 activation by micro-scale curvature promotes pro-angiogenic secretion of human mesenchymal stem cells

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Appendix V

Surface geometry of poly(ether imide) boosts mouse pluripotent stem cell spontaneous cardiomyogenesis via modulating the embryoid body formation process

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