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Biomaterials approaches to modeling macrophage–extracellular matrix interactions in the tumor microenvironment

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Abstract

Tumors are characterized by aberrant extracellular matrix (ECM) remodeling and chronic inflammation. While advances in biomaterials and tissue engineering strategies have led to important new insights regarding the role of ECM composition, structure, and mechanical properties in cancer in general, the functional link between these parameters and macrophage phenotype is poorly understood. Nevertheless, increasing experimental evidence suggests that macrophage behavior is similarly controlled by physicochemical properties of the ECM and consequential changes in mechanosignaling. Here, we will summarize the current knowledge of macrophage biology and ECM-mediated differences in mechanotransduction and discuss future opportunities of biomaterials and tissue engineering platforms to interrogate the functional relationship between these parameters and their relevance to cancer.

Introduction

Fibrosis, also termed desmoplasia, and macrophagic inflammation represent two hallmark features of the tumor microenvironment that serve as prognostic indicators for poor clinical outcome [1,2]. Interestingly, both phenomena are also notably present in various other conditions epidemiologically linked to cancer (e.g. obesity, chronic liver disease), and increasing experimental evidence suggests that they contribute to tumor development and progression under these conditions [3,4]. Indeed, it is widely accepted that fibrosis and macrophages promote cancer independently [5,6], and their reciprocal actions propagate tissue dysfunction in other conditions such as obesity [4]. Nevertheless, the functional connections between fibrosis and macrophage functions and the resulting effect on tumorigenesis remain unclear (Figure 1).

Tumor-associated and obesity-associated fibrosis can be attributed to increased presence of activated fibroblasts, which mediate changes in extracellular matrix (ECM) deposition. Activated fibroblasts are typically referred to as cancer-associated fibroblasts (CAF) or myofibroblasts and are characterized by excessive deposition of fibrillar ECM components

including collagen type I and fibronectin. The inherent contractile phenotype of CAFs and myofibroblasts leads to alignment and partial unfolding of individual ECM fibrils that, in turn, enhances fiber thickness, stiffness, and exposure of cryptic binding domains [7–9]. Collectively, these compositional, structural, and mechanical changes of the ECM promote increased tissue density and stiffness, which is used in cancer patients to detect a mass via radiography and/or palpation. Elevated tissue stiffness additionally impacts tumorigenesis by directly perturbing epithelial morphogenesis, promoting local invasion and metastasis [10], and driving further activation of fibroblasts and other stromal cells through altered mechanosignaling [11].

Mechanosignaling is the process in which altered physicochemical stimuli of the ECM are converted into biochemical signals to direct cellular behaviors (Figure 2b).

Mechanotransduction pathways are activated by altered engagement of adhesion receptors (e.g. integrins and CD44) that leads to varied cytoskeletal tension and downstream signaling ultimately controlling many processes relevant to tumor initiation and progression including cell proliferation, migration, and apoptosis [12–14]. Notably, adhesion receptors can also cross-talk with growth factor and cytokine receptors by sharing similar downstream signal transduction networks and thus, altered ECM characteristics can additionally modify cell behavior indirectly. Nevertheless, the role of these signaling processes in guiding macrophage behavior in tumor-associated and chronically fibrotic ECM microenvironments is frequently overlooked.

This review will explore current advances in tissue engineering and biomaterials to study the role of ECM physicochemical properties and mechanosignaling in macrophage behavior as it pertains to tumorigenesis. Breast cancer and obesity will be used as examples of tumor-associated and — permissive states linked to fibrosis and macrophagic inflammation, respectively. First, we will review current knowledge of ECM–macrophage interactions. Subsequently, we will discuss biomaterials-based model systems to study the role of ECM physicochemical properties in driving tumorigenic macrophage functions. Finally, we will identify future opportunities for tissue-engineered model systems to study the relationships of macrophages, the ECM, and mechanosignaling.

Macrophagic inflammation and fibrosis — partners in pathology

Macrophages are hematopoietic cells that have wide physiologic roles in the body ranging from innate immunity to trophic roles during health and disease [15]. Macrophages derive from either circulating monocyte or yolk sac-derived tissue resident pools and can be broadly classified into two phenotypes, referred to as polarization: (1) M1/classically activated macrophages that are pro-inflammatory in response to triggers like interferon gamma (IFN γ) and lipopolysaccharide (LPS) and (2) M2/alternatively activated macrophages which are anti-inflammatory, stimulated by the cytokines interleukin-4 and 13 (IL-4 and IL-13), and are involved in repair and remodeling of tissues (Figure 2a) [16]. Generally, M1 macrophages are considered tumoricidal whereas M2 macrophages are considered tumor promoting [17]. Hence, it is not surprising that tumor cells appear to educate the majority of tumor-associated macrophages (TAMs) to be biased toward an M2 phenotype [18].

During health, fibrosis and macrophagic inflammation are well-coordinated and self-limiting; for example, due to the resolution of mechanical [19] and biochemical stimuli upon completion of wound healing [20]. On the other hand, pathological fibrosis, as present in obese adipose tissue and the peritumoral microenvironment, is characterized by continued and unchecked pro-fibrotic and pro-inflammatory signaling [4,21]. This suggests that fibrosis and macrophages are functionally coupled and that ECM-mediated differences in mechanotransduction may play a role in this process. Indeed, fibrotic remodeling in obese adipose tissue correlates to macrophagic inflammation independently of body mass index [3] and increased stroma stiffness in clinical breast cancer samples positively correlates with the number of infiltrated TAMs, and this correlation is stronger in more aggressive tumor subtypes [22*].

While increasing experimental evidence suggests that ECM physicochemical properties directly affect macrophage polarization [23**,24], CAFs and myofibroblasts further potentiate this process by secreting soluble factors that modulate macrophage activation. For example, both cell types secrete CCL2, which stimulates macrophage recruitment and thus malignancy in both the tumor microenvironment and during obesity [25,26]. When combined, biochemical and mechanical stimuli can alter cell behavior in a manner that goes beyond their pure additive effects [27]. However, it remains to be tested whether such synergistic interactions may also apply to myofibroblast/CAF-derived biochemical and mechanical stimuli and their impact on the tumor-promoting effects of macrophages.

Relevance of mechanotransduction to macrophage phenotype

Although the mechanisms via which fibrotic/desmoplastic ECM remodeling may regulate macrophage polarization are not well understood, changes in mechanotransduction likely play a key role. Macrophages and their precursors are fully capable to adapt their behavior to a variety of compressive and shear stresses, strains, and tissue elasticities during tissue invasion (Figure 2a) [28]. Yet our knowledge of the cellular and molecular signals that may underlie differential macrophage responses to their mechanical environment is limited and currently mostly derived from extrapolating results obtained with other cell types (Figure 2b).

Studies with mesenchymal stem and tumor cells, for example, have revealed that ECM mechanical alterations mediate phenotypic changes by regulating integrin clustering, focal adhesion formation, and cell contractility in a manner that depends on Rho GTPase and Rho-associated protein kinase (ROCK) signaling [10,29]. Stiffness-dependent increases in cell contractility, in turn, cross-talk with growth factor receptor signaling and can up-regulate down-stream events such as phosphatidylinositol 3-kinase (PI3K) signaling, an important participant in oncogenic transformation [30–32]. Furthermore, cellular interactions with stiffer ECM promote nuclear localization of transcriptional regulators of cell-proliferative and anti-apoptotic genes including Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) [3,33,34]. As the self-renewing tissue resident macrophage pool can be recruited to become TAMs [35,36] or significantly contribute to obesity-associated macrophagic inflammation [37], these mechanosignaling pathways may play a role in the proliferation of this compartment.

Although it has yet to be elucidated whether macrophages are regulated through similar mechanisms, compelling evidence indicates that their phenotype is dependent on substrate stiffness. In fact, increased substrate rigidity decreases macrophage pro-inflammatory responses directly [38,39] but may also indirectly affect macrophages by modulating cell shape [40*]. Forcing macrophages into an elongated shape with micropatterned arrays of fibronectin upregulates M2 markers and decreases response to pro-inflammatory stimuli (Figure 3a) [40*]. These findings are further supported by the finding that ROCK represents a master switch in macrophage polarization with ROCK2 inhibition promoting M1 while decreasing M2 markers [41]. Finally, ECM stiffness-dependent changes in cell contractility can stimulate paracrine signaling of macrophage-modulating morphogens; for example, by enhancing mechanoactivation of transforming growth factor β 1 (TGF β 1) [42], a potent immunosuppressive cytokine that modulates macrophages to a more M2 phenotype and reduces pro-inflammatory cytokine secretion [43].

Biomaterials systems for studying fibrosis-associated macrophage mechanosignaling

Macrophages have typically been studied in monolayer culture or in vivo models. While two-dimensional (2D) cultures have led to important new insights, they cannot recapitulate the ECM compositional, structural, and mechanical changes that regulate macrophages during fibrosis. Mouse models on the other hand may not fully mimic human disease and isolated control of select parameters is difficult. We have previously shown that tissue engineered-model systems using biomaterials mimics of the ECM bridge the gap between 2D cultures and in vivo conditions [44,45] and thus offer potential to study macrophage behavior with fibrosis.

Natural biomaterials

Given their intrinsic biochemical and physical similarity to the native ECM, decellularized ECMs prepared by detergent-based extraction offer promise to study macrophages [46]. They can be prepared from cells or tissues prone to increased fibrotic remodeling [3] and are suitable to study macrophage responses to ECM-dependent changes in mechanosignaling or TGF β 1 mechanoactivation. However, decellularized ECMs frequently vary between batches, are difficult to scale up for higher throughput applications, and are mostly prepared from 2D cultures with their inherent limitations. Moreover, these ECMs are typically fragile during handling although improved protocols permit to prepare them more robustly by covalent coupling to their underlying sub-strates [47*]. Controlling functional properties of these ECMs selectively and across multiple time and length scales is still difficult, and due to the limited thickness of such substrates re-seeded cells may respond to the underlying culture plate rather than the ECM itself [48]. Matrigel® and collagen type I-based hydrogels are utilized to circumvent some of these limitations.

Matrigel® is a basement membrane mixture isolated from a mouse sarcoma whose composition varies widely between batches and does not permit mimicry of fibrosis-relevant ECM structural motifs and stiffnesses. The latter can be overcome by mixing Matrigel® with alginate to form interpenetrating networks whose stiffness can be adjusted through

calcium-crosslinks (Figure 3b) [31^{**}]. While this approach maintains constant adhesion ligand density and mimics physiologically more relevant visco-elastic rather than elastic matrix properties [49], it does not exclude an additional confounding factor of Matrigel®, namely that it contains growth factors that can interfere with macrophage phenotype. Collagen type I is devoid of growth factors, but stiffness adjustment is typically achieved by varying concentration, which independently alters cell behavior. Covalent crosslinking via non-enzymatic glycation overcomes this limitation [50], but simultaneously affects hydrogel pore size and thus possibly macrophage behavior [51,52]. Importantly, the source of collagen is important as bovine versus rat tail-derived collagen forms gels with thicker fibers, larger pore sizes, and higher elastic modulus [53]. Thicker or stretched ECM fibrils are typically stiffer than thinner or unstretched fibers [54,55^{**}], which may impact mechanosignaling at the fibril rather than bulk level (Figure 3c). The resulting functional consequences can be tested by altering collagen fibril characteristics independent of bulk stiffness; for example, by adjusting the gelation temperature or pH [53,56]. While both Matrigel and collagen type I offer many advantages including their remodelability by cells and ready adaptability across disciplines, their mechanical range is limited and spatiotemporal control of ECM properties is difficult.

Synthetic biomaterials

Synthetic biomaterials such as polyacrylamide (PA) or poly(ethylene glycol) (PEG) hydrogels allow for more controlled modulation of ECM physicochemical parameters. While PA and PEG hydrogels are inherently non-adhesive, they can be functionalized to enable cell binding by coupling purified ECM components, decellularized ECM, or peptide sequences thereof [57]. For example, PA hydrogels modified with fibronectin or RGD adhesion peptides and adjusted in their cross-linking density through altering the ratio of acrylamide to bisacrylamide are routinely used to analyze stiffness-dependent cell responses [58]. However, PA cannot be remodeled by cells nor can cells be encapsulated into these gels due to monomer toxicity limiting these studies to 2D formats. PEG-based hydrogels circumvent the dimensionality and toxicity shortcomings, but cannot be remodeled by cells either. Introducing and/or removing functionalities (e.g. integrin adhesion sites and matrix metalloproteinase [MMP]-cleavable sequences) through light-dependent mechanisms (e.g. photoinitiated addition, photocrosslinking, or photodegradation [59,61,62^{**}])) or via modular bioclick and bioclip reactions can help to address this shortcoming. Selectively adding or removing functionality at a desired timepoint [63,64] or in a specific location [60,61] additionally provides the opportunity to introduce temporal and spatial control over both chemical and mechanical properties of the respective hydrogel. Despite their advantages, these model systems are often amorphous and lack ECM structural entities that drive macrophage behavior.

To mimic the combination of amorphous and fibrillar components in the native ECM, fibers prepared through electrospinning or polymerization of native ECM components could be integrated into composite hydrogels [65]. In particular, incorporation of fibers into hyaluronic acid-based hydrogels of varying bulk stiffness [66] will be exciting as hyaluronic acid is increased with fibrosis and cancer [67,68] and has been shown to modulate macrophage behavior [23,69]. Integrating varyingly stiff and thick fibers [55^{**}] into

hydrogels of altered bulk mechanical properties will permit to decouple the effects of individual fiber versus bulk stiffness properties on macrophages. Differentiating between these properties will be critical as most current research focuses on bulk mechanical properties, but disregards that fiber mechanics are similarly important and may, in fact, lead to differential cell responses [55**]. Finally, being able to modulate the orientation of such fibers, for example, by unidirectional compression of the fiber-containing hydrogels [70] will enable new insights. This manipulation will allow assessing whether changes in collagen fiber orientation from parallel to radial alignment upon tumor progression can influence macrophage migration [71].

Conclusions and perspectives

While the above described biomaterials strategies allow for multicomponent analysis of macrophage function in response to fibrosis-associated ECMs, these systems are coupled to ever evolving complexity and thus future opportunities. For example, computational strategies may become necessary to analyze the large multifaceted data sets and distinguish individual influences on cell behavior in a dynamic network of exponentially increasing interactions. Studying macrophage behavior in 3D tissue-engineered models furthermore requires high resolution imaging modalities for real-time tracking of cells and subcellular-components, which may not be routinely accessible by many labs. Moreover, it has to be kept in mind that incorporation of macrophages will alter the physicochemical properties of the utilized ECM mimics over time. Assessing the spatiotemporal feedback of macrophage phenotype and ECM compositional, structural, and mechanical properties will be necessary to advance our knowledge of the underlying mechanisms. In addition to evaluating the influence of ECM-mediated stress and strain on immune cells, biomaterials models should reflect the multicellular composition of fibrosis-associated microenvironment. For example, fibrosis is characterized by altered vasculature and thus altered convective and diffusive transport properties. Microfluidic co-culture devices with integrated vascular networks could be used to evaluate the role of transport properties as well as shear and interstitial pressure on processes essential to macrophage function [72,73]. Ultimately, these model systems should be extended to investigate the physical control of other immune cells that play important regulatory roles in fibrosis and/or tumor-associated microenvironments (e.g. T helper cells). In summary, the rapid development of new biomaterials and tissue engineering strategies provides an opportunity to further our understanding of macrophage interactions with fibrotic ECM microenvironments and their relevance to tumorigenesis. Ultimately, this offers the potential to identify new targets for future preventative or therapeutic interventions in order to positively impact the lives of individuals at risk for or living with cancer.

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- of special interest
- of outstanding interest

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Figure 1.

Microenvironmental fibrosis and inflammation mediate cancer progression as well as tissue dysfunction in non neoplastic conditions such as obesity. The cellular mediators of fibrosis, activated fibroblasts (myofibroblasts or cancer activated fibroblasts), recruit macrophages by secreting pro inflammatory cytokines and chemokines and by depositing ECMs with varying composition, structure, and mechanics. Nevertheless, the role of obesity and cancer associated fibrotic ECMs in altering macrophage mechanosignaling and the relevance of these changes to tumorigenesis remain unclear.

Figure 2.

Mechanosignaling regulation of macrophage phenotype. (a) Macrophages (MΦ) derived from either the circulating monocyte pool or the tissue resident macrophage pool must adapt and respond to a variety of stresses and strains during their lifespan. Differentiation into either classically (M1) or alternatively activated (M2) macrophages is directed by chemokine and cytokine input, but also by varied mechanosignaling caused by changes in ECM stiffness and/or cell morphology. (b) Close up view of cell ECM interactions and mechanotransduction. Integrin attachment to ECM activates signal transduction via Rho GTPase mediated actin myosin contractility to direct cellular behaviors. Additional changes include stimulation of YAP/TAZ transcriptional regulators and downstream targets of phosphoinositide 3 kinase (PI3K) that regulate cell growth, FAK, focal adhesion kinase; ROCK, Rho associated protein kinase; YAP, yes associated protein; TAZ, transcriptional co activator with PDZ binding motif.

Figure 3.

Examples of biomaterials systems for studying macrophage phenotypic changes in response to fibrotic ECMs. (a) Geometric control of murine bone marrow derived macrophages (BMDM) by micropatterning drives macrophage polarization to an M2 phenotype with cellular elongation [40^{*}] suggesting that microenvironmental control of cell shape influences macrophage function. (b) Interpenetrating network of calcium crosslinked alginate in Matrigel® to control hydrogel stiffness independent of ligand binding density and porosity revealed that stiffness alone induces an invasive phenotype in MCF10A breast epithelial cells [31^{**}]. (c) Studies comparing cellular stiffness responses on flat hydrogels and synthetic fibrous materials revealed that they were inversely correlated. Unlike 2D hydrogels where greater bulk stiffness increased focal adhesion formation and proliferation, greater stiffness of individual fibers limited the ability of human mesenchymal stem cell (hMSC) to bundle fibers and form focal adhesion complexes [55].

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