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Review

# A review of the effects of the cell environment physicochemical nanoarchitecture on stem cell commitment

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ABSTRACT

Physicochemical features of a cell nanoenvironment exert important influence on stem cell behavior and include the influence of matrix elasticity and topography on differentiation processes. The presence of growth factors such as TGF-β and BMPs on these matrices provides chemical cues and thus plays vital role in directing eventual stem cell fate. Engineering of functional biomimetic scaffolds that present programmed spatio-temporal physical and chemical signals to stem cells holds great promise in stem cell therapy. Progress in this field requires tacit understanding of the mechanistic aspects of cell-environment nanointeractions, so that they can be manipulated and exploited for the design of sophisticated next generation biomaterials. In this review, we report and discuss the evolution of these processes and pathways in the context of matrix adhesion as they might relate to stemness and stem cell differentiation. Super-resolution microscopy and single-molecule methods for *in vitro* nano-manipulation are helping to identify and characterize the molecules and mechanics of structural transitions within stem cells and matrices. All these advances facilitate research toward understanding of stem cell niche and consequently to developing new class of biomaterials helping the "used biomaterials" for applications in tissue engineering and regenerative medicine.



## 1. Introduction

The construction of synthetic extracellular matrix-mimetic systems for programmed stem cell response is a field of topical interest [1]. Recent years have witnessed rapid advances in this field [2–4]. The engineered nanoenvironments are becoming increasingly sophisticated and have begun to approach the structural and functional complexity of the physiological environment *in vivo* around the cells. The importance of the artificially constructed ECM mimics can be dissected from two different aspects, which are interlinked. Firstly, such studies have elucidated fundamental understanding of the mechanisms of a myriad of biological processes like cell adhesion, proliferation, migration and differentiation [5–8]. These and a multitude of cell responses are governed by complex chemical and physical cues from the surrounding environment encompassing different length scales, from nano to micro [9,10], the mechanisms of which are poorly understood. Unraveling these interactions can have profound implications in eventual control and programming of various cell functions [11]. This brings us to the second aspect, the positive outcome of such understanding in the field of tissue engineering and regenerative medicine [12]. The control of differentiation of stem cells into specific cell lineages is vital in regenerating healthy tissues in the injured areas of the body [12]. Sprouting of blood vessels has to occur in order to transport nutrients to the differentiated cells, and finally, the recreated tissue has to integrate into the body, leading to eventual wound healing. Normally, the biomaterials serving as ECM mimetic scaffolds are programmed to present some specific chemical or/and physical cues and the response of the seeded cells is studied [13]. While this can be a good starting model to understand different facets of cell–material interactions, in reality, the stem cells *in vivo* are in a complex instructive 3D nanoenvironment which sends spatially and temporally controlled signals to the cells to elicit specific responses [14,15]. Besides, the interaction between different cells influences cell behavior [16,17]. Incorporating advanced functions such as precise spatio-temporal control of simultaneously present multiple physical and chemical cues in biomimetic constructs is a challenging task [18], but is essential to accomplish a gamut of stem cell functions (for example, maintaining the undifferentiated form (stemness), on-demand

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differentiation into specific tissues, successful vascularization *etc.*). This review will attempt to delineate the present state of the art progresses by discussing representative examples of the recent literature in this field. As it can be seen, the problem is not trivial, is essentially interdisciplinary, and the solution requires a collaborative approach from scientists across the disciplines of biology, chemistry and physics.

Stem cells can be classified according to their origin: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult stem cells [19,20]. Each of these classes has potentially important roles to play in tissue engineering and regenerative medicine [21,22]. However, the use of embryonic stem cells is complicated by ethical issues. On the other hand, iPSCs can be derived from somatic cells by forcing the expression of certain genes, and they can function, *e.g.,* as embryonic stem cells, surpassing ethical issues in the use of such types of cells [23–26]. Adult stem cells are present in somatic tissues, *e.g.*, bone marrow or adipose tissue, and play a vital role to repair and replenish dying somatic cells and damaged tissues [27]. Despite their immense potential in tissue engineering applications, ESCs and iPSCs have been found to differentiate into tumor cells [28], severely limiting scopes of their clinical trials in humans. This makes mesenchymal stem cells (a type of adult stem cells) a viable and practical alternative to use in stem cell research, as there is no literature report till date that hMSCs express cancer genes under any circumstances [11,27]. hMSCs are capable of differentiating into multiple cell lineages, *e.g.*, adipocytes (fat tissue), chondrocytes (cartilage), osteoblasts (bone cells), myoblasts (muscle tissue) and neuronal cells (nerve tissue) [27]. The instructions from the stem cell niche govern the fate of the stem cells, the maintenance of their multipotency and stem-ness [14,29], their survival and the choice of the phenotype of differentiation [30]. There are several aspects of these instructions, which can be broadly classified as: (1) physical cues that manifest in the form of, *e.g.*, ECM stiffness and the topographical features of the ECM [31–35]; (2) biochemical cues that come from growth factors (cytokines) [36–38] (Fig. 1). The growth factors may be presented in (a) soluble form [39], or (b) ECM bound form, the so called 'solid induction mode' [40]. For example, bone morphogenetic proteins (BMP) that play a critical role in bone cell formation, are believed to be sequestered in the ECM, since the bone tissue consists of a large amount of collagen that contains BMP specific interaction sites. Thus, BMP is presented to cells in ECM bound form [41,42]. *In vivo*, the factors (1) and (2) operate in tandem. The understanding and rational manipulation of these effects should be incorporated into the design of the ECM mimetic biomaterials, in order to achieve the desired control over the artificial scaffolds for stem cell growth and differentiation. In the following sections, the effect of these parameters on stem cell fate (in the context of emerging functional materials) will be discussed. The areas of controversy in regards to the mechanistic implications of these effects will also be highlighted.

## 2. Effect of substrate rigidity

In a seminal study, Engler et al. cultured hMSCs on polyacrylamide hydrogels of different stiffnesses, coated with collagen I (to provide adhesive surface for the cells, in the absence of any differentiation inducing media) [43]. It was demonstrated that on soft gels (0.1–1.0 kPa) mimicking the rigidity of brain tissues, the hMSCs showed neurogenic commitment, whereas, on stiffer gels (8–17 kPa), resembling the rigidity of muscle tissues, myogenic commitment was observed. When the stiffness of the matrix was further increased to mimic that of the collagenous bone tissues (25–40 kPa), osteogenic commitment was induced. Importantly, the hMSCs adopted morphologies similar to the eventual differentiated cells. For example, stiff matrices (25–40 kPa; inducing osteogenic differentiation) led to polygonal hMSCs, a morphology resembling osteoblasts. Likewise, softer gels mimicking muscle elasticity (8–17 kPa) induced spindle shaped morphology in hMSCs, similar to C2C12 myoblasts. This study also demonstrated an important role of the cytoskeletal motor non-muscle myosin II (NMMII) in cell differentiation. NMMII mediated actin-contractility was implicated in sensing the ECM stiffness, since blocking NMMII with blebbistatin completely suppressed any differentiation. Several important aspects of this work in the context of the subsequent literature reports deserve a detailed discussion.

### *2.1. The competition/compliance between biochemical signals and matrix stiffness*

Engler's work showed that in the absence of biochemical signals, the physical cues (in the form of stiffness) from the ECM were enough to determine the differentiation phenotype for stem cells [43]. Many other groups have reported similar stiffness dependent stem cell differentiation on 2D substrates [44,45]. This raises the important question: when biochemical instructions from the ECM are present, can they override the stiffness directed lineage specification? Which one of the two factors will be the determinant for the stem cell fate? The issue is important, for example, in stem cell-based therapies for medical conditions where there is extensive damage to the tissue, leading to local stiffening [46]. In a recent study, when hMSCs were injected into the heart of mice after artificially inducing myocardial infarction, they showed calcification (bone tissue formation) instead of the expected differentiation into heart muscle cells [47]. This result was attributed to a stiff environment created by scarred tissues, which no longer induced myogenic differentiation. These data suggest that transplantation of hMSCs into damaged tissues with the expectation that the instructive nanoenvironment of the tissue will control the differentiation of the stem cells and facilitate the healing, may have dangerous consequences.

Engler's work had indeed demonstrated that the presence of soluble induction factors could reprogram the lineage specification in the initial stages of cell culture, leading to mixed phenotypes [43]. Zouani et al. have recently shown that the substrate rigidity can be made to comply with specific biomolecular signaling provided by covalently grafted biomolecules present in the matrix [45]. They have cultured hMSCs on the surface of adhesion ligand (RGD) grafted co-polymer hydrogels of poly(acrylamide-co-acrylic acid). They could thus demonstrate that depending on the stiffness of the gels, which was controlled directly by varying the % of cross-linker bis-acrylamide, myogenic (13–17 kPa) or osteogenic (45–49 kPa) differentiation can be induced. This result is consistent with the findings in Engler's article. In Zouani's study, when the gels were functionalized with osteogenesis inducing BMP-2 mimetic peptides, the effect of the ECM stiffness was circumvented and osteogenic differentiation was favored over myogenic lineage even on soft gels (15 kPa), which were previously shown to induce myoblast formation in the absence of this ligand. The grafted BMP-2 peptide bound to the receptors on the cell membrane, thus activating the BMP-Smad pathway and leading to the expression of osteoblast genes. Moreover, it was clearly demonstrated that NMMII mediated actin stress generation was important for osteogenic differentiation, suggesting the role of cytoskeletal tension in regulating the BMP-Smad pathway. However, this pathway was not favored on very soft gels (0.76–3 kPa) and no nuclear translocation of the Smad proteins was observed on these gels which did not induce osteoblast differentiation [45].