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Review

Evolving insights in cell–matrix interactions: Elucidating how non-soluble properties of the extracellular niche direct stem cell fate

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ABSTRACT

The role of soluble messengers in directing cellular behaviours has been recognized for decades. However, many cellular processes, including adhesion, migration and stem cell differentiation, are also governed by chemical and physical interactions with non-soluble components of the extracellular matrix (ECM). Among other effects, a cell's perception of nanoscale features such as substrate topography and ligand presentation, and its ability to deform the matrix via the generation of cytoskeletal tension play fundamental roles in these cellular processes. As a result, many biomaterials-based tissue engineering and regenerative medicine strategies aim to harness the cell's perception of substrate stiffness and nanoscale features to direct particular behaviours. However, since cell–ECM interactions vary considerably between two-dimensional (2-D) and three-dimensional (3-D) models, understanding their influence over normal and pathological cell responses in 3-D systems that better mimic the *in vivo* microenvironment is essential to translate such insights efficiently into medical therapies. This review summarizes the key findings in these areas and discusses how insights from 2-D biomaterials are being used to examine cellular behaviours in more complex 3-D hydrogel systems, in which not only matrix stiffness, but also degradability, plays an important role, and in which defining the nanoscale ligand presentation presents an additional challenge.

1. Introduction

1.1. Introduction to mechanotransduction

As the field of biomaterials has evolved over the past decades, researchers have shifted from developing materials that were merely tolerated by the body to creating those that elicit a specific response. Nowhere is this more prevalent than in the fields of tissue engineering and regenerative medicine, in which researchers often aim to form scaffolds that, in addition to providing three-dimensional (3-D) structural support for tissue growth, also direct cell response. The means by which cellular behaviours are governed by soluble chemical messengers are well established. Signalling molecules such as growth factors interact with cells to trigger various pathways involved in stem cell differentiation and extracellular matrix (ECM) formation, among others. In addition to their interactions with soluble cues, however, cells are also influenced by adhesive interactions with their ECM, applying physical forces to it, sensing its deformation and even remodelling it. Like soluble factors, these interactions similarly affect cell behaviour.

The importance of cell–ECM interactions in relaying mechanical signals has long been recognized, but it was not until the early 1990s, when Ingber and colleagues attached magnetic beads to cells and applied a twisting moment, measuring the resistance of the beads to twisting, that the field truly expanded. It was these pioneering experiments that demonstrated that the cell cytoskeleton behaved like a "tensegrity" structure, an interconnected unit that could resist applied forces as an integrated structure [1]. These insights created tremendous excitement in the new field of cell mechanotransduction, which aimed to elucidate the role of mechanical forces in directing cell behaviour. The field of mechanotransduction concerns interconnected phenomena by which cells both respond to applied forces and exert forces on their surrounding ECM. Such physical forces result in changes in cell morphology and cytoskeletal structure, which fundamentally influence cell response.

The response of cells to applied and intracellularly generated forces in their interactions with the ECM, however, is only one aspect of how the ECM influences cell behaviour [2]. Among other effects, cells similarly respond via mechanotransductive effects

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caused by changes in nanoscale surface topography and ligand presentation. In 2004, seminal work by Spatz and colleagues [3] used non-adhesive polymeric substrates functionalized with precisely spaced adhesion peptides to reveal that cells are highly sensitive to inter-ligand spacing. Substrates patterned with ligands spaced up to 58 nm apart fostered cell attachment and spreading, whereas a distance of 73 nm or more was too great to support efficient adhesion. These and other nanoscale surface features appear to play important roles in directing stem cell differentiation and myriad other effects. Mesenchymal stem cells (MSC) have been shown, for example, to respond by triggering osteogenic differentiation when exposed to nanoscale pits that are slightly disordered as opposed to arranged in aligned, square patterns [4]. It is because of the complex interplay between these interrelated mechanotransductive effects that targeting the nanoscale features of the ECM remains an important means by which to direct cell fate.

Despite these important insights into the role of the ECM in directing cell behaviour, most knowledge in this field has been gained by studying cells cultured on two-dimensional (2-D) substrates. While 2-D systems have provided invaluable insights into cellular mechanisms, the unnatural morphology and polarity of cells residing as a monolayer, as well as the high stiffness of many cell culture substrates, artificially influences these behaviours, resulting in altered matrix synthesis and unphysiological cell migration and differentiation. For example, fibroblasts are spindle-shaped when cultured in 3-D collagen gels, with long extensions attached to matrix, whereas on 2-D collagen-coated surfaces, they form numerous stress fibres instead [5].

Since 2-D cell culture inherently misrepresents the *in vivo* behaviour of most cell types [6–8], there has recently been a shift towards 3-D tissue culture systems. These include hydrogels based on biopolymers such as collagen, hyaluronic acid and alginate [5,9]. However, as biologically derived systems are poorly defined in terms of their nanoscale architecture and are subject to batch-to-batch variability, a wide range of synthetic polymers have also been developed, including poly(ethylene glycol) (PEG), poly(caprolactone), poly(vinyl alcohol) and poly(glycolic acid), among others [9,10]. These systems, in which researchers are beginning to assess the effects of mechanotransduction and nanoscale ligand presentation in 3-D, are likely to be of great benefit to the fields of tissue engineering, regenerative medicine and stem cell biology [11]. The present review discusses these interrelated topics and addresses mechanotransduction and cell response in 2-D. It also examines how the 2-D cell response often lacks translatability to more *in vivo*-like 3-D situations, and discusses synthetic hydrogels, whose characteristics, including stiffness, degradability and ligand presentation, can be tuned to influence mechanotransduction in 3-D. The paper ends by discussing how the field might best progress to harness understanding of mechanotransduction to direct cell behaviour for therapeutic purposes.

1.2. Integrin-mediated cell–ECM interactions in tissue engineering and regenerative medicine

The emerging fields of tissue engineering and regenerative medicine seek to "apply the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [12], often using biomaterial scaffolds [13] to achieve these aims. Stem cells are widely used in these fields because of their ability to self-renew and differentiate into tissue-specific lineages [14,15]. This requires understanding of fundamental cellular behaviours, particularly the interplay between cells and their ECM. Since many biomaterials aim to simulate the ECM in order to direct cell differentiation and localized tissue formation, a more in-depth understanding of how the ECM directs cell behaviour is of critical importance.

Differentiation of stem cells in cell culture systems has typically been achieved using soluble chemical differentiation factors (e.g. dexamethasone for osteogenesis, insulin for adipogenesis and hydrocortisone for smooth muscle cell differentiation). However, ECM characteristics may also be harnessed to direct cell behaviour, in combination with [16], or often without the need for soluble factors [4,17,18]. Where ECM properties have been shown to induce terminal differentiation (as opposed to merely affecting transcript expression), mechanotransduction-mediated effects such as cell shape and cytoskeletal tension appear to be critical [19,20]. Approaches towards harnessing extracellular cues to precisely control stem cell fate therefore first require an understanding of and then an ability to exploit the cell's interactions with its ECM.

The ECM is a complex network of molecules that fulfils multiple roles within each tissue, the composition and resulting mechanical and biochemical properties of which vary considerably between different tissue types. In addition to providing structural support, strength and elasticity, it guides various cellular processes that influence metabolic activity, proliferation and differentiation, among others. The ECM accomplishes these functions by acting as a substrate for cellular adhesion, polarization and migration. Additionally, cells are able to remodel the ECM via enzymatic degradation [21] and by applying traction forces to it [22–24]. Some of the major components of the ECM are summarized in Table 1 [5,25,26].

Mammalian cells attach to the ECM via integrins, heterodimeric transmembrane proteins consisting of α and β subunits. In humans, 18 α and 8 β subunits exist in 24 possible conformations [27]. On the extracellular side, integrins recognize specific amino acid sequences, allowing them to adhere to various components of the ECM. Intracellularly, integrins attach to the cell's cytoskeleton via a series of linker proteins. As a result, integrins mediate cell–ECM adhesion through a complex feedback mechanism, acting both as mechanosensors and bidirectional signalling receptors, which pass environmental information across the cell membrane and intracellular information to the ECM. Because the direct mechanical link between the ECM and the cell cytoskeleton is mediated by integrins, mechanical signals are carried directly to the nucleus. As a result, this mechanism is more efficient and faster than other signalling methods, taking $\sim$2 μs to propagate 50 μm, compared with $\sim$25 s for small signalling molecules and $\sim$50 s for motor proteins [28,29].

Cell–ECM interactions are initiated by the binding of integrin receptors to ECM ligands (Fig. 1). This is induced by allosteric conformational changes on either the extracellular or intracellular end [30], followed by formation of protein aggregates called focal adhesions (FA) on standard 2-D surfaces. FA link integrin clusters to actin filaments, acting as cytoskeletal anchor points that transduce intracellular forces across the membrane to the ECM. This results in activation of the ERK/MAPK (extracellular signal-related-kinase/mitogen-activated protein kinase) [31] and RhoA/ROCK (Rho-associated protein kinase) pathways [32]. During clustering, actin polymerization occurs, radiating outward from the clusters. Myosin II molecules between actin clusters contract, and the force generated stimulates Src kinase-dependent lamellipodial extension and lateral separation of clusters. This enables cell spreading and is followed by subsequent retraction of myosin II, causing inward movement of clusters and increasing cytoskeletal tension [33]. It is across these stiff fibres that mechanical forces are transduced throughout the cell and across the membrane. Lateral integrin mobility and clustering is therefore required for efficient cell spreading and motility of anchorage-dependent cells on 2-D surfaces [34–37], and is a primary means by which cells interact with the extracellular environment.

In cells fully embedded within a 3-D collagen matrix, however, focal adhesion proteins such as vinculin, paxillin and talin, among

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