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High throughput approaches for controlled stem cell differentiation ☆

Hwan D. Kim [1], Eunjee A. Lee [1], Young Hwan Choi, Young Hyeon An, Rachel H. Koh, Seunghyun L. Kim, Nathaniel S. Hwang *

School of Chemical and Biological Engineering, Institute for Chemical Processes, NBio Institute, Seoul National University, Republic of Korea

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ABSTRACT

Stem cells have unique ability to undergo self-renewal indefinitely in culture and potential to differentiate into almost all cell types in the human body. However, the developing a method for efficiently differentiating or manipulating these stem cells for therapeutic purposes remains a challenging problem. Pluripotent stem cells, as well as adult stem cells, require biological cues for their proliferation and differentiation. These cues are largely controlled by cell–cell, cell-insoluble factors (such as extracellular matrix), and cell-soluble factors (such as cytokine or growth factors) interactions. In this review, we describe a state of research on various stem cell-based tissue engineering applications and high throughput strategies for developing synthetic or biosynthetic microenvironments to allow efficient commitments in stem cells.

Statement of Significance

Nowadays, pluripotency of stem cells have received much attention to use therapeutic purpose. However, a major difficulty with stem cell therapy is to control its differentiation through desired cells or tissues. In other words, various microenvironment factors are involved during stem cell differentiation, including dimensionality, growth factors, cell junctions, nutritional status, matrix stiffness, matrix composition, mechanical stress, and cell-matrix adhesion. Therefore, researchers have engineered a variety of platforms to enable controlling and monitoring bioactive factors to induce stem cell commitment. In this review, we report on recent advancements in a novel technology based on high-throughput strategies for stem cell-based tissue engineering applications.

1. Introduction

Numerous challenges remain for successful translation of stem cell-based tissue engineering into clinical practice, including fabrication of bioactive scaffolds that can provide a microenvironment for tissue-specific commitment, long-term survival, and engraftment. Because stem cells require a complex array of signals for differentiation, researchers have incorporated such signaling molecules into biomaterials to promote desired cell functions [1–5]. However, many of the complex signals that regulate stem cell commitment and differentiation remain unclear. In order to create these complex microenvironments, researchers have engineered a variety of platforms that enable bioactive factors to induce stem cell commitment. Traditional methods of assessing these various factors have involved iterative approaches that require a tremendous amount of time and effort. However, to address current challenges in stem cell-based tissue engineering, development of high throughput and combinatorial technologies has been utilized to optimize cellular interactions with synthetic microenvironments [6–8]. The high throughput analysis of cell–microenvironment interactions represents a critical step in the development of successful tissue. In particular, recent advancements in high throughput technology have enabled the creation of numerous different substrates for the identification of specific microenvironments determining stem cell fates [9,10]. This led to a particular emphasis on the interaction of synthetic and biosynthetic substrates with cell-surface receptors [11]. Recently, high throughput strategies for creating synthetic or biosynthetic microenvironments to allow efficient commitment in stem cells have been employed. An improved understanding of specific microenvironmental factors

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* Corresponding author at: 1 Gwanak-gu Gwanak-ro, Seoul 151-744, Republic of Korea.
E-mail address: nshwang@snu.ac.kr (N.S. Hwang).

[1] These authors contributed equally to this work.

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via high throughput approaches will enable controlled differentiation of stem cells for tissue engineering and regenerative medicine applications.

2. High throughput approaches for stem cell engineering

Stem cells reside within specialized microenvironments called stem cell niche that modulate their proliferation and differentiation, influence symmetric versus asymmetric division, and protect them from physiological stresses. During embryonic development, a complex array of signals regulates these physiological processes of pluripotent stem cells leading to their commitment to particular cell phenotypes. Complex arrays of signals via cell–cell interactions, cell–ECM interactions, and localized soluble factors result in tissue specification and differentiation (Fig. 1). In the field of biomedical engineering, researchers attempt to elucidate and recapitulate these diverse environmental interactions. In order to systematically probe mechanisms of stem cell functions, platforms for high throughput evaluation are desirable. There have been efforts to identify microenvironmental factors and downstream signaling pathways important in high throughput stem cell differentiation and commitment.

High throughput combinatorial techniques were initially used by the pharmaceutical industry to develop new small molecules and discover drug candidates [12]. Recently, researches in biomaterials field have adapted these methodologies to create biomaterial arrays for tissue engineering and drug delivery applications. A wide variety of chemical functional groups have been incorporated into an existing polymer library, and further expansion of chemical library could significantly enhance the capacity to direct adult stem cell behavior [13]. For example, the combinatorial synthesis of the biodegradable cationic polymer [e.g., poly (b-amino esters) (PBAEs)] has been utilized for drug/gene-delivery applications. Using this technology, Brey et al. developed a library of photo-polymerizable and biodegradable PBAEs that possess a range of tunable properties and identified most efficient PBAE combination for osteogenic differentiation of hMSCs [14]. This systemically fabricated library of photo-crosslinkable PBAEs can screen for a variety of biological behaviors by altering macromer chemistry and their molecular weight [15]. Furthermore, a library of tough elastomeric biomaterials composed of poly(polyol sebacates) was created by the Langer group [16]. Although much progress has been made in biomaterial design and application, the rational design of biomaterial cues to direct stem cell behavior and differentiation remains challenging. Due to the complexity of the signals that modulate cell behaviors, it is often difficult to optimize cellular microenvironments using conventional approaches. Alternatively, high throughput approaches have shown great potential in accelerating the discovery of materials that control stem cell behavior [5,6,11,17,18]. In contrast to conventional approaches, high throughput technology has enabled rapid screening of diverse materials crucial in controlling stem cell fate [6,11].

2.1. High throughput cell–ECM interactions

Physical interactions with the extracellular matrix (ECM) significantly influence stem cell behavior. Also, subsets of ECM components may influence cell shape, which has shown to be a potent regulator of cell growth and physiology [19,20]. Recently, Albrecht et al. utilized microfabrication technologies in *in vitro* cell culture model to decouple the complex spatiotemporal cues that cells experience *in vivo* [21]. They fabricated hydrogels containing 3D-microorganized cells using dielectrophoretic cell patterning (DCP) chamber. In addition, they tested combinatorial matrices of various natural ECM components on their ability to maintain functional hepatocytes and the ability to induce hepatic differentiation from murine embryonic stem cells (mESCs) [21] (Fig. 2). They manipulated cellular interactions with different combinatorial mixtures of ECM. In their study, 32 different combinations of Collagen I, Collagen III, Collagen IV, Laminin, and Fibronectin were created, and ES cells with a reporter for *Ankrd17*, a fetal liver-specific gene, were plated. Depending on the composition of the ECM, a 140-fold difference in reporter signals was observed between the least and the most efficient condition. This clearly demonstrated that a specific combination of ECM components can modulate early commitment of ES cells to a particular lineage phenotype.

In nature, signals are presented in a complex but in a spatially discrete manner. Thus, a novel protein deposition in a controlled, spatially discrete manner on a subcellular scale may closely mimic the native spatial organization of cues [22]. Currently, many methodologies for these applications exist. For example, dip-pen lithography is being utilized to deposit desired proteins in a well-controlled manner with the help of an atomic force microscope [23–25]. Multiple cantilever-based patterning approaches [26,27] and inkjet-printing approaches are currently being used to transfer bioactive molecules to the surface [28–30].

With these methods, growth factors or ECM components with varying concentration gradients can be immobilized as a combinatorial array on biologically relevant substrates for stem cell differentiation. Combinatorial protein displays for cell modulation have been achieved, and developments in micro- and nano-scale technology have enabled generation of ECM protein microarrays with well-defined geometries. Robotic potting technology has been utilized to fabricate microarrays composed of a mixture of proteins, morphogens, and other signaling proteins [26]. Bhatia et al. expanded the concept of protein-array platform by compartmentalizing protein arrays using a gasket to produce a multiwell plate [31]. This allowed simultaneous probing of ECM components and soluble growth factors influencing and interacting with mESCs fate. Furthermore, Soen et al. utilized primary neural precursors on printed protein arrays to explore its extent and direction of differentiation into neurons and glia. The signaling molecules were printed on a non-contact, piezoelectric 2D layer [32] (Fig. 3). In another study, LaBarge et al. utilized protein microarrays to dissect the instructive function of the microenvironment on human mammary progenitor cell regulation [33]. It was elucidated that cell fate decisions by human mammary progenitor cells are quite plastic in the context of hundreds of unique microenvironments in parallel. Furthermore, immobilization approaches that recapitulate the natural presentation mode of niche proteins have also received attention from researchers. This indicates that any choice of substrate can be selectively functionalized with engineered molecules to recapitulate the physiochemical characteristics of niches that may be important in controlling the behavior of stem cells outside their natural microenvironment. For example, Suzuki et al. showed that immobilization of an *Fc-chimeric Delta 1* fusion protein, in synergy with adsorbed fibronectin and soluble cytokines, led to *CD133+* positive cord blood cells in mice [34]. Also, Dread et al. utilized phage display to identify novel peptides that bind to the surfaces of pluripotent stem cells [35] and identified peptidic surfaces by generating arrays of self-assembled monolayers that support ES cell growth and self-renewal [36].

2.2. High throughput cell–biomaterial interactions

Creating of a library of polymeric materials for hydrogels and porous scaffolds can help researchers overcome current difficulties regarding the choice of biomaterials. High throughput microarray technology can have a wide variety of applications in stem cell differentiation. Such method of creating a library of scaffold material would be efficient and cost-effective for selecting the ideal scaffold

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