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High throughput screening for discovery of materials that control stem cell fate

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ABSTRACT

Insights into the complex stem cell niche have identified the cell–material interface to be a potent regulator of stem cell fate via material properties such as chemistry, topography and stiffness. In light of this, materials scientists have the opportunity to develop bioactive materials for stem cell culture that elicit specific cellular responses. To accelerate materials discovery, high throughput screening platforms have been designed which can rapidly evaluate combinatorial material libraries in two and three-dimensional environments. In this review, we present screening platforms for the discovery of material properties that influence stem cell behavior.

1. Introduction

The ability of stem cells to self-renew or to differentiate into specialized progeny makes them a valuable source for production of clinically relevant cells for regenerative medicine, disease modeling and biomedical applications. Stem cells broadly fall into two categories. The first, human pluripotent stem cells (hPSCs), include embryonic stem cells (hESCs) and have the potential to generate cells from any of the three germ layers that comprise all of the ~200 cell types found in the body [1]. Also included in this group are induced pluripotent stem cells (hiPSCs), which bypass the need for cultivation from a blastocyst by reprogramming somatic cells into a stem cell state using a cocktail of transcription factors [2]. The second group encompasses tissue specific or 'adult' stem cells whose role is to assist in repair or renewal of tissue. These cells are generally considered multipotent meaning that their differentiation potential is limited to the cell types of the tissue in which they reside.

The promise of stem cells in regenerative medicine is becoming reality with recent approval for the use of limbal stem cells for the treatment of ocular burns [3] and phase I clinical trials underway for the use of hPSC derivatives for spinal cord injury [4] macular degeneration [5] and heart failure [6]. To broaden the application of stem cells and their derivatives for wide ranging conditions there is a need for culture systems that enable controlled manipulation of these cells.

The first successful *in vitro* propagation of hESC was accomplished in 1998, this was over a decade after the culture of mouse embryonic stem cells (mESC) was achieved [7]. The culture conditions found to maintain mESC pluripotency could not be translated to the human counterparts, where pluripotency could only be maintained when the cells were cultured on a feeder layer of mouse embryonic fibroblasts (MEFs) [1]. It was later discovered that this MEF layer could be replaced with a basement membrane matrix extracted from mouse sarcoma cells such as Matrigel™ [8]. While these advances have enabled the culture of pluripotent stem cells outside of the body, the field requires culture conditions that are primed for clinical translation such as those that are scalable,

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defined, reproducible and xeno-free. High throughput screening strategies have been adopted to search for substrates that achieve these goals [9].

The critical role of the supporting substrate in maintaining pluripotency of human stem cells *in vitro* has been apparent since their derivation. Growth substrates to recapitulate the extra cellular matrix (ECM) such as Matrigel™ or its components, such as laminin, have been commonly used [8]. More recently, substrates bearing epitopes that are capable of interacting with cells have been developed, for example Synthemax™ is an acrylate substrate conjugated to RGD peptide derived from vitronectin that can support self-renewal of hESCs [10]. The RGD ligand is a cell adhesive peptide that interacts with cell surface integrins [11]. Integrins and other cell adhesion molecules (CAMs), such as cadherins have all been implicated in regulating cellular behavior from maintaining pluripotency to directing differentiation [12]. Advances in the characterization of stem cell interaction with their environment has demonstrated that material physicochemical properties including chemistry, topography, geometry and stiffness also play an active role in modulating stem cell fate, particularly demonstrated with mesenchymal stem cells (MSCs) [13–16] (Fig. 1).

In the body, stem cells reside in a complex niche and receive a multitude of cues from the surrounding ECM, cell–cell contact and soluble factors contained within the aqueous milieu. In addition, the same stimuli may trigger a different biological response depending on the stem cell type. This and other complex structure–function interrelationships, some of which are not fully known, hinder a rational approach in the design of stem cell culture substrates, as it is difficult to predict how a given material property or combinations thereof will bias stem cell fate. More recently, the discovery of naïve states of hESCs and the difficulty in optimizing their culture conditions emphasizes the need for methods to keep pace with the rapidly evolving field [17]. Therefore, researchers have adapted high throughput screening (HTS) strategies to identify culture substrates that are appropriate for stem cell culture [9]. HTS has been utilized in a pharmaceutical setting facilitating early stage drug discovery since the 1980s. Libraries of compounds can be assayed for activity against a biological target to generate lead candidates essentially when structure based design is not possible. Such approaches rely on innovation in robotics for automation, robust biological assays to minimize false positives and high content analytical tools [18]. Adoption of the HTS strategy to accelerate the discovery of materials that can direct stem cell fate began around a decade ago [19,20]. By applying combinatorial methods used in conventional HTS, the structural diversity of polymer libraries can be exponentially increased [20]. In addition, the design of material libraries can be guided by the outcome of biological activity. For this, a suite of high throughput materials characterization techniques is also required to generate comprehensive datasets that can be correlated to biological activity using statistical methods that identify structure activity relationships (SARs) in a systematic and unbiased fashion to enable a more rational approach to optimize materials identified from such screens [21–23] (Fig. 2).

Synthetic materials allow for greater manipulation and control of physical and chemical properties compared to biological substrates, lending to design of modular systems that can be simplified to uncouple substrate effects. In addition, for clinical applications, consistent material quality and function can be assured with fully characterized synthetic substrates, however it remains to be seen if these materials can recapitulate the complex nature of biological matrices. Nonetheless, HTS strategies can help to discover influential material properties to feedback into the design of robust differentiation systems, aid the isolation of rare or difficult to culture cell populations and begin to unravel complex molecular pathways underpinning the identified cell–material interaction.

This review will focus on an overview of the HTS systems designed to probe the interaction between material properties and stem cell phenotype, including surface chemistry, topography, elasticity and 3D micro-environments.

2. Substrate chemistry

Tissue culture polystyrene (TCPS) is a widely used synthetic growth substrate suitable for various cell types including human mesenchymal stem cells (hMSCs). However, simple substrates such as TCPS have limited cellular interaction and usually require coating with ECM proteins and/or soluble factors from the culture medium to modulate the behavior of adherent cells [24]. This has led to the development of a new wave of synthetic growth substrates that have a broad range of surface chemistries to elicit a particular cell response. The surface chemistry of materials has been used to achieve the desired biomolecular adsorption from the culture medium to control cell response and/or act in itself as a ligand for cellular interaction. HTS of proteins, peptide fragments or chemical moieties presented at the substrate surface, to invoke a desired response (*e.g.*, maintaining pluripotency or directing differentiation toward a specific lineage) have been widely explored and will be discussed in this section.

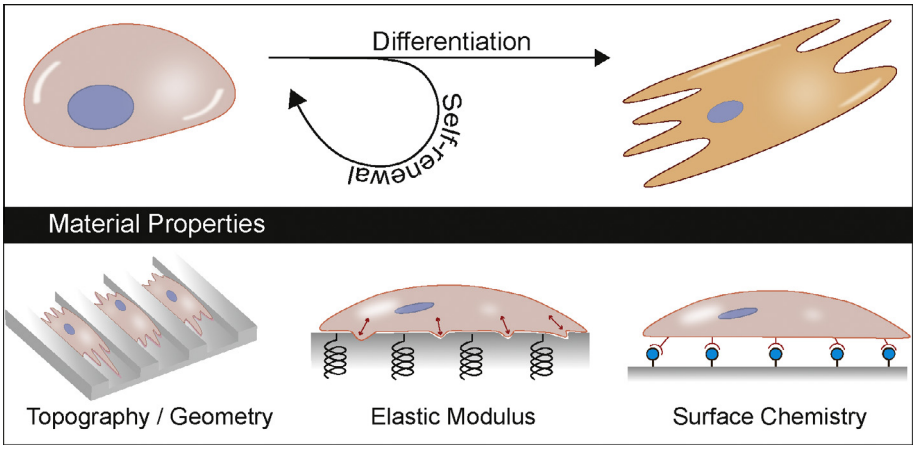

Fig. 1. The culture substrate that stem cells adhere to can harness material properties such as topography, patterning, elastic modulus, surface chemistry and combinations thereof, to influence stem cell fate.