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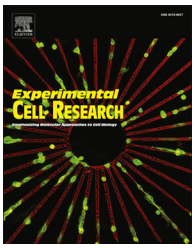

Review Article

Regulating tension in three-dimensional culture environments ☆

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ABSTRACT

The processes of development, repair, and remodeling of virtually all tissues and organs, are dependent upon mechanical signals including external loading, cell-generated tension, and tissue stiffness. Over the past few decades, much has been learned about mechanotransduction pathways in specialized two-dimensional culture systems; however, it has also become clear that cells behave very differently in two- and three-dimensional environments. Three-dimensional in vitro models bring the ability to simulate the in vivo matrix environment and the complexity of cell–matrix interactions together. In this review, we describe the role of tension in regulating cell behavior in three-dimensional collagen and fibrin matrices with a focus on the effective use of global boundary conditions to modulate the tension generated by populations of cells acting in concert. The ability to control and measure the tension in these 3D culture systems has the potential to increase our understanding of mechanobiology and facilitate development of new ways to treat diseased tissues and to direct cell fate in regenerative medicine and tissue engineering applications.

Contents

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Introduction

The processes of development, repair, and remodeling of virtually all tissues and organs are dependent upon tension at the cell level [1]. Genetic and soluble factors strongly regulate growth, but mechanical forces clearly guide the formation of structures [2]. Mechanical signals are also known to contribute to many pathological states including sclerotic diseases and cancer and have been implicated in determining lineage fate for stem cells [3,4] demonstrating our need for a fundamental understanding of mechanobiology for treating and hopefully preventing disease [5].

Tension acts both as a potent stimulus to the cell and a driver of extracellular matrix (ECM) reorganization. Understanding how cells transduce and generate tension has been studied intensely over the past two decades, predominantly in two-dimensional culture systems. Much has been learned about the importance of specific proteins involved in cell–cell and cell–ECM adhesions [6], the role of cytoskeletal tension [7], and mechano-specific signaling pathways [8]. Three-dimensional (3D) culture systems have been used extensively to study the "dynamic reciprocity" between cells and ECM involved in remodeling, but cell tension is quantified in only a limited number of studies. In Section 2 we provide a brief background of the study of mechanobiology in 3D protein matrices, the most utilized in vitro models systems consist of cells entrapped in reconstituted collagen and fibrin gels, and discuss the concept of "dimensionality." In these systems, the ECM transmits external forces to cells, and cells exert traction on the ECM [9]. The embedded cells establish a homeostatic tension level [10] with the surrounding matrix stress, and the tension level is, in turn, a critical regulator of cell motility, proliferation, phenotype, and ECM remodeling [11].

In this review, we describe the role of intrinsic and global stiffness in the regulation of cell-generated tension and cell behavior in 3D collagen and fibrin matrices. We highlight recent advances in the modulation and quantification of cell tension which facilitate more refined study of the effects of tension on cell behavior in these 3D systems. The focus of the review is limited to mesenchymal cells cultured within statically constrained gel systems; for cell and matrix responses to cyclic stretch please see the review by Elson and Genin in this volume; for endothelial cell network formation in protein matrices, please see the review by Morin and Tranquillo in this volume.

In these protein gel systems, the tension level is regulated by a variety of factors including the intrinsic stiffness of the matrix and local ligand density as discussed in Section 3, and whether the cell-populated gel is cultured rigidly attached or free-floating as discussed in Section 4. In Section 5 we provide a detailed discussion of methods for graded control of tension (level and direction) which involve more refined manipulation of the global boundary conditions. We also highlight the interaction between soluble and mechanical cues in the regulation of cell tension. In Section 6 we draw general conclusions and look to the future of mechanobiological studies in 3D gels.

Background of mechanobiology in 3d

In the 1980s, soft wrinkling culture surfaces [12], tunable stiffness substrates [13], and dynamic stretching devices [14] were developed which allow systematic study of how cells apply traction to their surroundings and how external loads affect cell behavior [15]. However, the importance of tension on cell behavior was actually highlighted in 3D matrix culture much earlier. Cell behavior has been studied in 3D matrices of clotted plasma and lymph since the early 1900s (see review by Grinnell and Petroll [11]), and over 50 years ago Weiss [16] demonstrated that cells locally reorganize fibers in a fibrin gel clot model between cell explants indicating long-range effects of traction forces. In the early 1970s, Elsdale and Bard [17] cultured cells on and in reconstituted collagen gels and found that they retain in vivo-like bipolar spindle morphology indicating lines of tension, and Bell [18] systematically studied the effect of free-floating and rigidly anchored boundaries on the structure of collagen gels compacted by fibroblasts of different proliferative potential. In the next decade, Stopak and Harris [19] found that fibroblast traction in 3D collagen matrices is sufficient to form patterns of tension, compression, and fiber alignment with similarities to wrinkling caused by cell traction on thin polymer membranes [12].

These biopolymer culture models provide a tissue-like spatial arrangement missing in 2D culture, and reveal many differences in cell behavior on flat surfaces and within a fibrous matrix. Most notably, cell morphology is markedly different in gels than on 2D surfaces [20–22]. Cell growth [17], motility [21,23], differentiation [24], tumorigenicity [25], and response to soluble factors are also altered when cells are extracted from 3D tissues and cultured on 2D substrates [26]. Reasons for discrepancies in cell behavior between 2D and 3D environments are an active topic of debate in the literature [27]. "Dimensionality" itself is not an independent stimulus, rather a complex set of factors that must be decoupled to better understand the critical determinants of cell behavior (see reviews [28] and [29]). Cell-generated tension in 3D matrices, the focus of the present review, is affected by many factors including cell morphology, adhesion, soluble factors, and the resistance of the cells surroundings to deformation (effective stiffness), all of which differ between 2D and 3D systems. Cell morphological states and migration are restricted by the fibrous meshwork such that cells spread and align along fibers and cells need to squeeze through (ameboid-like) or enzymatically degrade the proteins if the fiber mesh is dense and/or cross-linked [11]. Further, specialized cell–matrix adhesions [30] are formed in 3D systems with more symmetric adhesions over the surface of the cell which alters the concentration of ligands available for binding [31]. Diffusion of nutrients and growth factors is also limited in 3D gels in a protein density-dependent manner, and the matrix can act as a repository of factors which can be enzymatically released by the cell in a tension-dependent manner [32].

Combinations of ECM components and biological hydrogels (e.g., collagen and fibrin [33], collagen and agarose [34], etc.) are utilized as 3D models of varied complexity to mimic specific tissues and to facilitate particular cell–matrix interactions; however, single protein matrices of collagen or fibrin remain the most widely utilized model systems for the study of mechanobiology in 3D (as reviewed by Pedersen and Swartz [28]). Type I collagen is the most abundant protein found in interstitial tissue and is widely used in tissue engineering applications. Collagen monomers can be obtained as small aggregates by acidic digestion of connective tissues (e.g., rat tail tendon, bovine cartilage, and skin) and by pepsin extraction [20]. Collagen monomers form in vitro by self-assembly when the pH is brought to physiologic level, a

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