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Integrating concepts of material mechanics, ligand chemistry, dimensionality and degradation to control differentiation of mesenchymal stem cells

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ABSTRACT

The role of substrate mechanics in guiding mesenchymal stem cell (MSC) fate has been the focus of much research over the last decade. More recently, the complex interplay between substrate mechanics and other material properties such as ligand chemistry and substrate degradability to regulate MSC differentiation has begun to be elucidated. Additionally, there are several changes in the presentation of these material properties as the dimensionality is altered from two- to three-dimensional substrates, which may fundamentally alter our understanding of substrate-induced mechanotransduction processes. In this review, an overview of recent findings that highlight the material properties that are important in guiding MSC fate decisions is presented, with a focus on underlining gaps in our existing knowledge and proposing potential directions for future research.

1. Introduction

Mesenchymal stem cells (MSCs) isolated from bone marrow have great potential as a cell source for regenerative medicine due to both their relative ease of isolation and their ability to undergo differentiation towards multiple lineages [1–3]. Initially, the use of biochemical factors to induce controlled differentiation was seen as a key aspect of their effective clinical translation [4]. However, over the last decade there has been much research carried out on the role of the material properties of the substrates that MSCs are seeded onto, or embedded within, in guiding differentiation. While substrate mechanics has long been known to have an impact on cellular activity [5], a seminal manuscript by Engler et al. first provided evidence that MSC differentiation could be directed by substrate mechanics [6]. This discovery brought about renewed interest in the field of cellular mechanotransduction, with much of the research focused on characterizing the cellular signaling mechanisms involved in sensing and responding to two-dimensional substrate stiffness. In this review, we do not cover the various different cellular processes thought to be involved in mechanotransduction, but point the interested reader to several excellent reviews on this topic [5,7–9]. Instead, here we focus on the material parameters that are known to impact cellular mechanotransduction and present these as design choices that must be carefully considered when developing a biomaterials-based study. To illustrate the importance of these material parameters, in Section 2 we introduce the molecular clutch hypothesis, as it provides an excellent framework within which to explore the role of biomaterials design choices.

The majority of existing research has focused on the role of substrate stiffness in guiding cell fate. Conversely, the relative importance of other material properties and how they may interact with stiffness cues has seen less focus, leaving some gaps in our knowledge and presenting opportunities for further discoveries. It has become increasingly apparent that elastic modulus alone is not the sole material property governing the mechanotransduction response of MSCs and that there is significant interplay between mechanics and properties such as ligand chemistry and substrate degradation [10–14]. Additionally, the majority of existing research has been carried out using two dimensional (2D) substrates. Mechanotransduction is likely to be inherently different in the three dimensional (3D) environments employed in most therapeutic strategies using MSCs [15]. In the few studies that have investigated MSC response to 3D materials, there have been notable differences in comparison to behavior on 2D surfaces [11,12,16]. As a result, there is a need for investigation of MSC response to material properties in 3D substrates, particularly in macro-porous environments, which are considerably more com-

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plex from a topographical and mechanical viewpoint [17]. Furthermore, due to the complexity of separating material variables in such experiments, novel materials science approaches are required to enable single variable studies to reveal their relative importance and potential non-additive outcomes.

In this review, we summarize recent findings that highlight the importance of materials in guiding MSC fate decisions, underline gaps in our existing knowledge, and propose potential directions for future research. First, we describe the development of myosin-mediated traction, which is the basic mechanism underlying cellular mechanosensation of substrate mechanics. Following this, we highlight several specific material properties that recent studies have revealed to be important in directing MSC differentiation. To conclude the review, we discuss new materials strategies and experimental techniques that have the potential to lead to an increased understanding of these phenomena towards the ultimate goal of engineering effective regenerative medicine therapies.

2. Cellular mechanotransduction of material stiffness

In order to appreciate the importance of material properties in directing MSC fate, it is first necessary to understand the basic principals by which cells sense and respond to their local mechanical environment, a process which is termed mechanotransduction (Fig. 1). In this context, the dominant mechanism proposed in the field is that cells sense the stiffness or rigidity of the surrounding substrate through integrin–ligand attachments [8]. At the interface between cells and the substrate, ligands presented on the surface of the substrate are recognized and engaged by integrin receptors located at the cell membrane, which in turn enables the binding of these integrins to the actin cytoskeleton within the cell. This results in a tensile force at the cell–substrate interface, as the actin that forms the cytoskeleton is constantly flowing towards the center of the cell due to the action of myosin motors in a process termed retrograde flow [18,19]. The tension developed at this interface is proportional to the resistance provided by the substrate, and if the tension generated is high enough, the adhesion can mature into what is known as a focal adhesion complex. Both the development of tension and the maturation of focal adhesion complexes are believed to trigger the signaling processes that alter cellular activity including spreading, migration and differentiation [8,18].

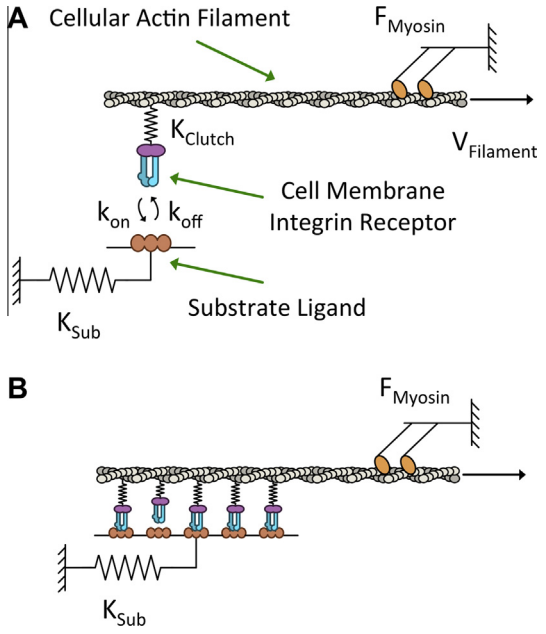

Fig. 1. The molecular clutch model of integrin–ligand interactions. (A) Forces applied by myosin motors (F_{Myosin}) result in the retrograde flow of actin filaments ($V_{Filament}$). This flow is resisted by the formation of bonds, termed molecular clutches, between actin filaments and the substrate. The formation of these bonds is initiated by the stochastic binding and unbinding, at rates k_{on} and k_{off} respectively, of integrin receptors present at the cell membrane to ligands presented on the substrate surface. This enables the substrate bound integrins to bind with actin filaments resulting in a connection between the substrate and actin filaments. Subsequent rearward motion of actin leads to the deformation of both the clutches and the substrate, with the tension developed in the filament proportional to the stiffness of both the clutches (K_{Clutch}) and the substrate (K_{Sub}). (B) As several molecular clutches can bind to a single actin filament, the mechanical resistance sensed by a cell is defined by the number of potential clutches, the clutch stiffness (K_{Clutch}) and the clutch binding rates in addition to the substrate stiffness (K_{Sub}). For a given cell type, the density and identity of the ligands presented by the substrate governs the clutch characteristics and are therefore important material parameters in studies of cell–substrate mechanotransduction. Schematic adapted from Refs. [22,42].

Importantly, several models have been developed to capture the complexity of the interactions at this interface and to provide a better understanding of the material properties that are key to defining the tensile forces developed by cells [20]. The most prominent of these models is based on the hypothesis that the integrin–ligand interface acts as a molecular 'clutch' [21–23]. In this model, myosin motors pull an actin filament rearward towards the 'center' of a cell with a force F_{Myosin} and at a velocity $V_{Filament}$ (Fig. 1). Integrin–ligand clutches reversibly and stochastically engage and disengage at rates k_{on} and k_{off}, respectively, and generate resistance to the rearward flow of actin. This causes stretching of the integrin–ligand clutches and their eventual failure at a force dependent rate k^*_{off}. The forces developed by this process are balanced by deformation of the substrate, resulting in a substrate strain X_{Sub}. Therefore, resistance to loading and resulting tension developed at the adhesion is defined by both the stiffness of the bound clutches K_{Clutch} and the substrate stiffness K_{Sub} [22]. In this framework, the stiffness of the clutches is defined by the number, stiffness and binding rates of integrin–ligand bonds, which in turn is determined by the density and identity of the ligands present on the material surface and the cell type. This results in a complex relationship between cellular tension and substrate stiffness, with several different modes of behavior [22,24]. However, from a materials perspective, the important variables defining cellular mechanotransduction can be identified as material stiffness, ligand density and ligand identity. It is also worth noting that additional factors such as dimensionality can have profound effects on the presentation of these properties to the cells, as will be further discussed in Section 3.4.

3. Material properties and MSC differentiation

In this section, we highlight recent studies that have advanced our understanding of the relationship between material properties, cellular mechanotransduction and MSC differentiation. Initially, we discuss intrinsic material properties, before moving on to discussing the changes brought about by transitioning dimensionality from 2D to 3D.

3.1. Substrate stiffness

Engler and co-authors were the first to demonstrate that MSC fate could be guided by substrate mechanics [6]. They seeded MSCs onto collagen-coated polyacrylamide substrates with different levels of stiffness and assayed for markers of differentiation (morphology, gene transcription and protein expression). The results revealed that MSCs show markers for neurogenic lineages on low stiffness substrates (0.1–1 kPa), myogenic lineages at intermediate stiffness (8–17 kPa) and osteogenic lineages at the highest stiffness